Multiple Memory Systems: Contributions of Human and Animal Serial Reaction Time Tasks.

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Abstract

Human memory systems have been divided into two broad domains, one responsible for 'declarative memory' and the other for 'non-declarative memory'. The evidence for multiple memory systems is reviewed with respect to the human SRT, a sensitive measure of non-declarative memory. A qualitative review of the human SRT literature concludes that damage to extrapyramidal brain systems disrupts SRT performance whereas limbic system neuropathology (LSN) leaves performance intact. However, a meta-analysis of the SRT literature with neuropathological patients revealed unexpectedly that patients with explicit memory disorders are impaired on the SRT task, although less severely than patients with extrapyramidal damage. Other evidence suggested that the apparent SRT impairment in humans with LSN might be due to the additional pathology (eg frontal) often evident in these patients. A brief review of the animal evidence for multiple memory systems concluded that, like humans, animals too have multiple memory systems but none of the animal tasks used to model non-declarative memory make good conceptual or behavioural contact with the corresponding human tasks. Thus a novel animal-analogue of the human-SRT task, the 'fan-maze', was developed. Although rats displayed a reasonable ability to perform the fan-maze SRT task it was abandoned due to technical and conceptual problems in favour of a better design. The second new SRT task used intra-cranial self-stimulation to promote prolonged, rapid and continuous responding. A control study determined that the optimal conditions for sequence learning was a single large (2820 trial) session. Intact rats that experienced a switch from the repeating to a random sequence under these conditions demonstrated a clear interference effect, the primary measure of SRT performance. A lesion study used these optimal conditions and showed that small caudate lesions impaired, whereas small hippocampal lesions facilitated, rat-SRT performance. Hence, this second task has proven to be a valid animal-analogue of the human SRT task, as rats performed it in a manner similar to that shown by humans and relied on the same neural substrate to perform the task as humans. In addition, this second task resolved the discrepancy of the LSN meta-analysis. Quantitative findings are reviewed in light of theories and studies presented earlier in the thesis. Limitations of the thesis are identified and suggestions are made as to future SRT research in animals or humans.

Chapter 1

An Introduction to Multiple Memory Systems

Note to the reader. Originally there was no single copy of the total thesis, instead individual chapters were stored in separate files and I simply printed them off as needed. This electronic version of the thesis has been assembled from those separate copies and may well have suffered for it in both the formatting and absence of several figures (that were always printed separately). For this reason the page numbers almost certainly do not match up with the numbers in the 'Contents'. My apologies if this makes it difficult to read. M Christie, 30-May-2007

General Introduction

Within the last 20-30 years considerable interest has been generated in the possibility that memory is not a unitary construct but rather is a collection of independent and (anatomically and functionally) dissociable systems. The concept of multiple-memory systems has in large part emerged due to two, now commonly documented, observations: that amnesic patients previously considered to suffer a global memory impairment, demonstrate some spared memory functions, and secondly that it is possible for neurologically normal subjects to demonstrate an experimentally induced dissociation between quantifiably distinct types of memory.

This thesis will examine both the likelihood and neurological basis of multiple memory systems, primarily focusing on a systems based approach predicated on the availability of critical information to conscious awareness ('declarative' and 'non-declarative' memory). This will initially involve a discussion of the general evidence for and against the likelihood of multiple memory systems before moving on to a consideration of the theory in light of the evidence provided by one specific human

non-declarative memory task, the Serial Reaction Time task (SRT). Both these sections will also address a number of serious concerns as regards some of the common conceptual and methodological assumptions made in the non-declarative memory literature and it will be shown that while such concerns are well-founded studies published in light of these concerns continue to provide good evidence for both the likelihood of multiple memory systems and the validity of the SRT as a measure of non-declarative memory. As part of both the general discussion and of that concerning the limitations inherent in the non-declarative memory literature a meta-analysis will be presented that addresses a number of questions / concerns introduced during the discussion. Thereafter the thesis will present experimental work that demonstrates an animal analogue of the Serial Reaction Time task developed in order to examine the neurological basis of non-declarative memory in rodent subjects.

This chapter briefly introduces the history of thinking about multiple memory systems. It also examines several theories concerning the likelihood, and possible nature, of multiple memory systems before discussing a recent critique of the paradigm. The chapter concludes with a summary of the aims and objectives of the thesis.

1.1 The History of Multiple Memory Systems

1.1.1 Multiple Memory Systems Prior To H.M.

Were you to ask the man-on-the-street when the possibility of multiple, independent, memory systems was first posited they would, I suspect, pick a point within the last 100 years, largely due to the influence of modern theorising about human cognition and recent advances in neuro-medical techniques (e.g. neuroimaging). It would, therefore, come as something of a surprise to our man-on-the-street to learn that the possibility of multiple memory systems has a long and distinguished (albeit somewhat sporadic) history stretching back to classical antiquity. The history of the study of memory contains a number of instances of philosopher-scientists who advanced and discussed the possibility of multiple memory systems, even as far back as Socrates and Aristotle who, while never specifically discussing the possibility, posited systems which either allowed, or suggested, multiple memory systems.

The first wave of systematic thinking on multiple forms of memory began in the mid 17th century. While no-one explicitly addressed the multiplicity or unitary nature of memory, the distinctions made within these discussions often suggest an unspecified assumption of multiple memory systems. For example, Descartes (1649) discussed a non-conscious form of memory in his *The Passions of the Soul.* Gottfried Leibniz (1704) described a similar (non-conscious) type of memory. Darwin (1794) distinguished between voluntary and involuntary recollections, again suggestive of multiple systems. Maine de Biran, in his 1804 monograph *The Influence of Habit on the Faculty of Thinking*, specifically postulated the existence of 3 independent forms of memory. In keeping with the popular theory of the time, phrenology, Franz Gall (c.1835) argued the need for a separate memory system for each 'specialised faculty' (eg. music, mathematics etc). However the idea of multiple memory systems fell into disuse shortly after Gall's efforts and didn't surface again until the second half of the 19th century. In 1861, Paul Broca not only reintroduced the concept but was the first person to advance a non-unitary memory hypothesis based on neuropsychological evidence. His seminal observations on selective loss of expressive linguistic abilities can be conceptualised in terms of damage to a particular kind of memory, and the sparing of other linguistic abilities as the ongoing function of different forms of memory: "this special memory [the memory "necessary" for the articulating of words] is in no way related to *other* memories" (emphasis added, see Rosenfield 1988).

In the subsequent decades a small flurry of work on the topic emerged. Carl Wernicke interpreted the symptoms of his eponymous aphasia specifically in terms of damage to a special memory centre distinct from that memory centre damaged in Broca's aphasia. The German physician Ludwig Lichtheim developed this idea at length. In a surprisingly modern assertion the French psychologist Theodule Ribot argued for multiple memory systems in his *Diseases of Memory* (1882) contending that "if, in the normal condition of the organism, the different forms of memory are relatively independent, it is natural that, if in a morbid sate one disappears, the others should remain intact". It is somewhat surprising therefore that it is difficult to find any further discussion of the subject until the 2nd half of this century. One reason for this neglect is undoubtedly the influential position enjoyed by the British associationists at this time who, while discussing memory at length, did so only in the context of a unitary system. However, all such discussions / hypothesise about the possibility of

multiple memory systems should always be seen as temporary and phenomenologically isolated events in a long period of an overwhelming focus on a unitary memory system.

1.1.2 Multiple Memory Systems During the 1950's and 1960's.

As with so much in the modern history of the study of memory the issue of unitary versus multiple memory systems owes a great deal to the impetus gained from the study of the patient H. M., who underwent a complete bilateral resection of the medial temporal lobes. Key among the findings by Scoville and Milner (1957) was the realisation that while H. M. exhibited profound amnesia for recent events his intelligence remained above average and other perceptual and cognitive functions were unimpaired. The well known conclusion from studies of H. M., and consequent animal models of his syndrome, was that the hippocampal formation and adjacent structures supported the acquisition of 'normal' memory. Moreover there appeared to be memory systems that do not depend on the hippocampus and related structures; the first convincing neuroanatomical evidence of multiple memory systems.

Although considerable work during the 1960's had been undertaken examining the distinction between short- and long-term memory this was largely within a unitary memory paradigm which regarded the different forms of memory as located at different positions on a continuum and therefore not independent systems. However, at the same time that the short / long-term distinction was being debated a number of distinctions within long term memory were also mooted that were suggestive of truly independent systems (See Reiff and Scheerer, 1959, and Bruner, 1969). However, much as 100 years earlier, none of this work produced a meaningful impact on the course of memory research and theorising and it wasn't until the early 70's / late 80's that the possibility of multiple memory systems was seriously considered.

1.1.3 The History of Multiple Memory Systems Post 1970

Schacter and Tulving (1994) identify 3 fundamental developments whose confluence gave rise to multiple memory as a major research topic post 1970. The first development was the surprising discovery that severely amnesic patients retained some learning and memory abilities. H. M was again at the forefront of this research, with Milner, Corkin and Teuber (1968) showing that H.M. demonstrated near, or near-

normal, motor skill learning, necessitating the dependence of motor learning on a system different to that for which he suffered such a clear and profound impairment. Similarly, Warrington and Weiskrantz (1968) showed that amnesics retained some ability to perform on fragmented-cue tests of previously encountered material despite being unable to recognise that very same material. This finding, and others like it, demonstrated differing susceptibility of memory tasks to the hippocampal system lesions usually found in amnesic subjects.

The second development was the conceptualisation of the distinction between remembrances and memoria as “two parallel and partially overlapping information processing systems” via the constructs of episodic and semantic memory (Tulving, 1972). Although the distinction would be seriously challenged in later years and undergo considerable revision it served to stimulate discussion and debate about the notion of separate memory systems.

Thirdly, and largely a consequence of the previous developments, was the revelations of remarkable dissociations between what are now referred to as declarative and non-declarative memory performances in neurologically normal subjects. These studies, motivated in large part by the observations of spared learning in amnesic patients, revealed that priming effects on non-declarative memory tests could be dissociated experimentally from performance on standard tests of recall and recognition. These results generated strong support for the contention that brain lesions in amnesic subjects, and experimental manipulations in neurologically normal subjects, demonstrate the existence of different and independent memory systems.

Consequently the field experience a boom such that an enormous amounts of effort is now devoted to answering questions concerned with the nature and substance of different memory systems. However, while the fact that there are different systems is these days almost taken for granted, due to the extensive body of work demonstrating dissociations between memory systems, it is actually far from a proven fact and the remainder of the chapter will explore some of the recent controversy concerning multiple memory systems.

1.2 Neurobiological Evidence for Multiple Memory Systems in Humans.

Despite substantial variation in terminology and detail, there is general agreement within the literature that memory is divisible into two broad domains. The first is responsible for the repetitive learning of common features which are typically unavailable to conscious awareness and deliberate recollection and instead influences behaviour via non-conscious predisposition. The second system is responsible for rapid (often 1-trial) learning of critical features which are available to conscious awareness at the time and may later be deliberately recollected.

A 'terminological maelstrom' (Reber, 1992) has developed to try and characterise this distinction: spatial learning vs. contextual (Nadal, 1994; Jarrard, 1993) and, earlier, general cognitive vs. noncognitive distinction (Hirsh, 1974; Nadal and O'Keefe, 1974; O'Keefe and Nadal, 1978; Mishkin, Malamut and Bachevalier, 1984) are but two of the characteristics. Some theorists have focused on a simple vs. configural / relational learning distinction (Sutherland and Rudy, 1989; Cohen and Eichenbaum, 1991; Shapiro and Olton, 1994, Eichenbaum, Otto and Cohen, 1994). Other theorists have focused on the involvement of conscious awareness with the memory trace, i.e. non-declarative and declarative memory (Graf and Schacter, 1985; Schacter, 1987, Schacter and Graf, 1986) or the declarative vs. non-declarative distinction (Squire and Zola-Morgan, 1988, Eichenbaum, Stewart and Morris, 1990).

Because this thesis focuses on contributions from the SRT task, which is characterised as a test of non-conscious / non-declarative memory, the thesis will have to employ terms associated with conscious awareness. However, this thesis will also focus on animal memory systems, and in particular how animal and human memory systems relate to each other. Thus there is a need for a set of terms that encapsulates both the aware / unaware status of the remembered information and are generalisable to animals. Therefore, the terms 'declarative' and 'non-declarative' memory will be used. Although these terms are not without their own problems when used to describe animal memory systems they are perhaps the least problematic of the various memory dichotomies in this regard. In particular, the use of the terms 'declarative' or 'non-declarative' in relation to animals automatically raises some very difficult conceptual and theoretical issues as regards conscious awareness in animals. Although the terms 'declarative' and 'non-declarative' do assume the presence /

absence of conscious awareness they do not focus on it to anything like the same degree as the 'explicit and 'implicit terms do. Furthermore, while the deliberate declaration of remembered information is usually associated with verbal delivery this need not be the case. It is perfectly possible for both humans and animals to 'declare' remembered information via behaviour. Thus this thesis will use the terms 'declarative' and 'non-declarative' to both avoid the major theoretical difficulty associated with any necessity to posit conscious awareness in animals, and to enable the relatively unconfounded generalisation between animal and human memory systems.

Regardless of terminology, assuming there are multiple memory systems, is their good evidence for any particular form of memory demarcation? The finding that H.M was severely amnesic yet able to demonstrate learning for which he had no memory produced a profound shift in thinking about amnesia, from global impairment to a more modular system within which different forms of memory operated independently of each other and in which damage to one module (system) did not necessarily impair a different one. Once it was understood that H.M.'s impairment (and other patients with 'organic amnesia') was a result of damage to the hippocampal system and related pathways considerable work was devoted to ascertaining what specific role the hippocampus plays in memory. However the concern of this thesis is not with the hippocampus *per se*, but rather with the opportunity that hippocampally based memory impairments offer for the demonstration of impaired and spared memory functions. While not neglecting the hippocampus based impairments, the following discussion focuses on those memories that are spared after hippocampal damage and in doing so demonstrates evidence of a functional dissociation between neural substrates responsible for different forms of memory.

1.2.1 Skill Learning

Many studies have demonstrated that amnesic patients can acquire a variety of skills at an apparently normal rate. Examples include motor skills (Brooks and Baddeley, 1976), perceptual-motor skills (mirror-tracing, Milner, 1962; rotary pursuit, Corkin, 1968; bimanual tracking, Brooks and Baddeley, 1976, Cermak et. al. 1973; Cohen, 1981; serial reaction time task, Nissen and Bullemer, 1987), perceptual skills (Cohen and Squire, 1980; Martone et. al,. 1984; Moscovitch et. al. 1986) and cognitive skills (Tower of Hanoi, Squire and Frambach, 1990; Saint-Cryer et. al. 1998; arithmetic

algorithms, Charness et. al. 1988; Kinsbourne and Wood, 1975; Milberg et. al 1988). Moreover the skills acquired can be based on novel material (Musen and Squire, 1991) which counters a common criticism of early studies of this type, namely that amnesics only showed spared learning for familiar (premorbid) stimuli. Similarly, skill learning is intact in monkeys with large lesion of the temporal lobe that fail tasks of object recognition (Zola-Morgan and Squire, 1984).

Recently, there has been interest in the possibility that more complex versions of skill learning, such as probability learning or artificial-grammar learning, might also depend on nondeclarative / procedural memory. In artificial-grammar learning (Reber, 1967) subjects first inspect a group of letter strings that adhere to a finite-state rule system. Subjects are then able to classify new letter strings as either grammatical or nongrammatical at well above chance levels. In a recent experiment amnesic patients were able to classify grammatical and nongrammatical letter strings as well as control subjects despite impaired recognition memory for the items they had encountered in the test (Nowlton, Ramus, and Squire, 1992). While the amnesic subjects in this study did score a little below normal subjects in artificial grammar learning (although not significantly so) amnesics in a more recent study displayed essentially identical performance to normal subjects (59.1% vs. 58.3%, respectively; Knowlton and Squire, 1994). Thus it appears that one kind of memory stores declarative information about the specific items that were presented, which is impaired in amnesics. However, the second kind of memory stores information non-declaratively either by abstracting information from the items in the form of rules or by assembling information from items as a collection of associations between item features and their grammatical category. If this division is valid, then it obviously provides good evidence of multiple memory systems.

1.2.2 Priming And Evidence Of Multiple Systems

Given its long history in the memory literature, no discussion of multiple-memory systems would be complete without some mention of priming. Priming refers to the improved facility for detecting or processing stimuli on the basis of recent experience with that stimuli (Cohen and Eichenbaum, 1993) and can occur in the absence of any conscious / declarative awareness of the stimulus (and thus is considered a form of non-declarative memory). Amnesic subjects can show robust

priming effects over a variety of stimulus modalities: word stems (Cermak et. al, 1987; Graf et. al. 1984; Squire et. al 1987, word fragments (Squire et. al. 1987), [stochastic] presentation (Cermak et. al. 1985, 1988) and visually degraded stimuli (Keane et. al. 1988). For example, H.M demonstrated priming for dot-patterns as a result of previous exposure to them, in the absence of any awareness of the episode during which he fist saw them (Gabrieli et. al. 1990). While there has been some uncertainty about whether amnesic subjects display normal or less-than-normal priming effects, there is now considerable evidence that, under some conditions, amnesic subjects can display normal priming effects (for a review see Tulving and Schacter, 1990). Under other conditions, however, amnesic subjects produce less-than-normal effects (Cermak et. al. 1985; Diamond and Rozin, 1984; McAndrews, Glisky and Schacter, 1987; Milner et. al. 1968; Warrington and Weiskrantz, 1968). A commonly accepted explanation for the variation in the degree of sparing of priming is that different priming tasks encourage the use of varying degrees of declarative memory (i.e. are not process pure), and in those tests that encourage the use of declarative memory (in conjunction with non-declarative priming) it is reasonable to expect that amnesic subjects would be impaired relative to controls (Cohen and Schacter, 1993).

Furthermore, unlike declarative memory tests (recall and recognition) priming effects are independent of levels of processing and are generally not influenced by secondary / distracter tasks (Parkin et al 1990; Parkin and Russo 1990). Also unlike declarative memory tests, priming is greatly affected by presentation modality (Tulving and Schacter‘s “hyperspecificity of access”, 1990) such that prior experience of a source picture primes a picture fragment but not a (relevant) word fragment and experience of a word primes a word fragment but not a (relevant) picture fragment. As a result Roediger and Srinivas (in Graf and Masson, 1993) characterise declarative memory tasks as being sensitive to secondary task interference, levels of processing and retention interval, whereas non-declarative memory tasks, (including priming) are deemed to be more sensitive to perceptual or surface features.

There has also been considerable argument as to whether or not spared priming in amnesics is limited to familiar stimuli only. The activation / integration approach contends that priming is due to the activation of pre-existing representations and therefore predicts that amnesics would not be capable of non-declaratively

learning novel stimuli / associations. The memory systems view however suggests that only those memories which are dependent on episodic / declarative memory are subject to consolidation failures (a failure to encode a memory trace in long-term / reference memory) and therefore amnesics would be capable of learning any novel stimuli / associations that did not require these systems. Overall, the evidence supports the memory systems view, especially for novel nonverbal priming capabilities. Studies attempting to demonstrate priming of novel verbal associations are far less conclusive (Cermak, 1988; Shinamura and Squire, 1989). However, learning verbal associations likely also requires semantic processing, which may require episodic memory (Tulving and Schacter, 1990). A compelling demonstration of the variable nature of priming in amnesic subjects is the finding that a pairing-specific reduction in a Stroop task was normal in amnesic patients when the conflicting information was conjoined in the same way (i.e. when each individual stimulus item was both a colour name and had a display colour), but when the conflicting information was not conjoined the amnesic subjects failed to demonstrate a priming effect (Musen and Squire, 1991).

In light of these, and similar, results Tulving and Schacter (1990) offer 5 points of evidence to demonstrate the independence of priming and declarative memory:

1) Amnesic subjects: while having no recollection of the priming episode or the primed material they nevertheless show priming of it.

2) Developmental: while recognition memory improves with age priming is as strong in young (3-year olds) subjects as in older (university student) subjects (but see Rovee-Collier, Hayne and Colombo, in press).

3) Drug dissociation: drugs that impair declarative memory have little or no effect on priming.

4) Functional independence: the two systems appear to operate by different, sometimes opposing, rules, e.g. semantic elaboration has little effect of priming but improves declarative recall, and declarative memory is not modality specific whereas priming is.

5) Stochastic independence: Priming effects are as large for words that subjects declaratively recognise as for words they do not.

1.2.3 Interim Conclusions of Human Studies

Although not all of these arguments discussed above are conclusive in isolation they all indicate the same empirical conclusion. Taken together they provide strong circumstantial evidence for the presence of multiple memory systems. Furthermore, although most accounts of multiple memory systems posit two distinct forms there is some evidence that priming is not only dissociable from declarative memory but also other forms of non-declarative memory, especially motor-skill learning. Studies with Alzheimer's patients show they suffer impaired lexical and pictorial priming but perform a motor skill-learning task (rotor-pursuit) at control levels, whereas Huntington's patients are impaired on motor-skill learning but perform like controls on lexical and pictorial priming (see Butters, Heindel and Salmon, 1990). Similarly, Tulving and Schacter (1990) report the case of a subject with complete global amnesia (unable to remember anything from his previous life, either before the injury or after it) who nonetheless displays evidence for both perceptual and conceptual priming but whose performance on these tasks is stochastically independent. Further compelling demonstrations of the dissociation of memory systems is found in the SRT literature but due to the particular importance of this material to this thesis it will be discussed separately, and in some detail, in the following chapter, and Chapter 4 will discuss the evidence for multiple-memory systems from animal research.

1.3 The Evolutionary Likelihood of Multiple Memory Systems

While the focus of efforts to answer the question of multiple memory systems is largely confined to the neurocognitive and neurophysiological domains there have been contributions from other fields, including work from the field of evolutionary psychology. The following section will briefly review and discuss two such contributions and raise some criticisms of the first contribution in particular, especially that the authors failed to fully extend their promising discussion of the animal evidence into the human domain. However, the ultimate conclusion that multiple memory systems are evolutionarily likely is not seriously threatened by these criticisms and thus serves to strengthen the argument that multiple memory systems are possible, even likely, and in doing so provides *a priori* theoretical grounds to justify an attempt to

investigate the possible existence of multiple memory systems in rodent subjects.

1.3.1 Sherry and Schacter and the Evolutionary Likelihood of Multiple Memory Systems.

Sherry and Schacter (1987) contended that rather than the notion of general laws and a unitary memory system (in humans) being incorrect they are instead incomplete. In that an evolutionary analysis predicts that both generality and specificity are expected characteristics of memory. Sherry and Schacter were concerned with the key question of "whether the evolution of qualitatively distinct memory systems would be expected to occur or whether a single memory system that is characterised by increasing complexity and flexibility is the expected evolutionary outcome". Obviously, evolutionary adaptations need not necessarily lead to multiple memory systems. Selective adaptation could occur within a unitary memory system as long as the system is sufficiently flexible to accommodate any demand placed on it. The primary impetus therefore for the evolution of a multiple memory system is that the environment can impose demands that, by their nature, are not amenable to resolution by a single memory system, regardless of how flexible it is.

Sherry and Schacter's argument is that memory systems necessarily develop with a degree of specialisation, primarily because they arise in response to specific problems, and this intrinsically produces limitations on what the system can do. Therefore an environmental problem that is solvable by one memory system inevitably results in a system that, by virtue of its prior specialisation, is incapable of solving other problems. Consequently new environmental challenges may necessitate the development of a novel and separate memory system. Schacter and Sherry coined the term '*functional incompatibility*' to describe the situation where a pre-existing memory system is unable, by virtue of its specialised nature, to accommodate a novel environmental challenge. Thus the memory demands of a novel situation are functionally incompatible with the behavioural range of the pre-existing system. Their conceptualisation of *functional incompatibility* is a modified version of the idea advanced by Rozin and colleagues (Rozin, 1976, Rozin and Kalat, 1971) who argued that memory (and learning) is an adaptive specialisation shaped by natural selection to solve problems posed by the environment.

Not all novel environments result in new, and independent, memory systems. Some, in fact most, memory problems may be sufficiently similar that they can be effectively solved in similar ways and therefore by the same system. In keeping with this point Sherry and Schacter note that most memory systems are extremely generalisable and deal perfectly well with many different problems either as a result of adaptation or fortuitously (perhaps via the mechanism of exaptation developed by Gould, 1991, but see Buss, et. al., 1998 for a recent critique of exaptation). The consequences, according to Sherry and Schacter, is that while there will be multiple memory systems there will not be altogether that many of them, and those that do exist will deal with a variety of environmental demands. It is only when the pre-existing systems are incapable of being modified to handle a novel situation that a totally new memory system will arise.

Functional Incompatibility and Processing Variant and Invariant Features of an Episode.

In support of their thesis of the evolutionary likelihood of multiple memory systems in humans (and other primates) Sherry and Schacter develop a dichotomous memory system which they label memory systems I and II. Essentially, the systems are a somewhat standard non-declarative vs. declarative memory dichotomy as *System I* supports the gradual acquisition of skill / habit learning, and *System II* supports the rapid (one-trial) learning of specific situations and events. Sherry and Schacter focus on the type of variance extracted by the two different memory systems from the features of the stimuli event to delineate the dichotomy. In this manner they distinguish between System I (non-declarative) memory which detects and preserves *invariances* across episodes and System II (declarative) that detects and preserves *variances* across episodes.

In terms of the functional incompatibility of the systems it will be readily apparent that the preservation of variant and invariant features are contradictory processes and therefore likely to be best handled by functionally independent systems. It is therefore Sherry and Schacter's contention that the data demonstrating dissociations between habit / skill learning and episodic / representational memory indicate the existence of separate memory systems due to one system's inability to handle the critical information of the other system. In order to support this argument, it

is necessary to show that the habit / skill learning system is incapable of handling *variant* features of successive episodes (not just unlikely to, or inefficient at it), and that the episodic / representational system is likewise incapable of handling *invariant* features of successive episodes (again not just unlikely to, or inefficient at it).

Functional Incompatibility in Animals

Sherry and Schacter offer song-learning and food-caching in birds as an example of functionally incompatible environmental demands requiring the evolution of multiple memory systems. There are few grounds for argument against the contention that these two systems are functionally incompatible and the use of one system to drive the behaviour of the other system would result in a serious impairment of reproductive fitness. However, as Schacter and Tulving discuss in the introductory chapter of their book 'Memory Systems' (1994), each species has environmental pressures unique to it (in conjunction with pressures common to all species) and thus species (by definition) evolve differently and this makes it difficult to draw behavioural and cognitive comparisons between species due to the influence of even subtle differences in neural architecture. That brain / behaviour relations in animals are often different (in varying degrees) to human brain / behaviour relations and thus is problematic for the purpose of modelling human behaviour in animals, and especially so for uniquely human behaviours. Although memory is demonstrably not unique to humans there may well be aspects of it that are. It may be that, for example, humans create complex interrelated memory traces which other species cannot and thus while song learning and food-caching are strong evidence for multiple-memory systems in birds, it is only useful as a demonstration of the possibility that multiple-memory systems can evolve and acts only as circumstantial evidence for the likelihood of this in other species, and especially in the 'higher' organisms.

Conclusions on Sherry and Schacter

This thesis is fundamentally predicated on the theory that dissociable / independent memory systems exist, and is willing to accommodate the likelihood that different memory systems evolved due to the inability of one system to handle a novel environmental challenge, necessitating the evolution of an new and independent system. However, while offering evidence of behavioural dissociations in memory (see original article for list of studies), Schacter and Sherry offer no direct evidence that

human declarative / representational memory systems functions by extracting variant features from successive events, nor that human non-declarative / skill-learning system operates by extracting invariant features from successive events. They may well actually do this, but none of the evidence they present addresses those issues, instead the evidence is concerned with demonstrating the dissociation and only provides indirect support for why the separate memory mechanisms evolved.

Sherry and Schacter suggest, as a result of the examples they present, that there is “compelling psychological evidence that gradual learning of certain habits and skills can proceed independently of the ability to remember specific episodes” and that skill-learning and episodic remembering are mediated by different brain structures. While both points have been convincingly demonstrated in the literature this does not, as they themselves noted, necessarily demand the postulation of separate memory systems. The fact that they depend on different brain structures and that they function independently of one another certainly suggests different systems, but does not conclusively prove it. In fact it is entirely possible to postulate that this could be achieved (to quote the authors themselves) by a “single memory system that is characterised by increasing complexity and flexibility”.

Furthermore, Sherry and Schacter’s model is almost exclusively ‘top-down’ in that it is driven by environmental changes and ignores both the ‘bottom-up’ contribution provided by continual mutations in the phenotype and the incessant interaction between these two processes. One of the primary advantages of sexual reproduction is the constant mixing of genotypes it produces. The particular advantage of this is that it produces slight changes in the reproductive fitness of the offspring which allows evolution via natural selection. In contrast Sherry and Schacter’s top-down approach regards natural selection in terms of more abrupt events with relatively dramatic consequences. However, instead of multiple-memory systems appearing relatively quickly as a result of the sudden imposition of environmental demands that a single-memory systems was ill equipped to handle it is possible that very gradual increases in flexibility and function occurred simply due to random genetic mutation over generations. The point that declarative memory is largely cortically based, and non-declarative memory is largely sub-cortically based, supports this hypothesis. Non-declarative memory is a phylogenetically older system and thus depends on

commensurately older brain structures. In contrast declarative memory is evolutionarily more recent and thus depends of more recently evolved brain structures, as well as being more frequently expressed, and of a higher 'quality', in the 'higher organisms' i.e. those with neo-cortices.

Thus while Sherry and Schacter's approach is not wrong it is incomplete. Undoubtedly relatively sudden changes in the environment influenced the development of multiple memory systems, be it by directly encouraging it's development and / or by encouraging the utilisation of 'spare' potential inherent in the system but hitherto unused. However, the majority of the changes are likely to have occurred at a more gradual pace in a 'brick-on-brick' manner. Nevertheless, both these approaches assume an identical reason for why multiple-memory systems developed, because the two forms of memory provide a reproductive advantage over that of a single system. And thus the top-down and bottom-up processes are not competing approaches but are rather complimentary. Therefore, the argument here is not so much with Sherry and Schacter's contention as to the likelihood of the evolution of multiple memory systems but rather with their almost exclusive focus on a top-down approach at the expense of a more balanced account.

In conclusion, systems that seek to winnow either the variant or invariant features of an episode are obviously incompatible in that they process information in a diametrically opposed fashion. It is Sherry and Schacter's failure to clearly show that this is how human memory actually functions that substantially weakens their argument. If, however, it can be shown that skill-learning and episodic memory do function in this manner then it will be a perfectly reasonable consequence to assert that they are functionally incompatible and therefore provide convincing evidence of the need for evolutionary independent memory systems in humans. Thus the current state of their theory of the evolutionary likelihood of multiple memory systems is "not so much incorrect as incomplete"!

1.3.2 Reber and the Evolution of the Cognitive Unconscious.

Although more concerned with an evolutionary perspective of the "cognitive unconscious" (Rozin, 1976) than multiple memory systems *per se'* Reber's (1992) article necessarily rests on the premise that there are independent and dissociable

systems. In particular Reber claims that conscious cognitive processes are phylogenetically far more recent than unconscious processes and thus non-declarative memory functions are not only dissociable from declarative cognitive functions but likely to enjoy a number of advantages over them.

Working from general principle of evolutionary development, Reber distils the work of the 19th century scientist Hughlings Jackson, the pre-Darwinain embryologist Karl Ernst von Baer and the more recent work by Schank and Wimsatt (Schank and Wimsatt 1987; Wimsatt 1986) into four principles from which he develops a general axiom about consciousness and five properties of a non-declarative memory system.

The principles, the axiom and the subsequent properties are worth discussing briefly as they provide additional support for the evolutionary likelihood of a dissociation between memory functions, and thus strengthen the argument presented in the preceding section. Furthermore, several of the points Reber makes will have a direct bearing on arguments made later in the thesis and are introduced here for that reason.

The 'operating' principles, or heuristics, Reber develops are, firstly, the principle of success: successful[1] forms become the basis for later forms. Secondly the principle of Conservation: developmental processes are conservative and once successful forms are established they tend to become relatively fixed and function as the basis for developing forms. Thirdly, the principle of Stability: successful forms will tend towards stability, showing fewer successful variations than more recent forms. And finally the principle of Commonality: evolutionarily earlier forms and functions will be displayed across species.

In order to apply these principle to the cognitive unconscious Reber posits a simple axiom about consciousness, that it "is a late arrival on the evolutionary scene". As Reber notes this axiom is sufficiently well supported and accepted to constitute a virtual truism. Irrespective of how you characterise consciousness (to borrow Reber's example) humans have it and protozoa do not. Therefore somewhere along the evolutionary progress that led from the latter to the former, the capacity for reflection and the ability to modify cognitive functions developed. Regardless of when

[1] Successful in the sense of the establishment of either a viable species or an individual organism.

consciousness actually evolved it was undoubtedly preceded by “cognitively sophisticated, adaptive functions and forms” which are now clustered under the general rubric of the ‘cognitive unconscious’.

As consciousness is presumed to be a relatively recent evolutionary event and because of generalisations about the properties of forms that develop evolutionarily early Reber is able to propose some rather specific properties that non-declarative cognitive system can be expected display. And in particular these properties can be contrasted with those of a declarative cognitive (i.e. evolutionarily recent) system can be expected to have and in doing so provide further argument for the likelihood of the two systems being independent and dissociable.

The first property Reber proposes for a non-declarative cognitive system is that of robustness. Because non-declarative processes are reliant on phylogenetically older forms then they should be more stable and more resilient. Specifically non-declarative cognitive systems should be less vulnerable to disruption from insults, injuries and diseases than declarative cognitive systems. In support of this property Reber notes that Alzheimer’s patients have extensive cortical (and some subcortical) dysfunction and consequently substantial declarative cognitive impairment, and that those relatively few cognitive functions that are spared by the disorder are those that rely on evolutionarily older sensorimotor tasks (e.g. motor skills). Another reason for the resilience of non-declarative cognition is that the course of cognitive disorders is inversely related to the phylogenetic age of the function. The more evolutionarily recent a function the earlier it is disrupted in the course of cognitive deterioration. For these reasons declarative cognitive functions will tend to be disrupted sooner and more severely than non-declarative cognitive functions, and this is borne out in the amnesia literature (see Chapter 2).

The second principle is that of independence from age. Because they are phylogenetically older non-declarative cognitive functions should be relatively independent of age. For the same reason they should also develop earlier in the life-cycle than declarative functions, and Reber offers evidence that human infants develop non-declarative cognition functions substantially earlier (at around three months after birth) than they develop declarative cognitive functions. Similarly non-

declarative functions should show less decline with age (albeit while not being immune to ageing effects), and there is considerable evidence that non-declarative cognitive functions do not decline as quickly, as soon or as far as general (declarative) cognitive functions do due to normal aging (but see Rovee-Collier, Hayne and Colombo, 2001).

Thirdly non-declarative functions should demonstrate lower population variance, in that they should show less individual-to-individual variation compared to declarative functions (by virtue of the principles of stability and conservation).

Fourthly non-declarative cognitive functions should be relatively independent of intelligence. Standard intelligence tests focus almost entirely on measuring overt, conscious and declarative cognitive functions and thus are only peripherally influenced by non-declarative cognition.

Finally, the principle of commonality holds that the "underlying processes of non-declarative acquisition and memorial representation [should] show across-species commonality". Thus there should be very real and very similar principles in manner in which information is acquired and stored across species, to the extent that such functions are all but independent of the phyla. To support this point Reber employs a modified Rescora-Wagner (Rescora, 1998; Rescora and Wagner, 1972) thesis that organisms acquire associative knowledge based on their ability to detect 'true' covariations between events. Reber goes on to note that "simple co-occurrence is not sufficient to produce stable learning, organisms ultimately key on a kind of Humean causality". In particular Reber claims that such an associative learning system forms the epistemic basis for the "induction of tacit knowledge" by capturing patterns of stimulation inherent in the environment (which harkens back to Sherry and Schacter's thesis that the organism parses (in)variant features of events). Reber's argument is that such an associative learning system can be observed across the "full panoply of species" and as such is likely to function in an extremely similar manner across species. This point in particular is critical to this thesis as it will be argued later that humans and rats share similar neuroanatomical and functional characteristics of a 'non-declarative' memory system (see Chapter 4) and thus we can model human non-declarative learning in rats. According to Reber this is a perfectly reasonable position to take, in particular because the forms and functions that the phylogenetically older

non-declarative cognitive systems rely on are, by virtue of their phylogenetic antiquity, common across most species.

Thus not only does Reber's theory provide support for the central behavioural tenant of this thesis but it also serves to strengthen the likelihood that there are independent, and dissociable memory systems. The cognitive unconscious developed far earlier than, and separately from, the cognitive consciousness and is thus largely independent of it and for this reason enjoys a number of functional advantages to cognitive consciousness, and the cognitive unconsciousness is therefore far more similar across species than 'cognitive consciousness'.

1.3.3 General Conclusion on the Evolutionary Support for Multiple Memory Systems

The conclusions from the work discussed above is clearly that multiple memory systems are evolutionarily more likely than a unitary memory system. Firstly it is unreasonable to expect a unitary system to be sufficiently flexible to accommodate the large variety of demands placed on memory by the environment, particularly the demands of a dynamic and ever-changing environment. Secondly, there are good conceptual grounds for positing that the different memory systems evolved at a different point in time and for this reason are very likely to be largely independent dissociable (although capable of complementary / convergent activity). Thus these accounts provide another strong avenue of evidence for the multiple memory systems theory.

1.4 Criticisms Of The Multiple Memory Systems Approach; Shanks And St. John And The Question Of What Is Learned During Supposed Implicit Learning.

Among the many theories of multiple memory systems there is often an almost non-declarative assumption that that any evidence supporting a dissociation between memory systems or demonstrating the stochastic independence of such systems is good evidence. However, the fact is that some of this evidence, especially those studies undertaken in the early part of the current period of research often rest on weak methodological and conceptual grounds. This criticism is especially relevant to

studies employing the non-declarative and declarative dichotomy by virtue of the complex and often ill-defined nature of its key distinguishing feature: conscious awareness. As this thesis is both expressly concerned with this dichotomy and heavily based on the literature in this field it is appropriate to consider criticisms of the paradigm and the following section will explore some these issues in detail. One of the more comprehensive assaults on the assumption of multiple memory systems has come in the form of a detailed refutation of the evidence for non-conscious learning by Shanks and St. John's (1995) "Characteristics of dissociable human learning systems". It will be shown that while many of the criticisms raised by Shanks and St. John are valid and appropriate their ultimate conclusion, that there is no good evidence on non-conscious learning, is both incorrect and premature. The reasons for disagreement with Shanks and St. John's position are: 1) their unreasonably stringent application of criterion, 2) their unjustified dismissal of the entire *corpus* of amnesia studies, and (in Chapter 2) 3) the presentation of more recent studies, designed specifically with their criticisms / criterion in mind, that demonstrated non-declarative learning in the absence of declarative knowledge of the information critical to task performance.

1.4.1 Single Dissociations, Awareness and The Information and Sensitivity Criteria

Shanks and St. John developed a dual-criterion system for assessing the validity of non-declarative -learning studies and consequentially come to conclusion that there is little unequivocal empirical evidence that learning can proceed in the absence of conscious knowledge. Instead they propose a distinction between instance- and rule-learning to account for the apparent dissociation in human learning processes. Although their ultimate conclusion is at odds with this author's there is much to be commended in Shanks and St. John's thoughtful and comprehensive examination of the material which justifies an equally careful consideration herein.

There can be no question that learning and memory are inherently interrelated (i.e. very seldom process-pure) and while it is redundant to note that memory requires learning the reverse, that learning requires (conscious) memory, is at the heart of the declarative / non-declarative memory debate. Thus while Shanks and St. John specifically address their attention to *learning* without awareness (and rightly chide

those critics who premise their criticisms of their article on points strictly relevant only to memory) there remains much of what they say which is directly applicable to memory research. This is reinforced by the point that in order to memorise something (especially skills) it is *necessary* to first learn it. Therefore learning is a prerequisite for memory and thus a consideration of the former greatly informs our understanding of the latter.

To test the validity of various tests of non-declarative learning Shanks and St. John first note that most experiments of this type typically rely on the logic of dissociation to demonstrate non-declarative learning can take place in the absence of awareness. However they cite two main problems with this approach as used in most such studies. Firstly such studies commonly use single dissociations which offer relatively weak proof of independence and secondly they note most non-declarative - learning studies employ a "constrained version of the logic of dissociation" within which separate indices of learning and awareness are used (see below). At first glance using separate indices of learning and awareness makes good logical sense but in practice it quickly runs foul of serious difficulties with quantifying awareness and devising tests that are sufficiently sensitive to both contamination by unconscious information and exhaustive detection of all relevant conscious knowledge.

Having identified the problem Shanks and St. John then go on to detail an experimental template that conceptually underlies almost all studies of this sort. While discussing this template they note that "subjects may be unaware of the relationships between stimuli even though they are aware of the stimuli themselves". This description neatly encapsulates what non-declarative learning / memory is. It is not so much a lack of conscious knowledge of the stimuli themselves but rather of the relationships between them that, once learnt, provide the subject with a behavioural advantage. However, the quote above brings us to the first concern with their article (and of others that do similar) which is that their use of the term 'awareness' is rife with possibility for confusion. While somewhat pedantic it is nonetheless worth noting that even in order to learn something non-declaratively the learner must, in some fashion, be aware of the stimuli. Thus to describe somebody as aware or unaware risks conflation with the, relatively, less ambiguous terms 'conscious' and 'unconscious' (see Catania, 1994 and below, and Terrace and McGonigle, 1994). Obviously Shanks

and St. John and others often use the term '(un)aware' as shorthand for 'consciously (un)aware' but given that they also talk of 'awareness' in the sense of subjects having differing degrees of awareness of stimuli, relationships etc. it would seem useful to constrain the use of these conceptually similar terms to deliberate, specific, and singular meanings. To this end this thesis will describe subjects as being either 'conscious' or 'unconscious' of something or as having (varying degrees of) 'declarative' or 'non-declarative' knowledge or information. [Like many authors I will not offer a definitive quantification of conscious or unconscious and will simply employ these terms in their common-use sense, except to note that, in humans a necessary property of conscious knowledge is that it is available to deliberate / intentional and (not necessarily verbal) declaration whereas unconscious knowledge is not].

Shanks and St. John state that non-declarative retrieval is defined as occurring when information from some prior episode can be retrieved and influence processing in the absence of conscious recollection of the prior episode, and state that "non-declarative retrieval requires the absence of conscious re-experience of the study episode". Thus Shanks and St. John are not only concerned with a subjects ability to consciously recollect the learning episode during test but also the subjects state of conscious awareness during the learning episode itself and insist that the subject must be unaware of the relevant relationship *in addition* to being unaware of the episode itself. Here perhaps is the first and most obvious disparity between concerns of learning and of memory, that no recourse to the subject's state vis-à-vis recollection of the learning episode is required for a demonstration of non-declarative memory, simply that the subject be unconscious of the specific information that influences their behaviour whilst they are expressing it. While it is not impossible for a neurologically normal subject who is overtly conscious of the critical information whilst learning to be unconscious of it during testing it is however unlikely and thus when testing non-declarative memory it is methodologically preferable that such subjects also be unconscious of the specific information during learning.

This thesis agrees with those authors who criticise Shanks and St. John for making their necessary conditions for non-declarative learning so strict that it is essentially unprovable (e.g. Cleeremans, 1994). It seems almost redundant to note that it is not recollection of the learning episode *per se'* but rather of the particular

information learnt during that episode that facilitates subsequent performance which is germane to an examination of (un)conscious learning and memory. Thus to insist that a subject be unconscious of not just the critical information learnt during the episode but that of the learning episode itself strikes this author as unnecessary. What does it matter if a subject can consciously recollect the learning episode and even the process by which they learnt the information as long as they are unconscious of the relationships between the stimuli that go to make up the information that facilitates subsequent performance? Conscious recollection of the episode and it's details (type of stimuli, procedures etc.) confers no advantage during test (assuming good methodological rigour). In fact an argument could even be made that conscious recollection of the episode in the absence of conscious knowledge of the critical information might interfere with subsequent performance, but this is clearly not Shanks and St. John's reason for insisting on its absence. As Stadler and Frensch (1994) note in their peer-review of Shanks and St. John's article, there are circumstances when learning is declarative and memory non-declarative, such as the development of automaticity for example, but which Shanks and St. John would deny as being non-declarative by virtue of the subject being conscious of the original learning episode(s). Given no clear methodological or conceptual requirement for subjects to be unconscious of the actual learning episode Shanks and St. John run the risk, with such over-zealous criteria, of creating a 'stone-man' (proposed in opposition to the concept of a 'straw-man') hypothesis that is practically unprovable and thus their examination of non-declarative learning runs the risk of degenerating into a case of *reduction ad absurdum*.

1.4.2 The Dangers of Overly Stringent Application of Criteria.

While their emphasis on a subjects' conscious state during learning is unnecessarily strict the criterion Shanks and St. John develop from this are extremely useful. The first of these, the 'information criterion', addresses the concern that there may be a disparity between the information that is responsible for performance and that which is revealed by tests of conscious awareness of the information, essentially an issue of test validity. Shanks and St. John note that in order to conclude that a subject has non-declarative knowledge of the critical information any test of conscious knowledge must actually test for conscious knowledge of the precise information that produces the performance advantage,

and they go on to conclude that many tests of conscious knowledge actually test for something other than this and therefore fail to demonstrate the necessary absence of conscious knowledge of the critical information.

Their 2nd criterion, the 'sensitivity criterion', is predicated on the point that in order to demonstrate the independence of an declarative and an non-declarative system any test of conscious knowledge must be sensitive to *all* relevant conscious knowledge (and is thus reminiscent of Schacter, Bowers and Booker's (1989) retrieval intentionality criterion; and Jacoby, Ste-Marie, and Toth's (1993) process-dissociation procedure), and as such is primarily an issue of test sensitivity. Shanks and St. John note that unless this criterion is met the fact that a subject displays improved task performance may simply be due to the fact that the behavioural task is more sensitive to the conscious information the subject has than the test of conscious is. Shanks and St. John conclude from this that there must be either some independent reason to believe that the test of conscious knowledge is exhaustive or that there is some reason to believe the test of conscious knowledge is equally as sensitive as the performance test. Given the practical unlikelihood of demonstrating exhaustive knowledge Shanks and St. John chose the 2nd approach, although some of their critics, Jeminez et al (1994) and Cleeremans (1994), for example, condemn them based on the former definition, instead noting "if the retrieval contexts… are approximately matched , then the Sensitivity Criterion may be met". When applying this criterion Shanks and St. John come to the conclusion that none of the variety of tests for conscious knowledge in non-declarative learning / memory tests extant in the literature satisfies this criteria and they therefore conclude that there is no good evidence for unconscious learning. While this criterion is now widely accepted in one form or another, Shanks and St. John have been heavily criticised for what is seen as an extremely stringent application of what they accept as 'approximately matched' and in doing so erroneously failing to reject the null hypothesis (of no independence between systems) due to excessively strict criteria. (Berry, 1994; Lindsay and Gorayska's, 1994; Merikle, 1994; Overskied, 1994; Reber and Winter, 1994; and Willingham, 1994).

In response to critiques of this nature Shanks and St. John note that relaxing the criteria increases the danger of false-positives due to "differential sensitivity or a

mismatch in the type of information examined by the two tests". Whilst true this is also somewhat disingenuous, in that the criticism of Sherry and Schacter's criteria acknowledges the necessity of the sensitivity criterion and attacks it on the grounds of it's application rather than it's utility. Moreover it is worth noting that given the very stringent criteria a single demonstration of dissociable learning that satisfies Sherry and Schacter's criterion is sufficient to invalidate their hypothesis of no difference, as the studies that fail to satisfy the criteria and thus fail to demonstrate dissociable learning systems simply produce an absence of evidence, and are not evidence of absence.

1.4.3 The Utility of Amnesic Subjects in Memory Studies

The second prime criticism levelled at Shanks and St. John is their abrupt and total dismissal of the evidence for multiple-memory systems demonstrated by subjects with a variety of amnesic etiologies. In the original article they state that "We know of no convincing data that would suggest that amnesics are capable of unconscious learning". Specifically because although such subjects may be unable to recall the learning episode Shanks and St. John contend there is no good evidence to suggest that they were unconscious of the relationship between stimuli during the learning episode. However, they appear to directly contradict themselves in their response to the peer-reviews when they state "if.. information is unconscious at the time of retrieval, then it is plausible to assume that it was registered unconsciously"!

It will come as no surprise to the reader to learn that Shanks and St. John have been roundly criticised for so casual a dismissal of the amnesic related evidence for multiple learning / memory systems. In their defence Shanks and St. John contend that data from amnesics subjects is unlikely to be useful because the fact that amnesic subjects are incapable of verbalising information does not necessarily mean they do not have that information available to consciousness during testing (Shanks and St. John are also highly critical of the validity of verbal reports as a measure of conscious knowledge). If amnesics are *selectively* (their emphasis) impaired on declarative tests such as recognition then the deficit displayed by amnesic subjects on declarative memory tasks doesn't necessarily denote the absence of declarative knowledge, merely an inability to express it. Shanks and St. John have a good point, care must be taken to ensure that impaired performance on declarative tests actually tells us

something about a subject's ability to use declarative memory during learning and isn't simply an indication of a subjects inability to perform the test irrespective of their ability to employ declarative memory during learning (i.e. studies with amnesic subjects fail the information criterion). During their response to the peer-review of their article Shanks and St. John defend their decision to discard the evidence from amnesic subjects in some depth and make a number of good points about typical studies supposedly demonstrating non-declarative learning in amnesic subjects and a brief summary is warranted.

Shanks and St. John point out that the commonly-held theory that amnesia is a deficit in conscious declarative memory is but one of many theories and not without it's criticisms. Thus if amnesia turns out to be something other than this (for example: a selective deficit of contextual processing, Mayes, 1998) then its relevance to the unconscious learning is very limited.

Like a number of other authors (e.g. Curran ,1997) Shanks and St. John are concerned with the fact that amnesic subjects always show impaired baseline performance in comparison with normal subjects. Although this difference is typically slight (and not statistically significant) it does raise a number of issues (e.g. baseline differences can help to obscure / emphasise group differences; Curran, 1997) Shanks and St. John are specifically concerned with the point that if amnesic and normal non-declarative learning performance is not truly equivalent "it is possible that the extra conscious knowledge the normal subjects have (as indexed by superior performance on the declarative test) is what explains their superior performance on the non-declarative test". While this is undoubtedly true they then conclude as a direct result of this point that there are no grounds "for concluding that unconscious knowledge is playing *any* role at all" (emphasis added). Thus they suggest any difference in behaviour is due solely to the relative difference in conscious knowledge. This however is an unwarranted conclusion in that while different degrees of conscious knowledge could produce superior non-declarative test performance (and see the next chapter for some empirical evidence for this) this point in no way excludes the other possibility, that improved performance on an non-declarative memory test may be due to non-declarative memory. Before leaving this topic the reader's attention is drawn to Sherry and Schacter's point that one way to employ amnesic subjects to demonstrate

multiple learning systems would be to show that amnesics can perform better than normal subjects on an non-declarative task while performing worse on an declarative task, but that there "appear to be no published cases of this sort". They go on to conclude that studies with shorter retention intervals for amnesic subjects which might provide related sorts evidence (e.g. Schacter et al. 1984) are very difficult to interpret, but that "A crossover interaction would be *much* more persuasive". (Emphasis added, the relevance of which will become apparent in Chapter 2).

Finally they also note that a single source (system) model of conscious information which is used for both declarative and non-declarative learning-and-memory would predict amnesics would be impaired on both declarative and non-declarative tasks, and indeed they sometimes are (see Chapter 2), but to vastly different degrees. Once again Shanks and St. John are being somewhat disingenuous as any non-declarative impairment demonstrated by amnesic subjects is substantially less than their declarative impairment, and any recourse to arguments about degrees of impairment (as Shanks and St. John would be forced to undertake in order to continue arguing their point) intrinsically allows the *possibility* that amnesics do provide good evidence of multiple systems by virtue of having a relative impairment of one system compared to the other. While there is some evidence that both memory systems are impaired in amnesic subjects there is also good evidence that some amnesic subjects display completely normal non-declarative memory in the presence of a serious declarative memory deficit (i.e. the priming studies cited above).

Given their emphasis on learning as opposed to memory and that amnesia is typically thought of as a memory (as opposed to learning) deficit their insistence that amnesic subjects be held to the same strict criterion as normal subjects for the availability of the critical information to conscious awareness during learning is reasonable. In fact, in light of what is discussed above the use of amnesic subjects may actually make demonstration of non-declarative learning more difficult. As using of the presence of an declarative memory deficit during testing to reason back to one during learning is logically vulnerable (although logic of this form is commonplace).

Another issue raised in the peer-review is that Shanks and St. John argue their case from the assumption that conscious processing is the default condition. As Berry

(1994) points out not everybody would accept this position, and in fact some (e.g. Reber, 1990) argue precisely the opposite. Several commentators (Holyoak and Gattis, Reber and Winter, and Terrace; 1994) note that this is as much an assumption as the reverse which Shanks and St. John themselves criticise.

1.4.4 Conclusions on Shanks and St. John

It is not the intention of this thesis to provide a comprehensive discussion of all the points raised in the original article and subsequent commentary. Furthermore Shanks and St. John's alternative to conscious / unconscious learning, instance and rule learning (which is due a chapter in it's own right) has deliberately not been discussed in order to focus on those issues directly related to the validity of multiple memory systems (Shanks and St. John consider their alternatives to be complementary operations of a unitary system). Therefore, the examination of their article will conclude with a few final points, while acknowledging the wealth of material left untreated and with the promise to visit a subtopic (hitherto unmentioned) of this area in detail in the next chapter.

While this thesis does not concur with Sherry and Schacter's conclusion that there is no good evidence of dissociable learning / memory systems in adult humans it acknowledges the debt the field owes them for drawing attention to considerable methodological and conceptual complexities hitherto often confused / ignored. What is taken issue with, however, is how these criteria are applied. Specifically that Sherry and Schacter employ the information criterion too stringently to the point of making it all but impossible to satisfy, and their cavalier and dismissive treatment of the amnesia literature and their reasoning for which is weak and unconvincing. Other concerns such as their non-declarative assumption of conscious processing as the norm, and the somewhat artificial separation of learning and memory in light of the point that declarative / non-declarative dichotomy rests on several strands of evidence and "does not stand or fall according to the status of aware versus unaware learning" alone (Squire, Hamann and Knowlton, 1994; also see Willingham, 1994) strengthen this conclusion.

General Conclusions On The Evidence For Multiple Memory Systems

Totally conclusive evidence for or against multiple memory systems is missing.

However the evidence presented in this chapter strongly favours the multiple-system hypothesis (see also Kinsbourne, 1987; Weiskrantz, 1987; and Gershburg and Shimamura, 1998). The demonstration of functional independence of memory tasks in subjects, amnesic or neurologically normal, cannot easily be explained by a unitary memory system. What is more, the results from the animal literature discussed in Chapter 4 provides convergent evidence that there are similar neurologically independent and functionally dissociable forms of memory in both animals and humans. That said Shanks and St. John's conclusion is based on a thoughtful analysis of the literature and cannot be simply ignored. In response to this the next chapter will examine the evidence for multiple memory systems as provided by a specific behavioural paradigm, the serial reaction time (SRT) task, both in general and with specific consideration for Shanks and St. John's critique of the task, and arrive at firm conclusions as a result of the evidence discussed therein.

1.5 General Aims of the Thesis

The remainder of this thesis will be concerned firstly with a qualitative review of a specific human non-declarative memory task, the serial reaction time (SRT) task. This review (Chapter 2) will focus on how the SRT is used to provide evidence for non-declarative memory and its utility a source of evidence for multiple memory systems. This review will discuss the SRT, the contrasting abilities of subjects with different amnesic etiologies to perform the SRT task, and the capacity for experimental dissociation between this task and declarative memory tasks. Shanks and St. John will also be revisited in light of the SRT literature and some recent studies introduced which address the concerns of their critique while still demonstrating good evidence of non-conscious learning / memory.

Chapter 3 will present a meta-analysis of studies from the SRT literature to address two important questions: 1) Do those neuropathological subjects with limbic system damage (i.e. organic amnesia, Korsakoff's syndrome, *mildly* dementing Alzheimer's patients, etc.) actually display SRT performance that is not different to control subjects in contrast to what SRT studies usually claim, and 2) do various neuropathological groups show reliable SRT performance.

Chapter 4 will present a review of the limited animal literature on non-

declarative learning and in doing so demonstrate that there is, as yet, no good animal analogue of a human non-declarative memory task. It will also be shown that what few tasks there are that supposedly demonstrate human-style non-declarative learning in animals involve substantial methodological and conceptual differences to the human tasks they attempt to mimic. A possible animal analogue of the human-SRT will be introduced and its utility and validity discussed.

In response to the argument in the Chapter 4, Chapter 5 will introduce a novel rat-SRT task which has a reasonable degree of similarity to the human-SRT task, and will show that rats performing this task display behaviour very similar to that of human behaviour in the SRT task. A novel apparatus, the 16-arm Fan-Maze, will be introduced the development an animal-SRT task reported. However, a number of serious limitations that became apparent with this approach will be presented and an alternative, the Intra-Cranial Self-Stimulation SRT (ICSS-SRT) task, will be suggested.

The following chapter (6) will report a control study designed to demonstrate the ideal conditions for SRT learning in rats. Manipulations of the type of sequence (repeating or random) and the number of sessions / days (one or three) are examined and conclusions presented as to the best combination of these variables for SRT learning. This study will also provide firm empirical support for the ability of animal subjects to demonstrate SRT behaviour of a very similar type to human behaviour, and thus the likelihood of having some form of non-declarative memory.

Thereafter the next chapter (7) presents an experiment designed to demonstrate a dissociation between lesion site (dorso-lateral caudate nucleus and dorsal hippocampus) in the SRT memory task. These results will demonstrate that the rat-SRT relies on the same neural substrate as human SRT performance does and, furthermore, that the rat-SRT is not impaired by hippocampal dysfunction.

The final chapter (8) will present a discussion of the experimental results in light of the theoretical arguments presented in the thesis and as the empirical results relate to the meta-analysis. This chapter will also discuss the validity and utility of the rat-SRT and several limitations of the current research will be discussed. Possible avenues for future research will also be suggested before presenting general conclusions.

Chapter 2

The Serial Reaction Time Task: A Non-declarative Memory Test

General Introduction

This chapter will initially present a brief overview of a common test of non-declarative memory, the Serial Reaction Time task (SRT), before progressing to a discussion of the pattern of impairment and spared-performance found in different neuropathological populations. It will be shown that those subjects with a limbic-system amnesia appear to display normal, or near normal, SRT behaviour, even though suffering a substantial explicit memory deficit. In contrast subjects with non-limbic-system disorders (specifically those suffering basal ganglia based syndromes) are impaired in the SRT whereas their explicit memory performance is unimpaired. Thereafter a number of critical methodological issues are discussed. A large part of methodology discussion will be devoted to a consideration of the arguments made by Shanks and St. John (1994) that pertain to the SRT. Thereafter several studies will be presented that address these concerns while still demonstrating good evidence of non-declarative learning in the SRT task. As a result of this discussion conclusions will be presented as regards those questions surrounding multiple-memory systems first raised in Chapter 1, including the concerns raised by Shanks and St. John, and thus the relevance and validity of the SRT task as a measure of non-declarative memory.

2.1 Introduction to The Serial Reaction Time Task

First introduced by Knopman and Nissen in 1987 the SRT has enjoyed considerable use since and is often employed in attempts to demonstrate a dissociation between explicit and non-declarative memory. In the prototypical SRT study subjects perform a visual serial reaction time task with an embedded (and uncued) repeating sequence. The task is considered to be non-declarative by virtue of the fact that subjects are, supposedly, not consciously aware of the repeating sequence. Implicit sequence learning in this task is demonstrated in two ways. The first, the 'learning effect' is the reduction in reaction times (over and above that which is attributable to sheer motor improvement alone) across the session demonstrated by subjects who experience a repeating sequence. The second, and more substantive, measure of learning is the 'interference effect' which is a rebound increase in reaction time that occurs when subjects are (unknowingly) switched from a repeating to a random sequence.

2.1.1 Standard Methodological Features of SRT studies.

In a typical SRT study subjects are seated in front of a computer monitor immediately below which is a response board with four buttons. An asterisk appears in one of four positions spaced horizontally across the bottom of the screen and aligned with the positions of the response buttons. The reaction time to each stimulus is determined and (typically) the median for a block of trials calculated. The stimulus remains onscreen until the correct button is pushed and, following a short delay, the next stimulus appears. Stimuli appear in either repeating or random sequences. The most common repeating sequence is a 10-trial repeating sequence of light positions which repeats ten times during a 100-trial block and most studies employ 5 or 6 blocks. So called 'random' sequences are in fact quasi-random in that they are at least constrained by the rule that a stimuli cannot immediately reappear in the same stimulus location on the next trial. Blocks of random (sic) sequences usually contain as many trials as the relevant repeating sequence blocks (i.e. 100 trials). The beginning and end of any sequence (random or repeating) is not marked and the subjects are not told that learning or memory is being assessed or that a repeating sequences is present, but rather are instructed to simply respond to the stimulus as fast as possible.

The prototypical procedure is for a subject to perform the SRT for five blocks.

The first four blocks contain the repeating sequence and the fifth block the random sequence(s). The expectation is that the subject will show a marked decrease in reaction times (RTs) over the course of the first four (repeating) blocks (the learning effect) and a sudden increase in reaction times in block five when switched from the repeating to the random sequence (the interference effect). In contrast subjects who only ever experience the random sequence throughout all five blocks are expected to demonstrate a smaller decrease in reaction times (due simply to an improved ability to perform the motor requirements of the task) and, critically, no increase in reaction times between block four and five.

Although both the learning and the interference effects are measured during a typical SRT experiment the literature has tended to focus on the interference effect due to its ease of quantification allowing simple and prompt comparison between groups and because it is not confounded by sheer motor-skill improvement. As a result the following discussion (and indeed the thesis as a whole) will concentrate on this measure of non-declarative learning and typically only discuss the learning effect in terms of offering support for, or contradicting, conclusions based on interference effect.

2.2 Neuropathology and the SRT

One of the great promises of the SRT is that as a test of non-declarative memory it will differentiate between types of neuropathology. Specifically it will reveal spared / preserved non-declarative memory in subjects with impaired explicit memory while revealing impaired non-declarative memory in other aetiologies. Thus those subjects that are typically thought of as ‘amnesic’ should be able to perform this test in very similar manner to that of non-amnesic subjects.

2.2.1 Limbic System Amnesia and the SRT

This section will present evidence that those subjects with limbic system amnesias are not (or only mildly) impaired on the SRT while suffering varying degrees of explicit memory impairment. This suggests that the SRT is both a valid measure of non-declarative memory and that the two forms of memory are dissociable and independent of each other. Knopman and Nissen’s (1987) article which originally introduced the SRT will be presented first. This is a fortuitous coincidence for three

reasons: this article was the first to introduce the SRT in its current form and is thus the foundation article for this field and the main impetus for the subsequent development of SRT-based non-declarative memory research. Secondly, while some of the methodological detail has changed the broad approach to SRT work remains very similar to that first reported in this article and thus this article is a good primer for discussing later SRT work. And lastly because it demonstrates preserved SRT ability in limbic system amnesiacs (Alzheimer's disease subjects).

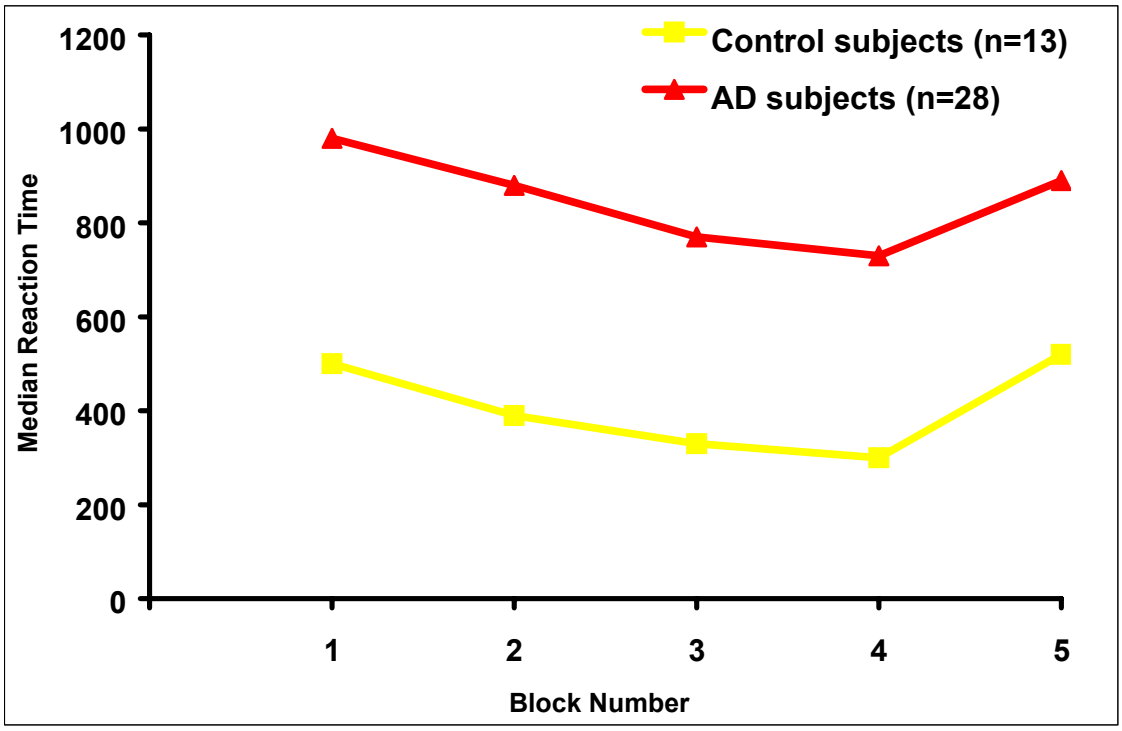
Alzheimer's Disease

In their 1987 study Knopman and Nissen compared elderly control subjects with mildly dementing Alzheimer's disease (AD) subjects. Both groups experienced five 100-trial blocks, the first four blocks employed a 10-trial repeating sequence (ten repetitions over a block) while the fifth block contained random-sequence stimuli. Both the Alzheimer subjects and the control subjects displayed a decrease in reaction times over repeating-sequence blocks (the learning effect) and an increase in reaction time when switching from a repeating- to a random-sequence (the interference effect), suggesting the AD subjects had some preserved (implicit) memory ability.

Nonetheless it is readily apparent (see Fig. 2.1) that the reaction time for the AD subjects was markedly slower than controls, in fact by more than twice (see original text). However, what is also clear is that the Alzheimer patients display a near identical *pattern* of behaviour as the control subjects. Thus irrespective of their generally slower reaction times AD subjects displayed both a learning and an interference effect of a very similar nature / strength as that shown by control subjects. Knopman and Nissen emphasise the point that acquisition of knowledge about the repeating sequence occurred despite the patients markedly slower reaction times compared with the control subjects and in contrast to the AD subject's significantly impaired explicit memory.

Somewhat surprisingly however Knopman and Nissen found that the sequence learning ability of the Alzheimer patients' was not related to severity of their dementia as indexed by the mini-mental state exam. This is at odds with what was expected and what is reported in other studies (especially Ferraro, Balota & Connor, 1993, see below).

Fig. 2.1, The Performance of Alzheimer's Disease and Control Subjects in a SRT Task (Data drawn from Knopman and Nissen, 1987).



As will be seen in the following section this study is typical of most SRT studies employing subjects with limbic system damage in that these subjects show a degree of spared sequence-learning ability in the presence of an explicit memory impairment. However, subsequent studies often differ from this one in that while Knopman and Nissen only tested for general explicit memory ability later studies often deliberately test their subject's explicit knowledge of the actual repeating sequence in order to demonstrate amnesic subjects can perform the SRT without explicit knowledge of the actual information that bestows a behavioural advantage.

Nevertheless, virtually all SRT studies with limbic systems amnesiac subjects conclude that their neurologic subjects perform the SRT in a control-like manner, irrespective of any generally slower reaction times demonstrated by the neurologic subjects. However, given that all subsequent SRT studies with limbic system amnesiac subjects show a non-significant difference in the same direction (limbic system amnesia subjects always have slower reaction times than controls) it is possible that individual studies do not have sufficient statistical power (by virtue of

small sample and effect sizes) to demonstrate the difference between neurologic and control groups, rendering the conclusion of equivalency unreliable. For this reason Chapter 3 will present a meta-analysis designed to test the assumption in the literature that limbic system amnesiacs are not different to controls.

In a study designed to test both the ability of Alzheimer's disease subjects (AD) to perform the SRT and to examine how well they retained sequence information over long delays Knopman (1991a) tested AD subjects on the standard SRT task and then repeated the test 1 or 2 weeks later. In contrast with the result from the earlier study (above) he found that AD subjects' degree of 'sequence-specific learning' (interference effect) was significantly weaker than controls (80ms vs. 128ms respectively; first session). However, Knopman then went on to divide the AD group into 'learners' and 'non-learners' *post hoc* (to be classified as a 'learner' subjects had to have an interference effect of greater than 50ms). Once 'non-learners' were removed from the analysis there was no difference in sequence-specific learning between groups (AD: 119ms, Controls: 146ms), but as with the original 1987 study the AD subjects responded significantly slower than the control group.

In the second session, delayed by 1 or 2 weeks, both the AD and control subjects demonstrated considerable sequence retention in that both groups had much lower reaction times in the first block of the second session than in the first block of the first session when they were naïve to the task. As in the first session once 'non-learners' were removed from the analysis there was no difference in the strength of the interference effect displayed by either group, although AD subjects were again significantly slower than the control.

While additional general psychometric testing showed that the AD subjects were overall "markedly impaired to the elderly control group" the results of a generate task (a test of explicit sequence knowledge) showed that the somewhat less-accurate AD subjects were not significantly different from the control group (36.2% and 48.1% accurate respectively). Furthermore, the chance-level of behaviour on the generate task is 33% and thus the AD subjects appear to have been performing this task virtually at chance, suggesting they had very little explicit awareness of the repeating-sequence. Similarly a generate score of 48% for the control group is lower than seen

in most studies employing similar conditions (typically ~70-80%, see below) and suggests these subjects had only partial awareness of the sequence.

The results of this study suggest, that with use of judicious selection criterion, AD subjects are capable of demonstrating non-declarative sequence-learning and retaining that learning over a delay, in the absence of any explicit awareness for the sequence, and in the presence of a significant explicit memory impairment. This study is also interesting in that it provides evidence that control subjects are able to demonstrate sequence-learning with a much weaker degree of explicit awareness of the sequence than is common.

There is further corroboration that AD subjects can perform the SRT, providing evidence for both multiple memory systems and the validity of the explicit / non-declarative memory distinction. In a study of mixed amnesic etiologies Ferraro, Balota and Conner (1993), tested: non dementing healthy aged subjects, non dementing Parkinson's disease (PD) subjects, very mildly demented AD subjects, and mildly demented AD subjects. The task characteristics were typical in that they used 4 blocks of a repeating sequence before switching to one block of random sequences. The result that stands out is that the mildly dementing AD patients are significantly impaired to all other groups as they display both much slower reaction times *and* a much weaker interference effect.

In contrast the very mildly dementing AD subjects are extremely similar to controls in terms of both their actual reaction times and the strength of their interference effect. The pattern of results for Parkinson's disease subjects is different again in that while they showed similar reaction times to both the controls and the very mildly dementing AD subjects they had a significantly weaker interference effect than both these groups. A comparison between the mildly dementing AD group and the PD group revealed the mildly dementing AD group was significantly slower than the PD group and had a significantly weaker interference effect, indicating it was more impaired than the PD group. Although no test of explicit sequence knowledge was employed subjects did undergo a battery of psychometric tests which produced results consistent with group classification (i.e. as regards dementia and explicit memory impairment). What is more interesting is that when the scores on the psychometric tests were correlated with a degree of non-declarative learning (interference effect)

only the very mildly dementing AD group revealed any reliable correlations. On closer examination it is evident that the tests that do produce significant correlations rely more on perceptualmotor aptitude. Interestingly those tests that do not correlate with a degree of non-declarative memory are considered tests of explicit memory. Therefore the lack of a reliable correlation between tests of explicit memory and a measure of non-declarative memory is important as it provides divergent validity for the separate constructs.

It is apparent from these studies that while AD sufferers can demonstrate good SRT performance in the presence of an explicit memory impairment this is only true in the early stages of the disease (most likely while the neuropathology is still relatively limited). However, given the pervasive and progressive nature of this disorder, it is all but impossible to determine if the impairment evident in more dementing subjects is due to a mildly impaired non-declarative memory or a consequence of more general neuropathology.

Mixed Amnesics

Given that organic amnesia is, by definition, produced by damage in the limbic system the first experiment in Reber and Squire (1994) was designed solely to demonstrate the ability of amnesic subjects to perform the SRT in the absence of any explicit memory for the repeating sequence. During the first of two session (sessions were separated on average by 73 days) the amnesics and first control group subjects experienced a 10-trial, 40-block (for a total of 400 trials) repeating-sequence before moving onto a verbal report phase and then a prediction phase (both are tests of explicit knowledge of the repeating sequence). In contrast the ‘random’ control group experienced a quasi-random sequence for the full 400 trials before going onto the verbal report and prediction phases. As expected the amnesic subjects demonstrated an improvement in reaction time (RT) over blocks consistent with that shown by the (‘first’) control group that experienced the repeating sequence. Furthermore the random-control group did not display any improvement in reaction times over blocks and both the amnesic and control groups were significantly different from this group.

The second session revealed a similar pattern of results and included an interference test. All subjects experienced four blocks of the repeating sequence (for a

total of 400 trials) before completing explicit sequence knowledge tests. After these tests subjects returned to the SRT and performed a further repeating sequence block before being switched to a random sequence block in the sixth and final block. All subjects, control and amnesic, demonstrated; a significant reduction in reaction times over blocks, similar reaction times in the blocks immediately prior to, and after, the explicit memory tests, and (most importantly) a similarly strong increase in reaction times when switched from a repeating to a random sequence. While the amnesic subjects demonstrated good SRT performance their sequence recognition test scores were indistinguishable from the random control subjects, and both groups were not different from chance and significantly impaired in comparison to the first control group. Thus amnesic subjects demonstrated sequence learning in a similar fashion to control subjects while having no measurable explicit memory for the repeating sequence.

In their second experiment Reber and Squire attempted to address the concerns of Shanks and St. John (1994) with respect to a number issues to do with stimulus presentation (see below for a detailed discussion of these concerns). To this end they substituted the original 10-trial sequence for a 12-trial sequence that allowed both equal frequency of stimulus presentation and transition frequency (i.e. the frequency with which one particular stimuli preceded another particular stimuli) between the different sequence types. Otherwise the study was identical to that in the second session described above. Once again both groups demonstrated good reduction in reaction times over repeating-sequence blocks and a strong increase in reaction times when switched from a repeating to a random sequence. Thus there remains good evidence that amnesic subjects can perform the SRT in a manner near identical to controls when nothing but actual sequence information is allowed to vary between the repeating and random sequences (see below for a discussion on this issue). Furthermore the validity of the SRT as a test of non-declarative memory is strengthened by the finding that both the amnesic and control groups performed at chance-levels in the recognition memory test and thus both groups demonstrated good SRT performance in the absence of any explicit memory of the 12-trial sequence.

In a similarly comprehensive study Reber and Squire (1998) attempted to

demonstrate a crossover in SRT performance between amnesic subjects and a wide variety of control conditions. The rational behind the attempt is that if the multiple memory system view is correct it should be possible to generate circumstances whereby one group (amnesiacs) exhibits significantly more non-declarative memory, and significantly less explicit memory, than another group (controls).

However, analysis of the 'pretraining' data revealed an identical, and significant, decrease in reaction times across the first portion of the 'pretraining' session for all groups including those subjects who only ever experienced random sequences. Fortunately the 'random only' subjects failed to demonstrate any interference effect at all, unlike the various groups that experienced repeating sequences during this phase who all demonstrated strong interference effects. Thus the practise of preferring the interference-effect over the learning-effect that is common in the literature has some experimental support.

Both the amnesiac and the "CON" groups (the primary control group for amnesiac subjects) display a significant increase in reaction times when switched to random stimuli in this study. However the Reber & Squire report that the CON group displayed a (non-significantly) greater interference effect than the amnesiac group, and the author's suggestion that the CON group's greater score "probably reflects a contribution of explicit sequence knowledge" seems reasonable. Nevertheless, the amnesiac subjects demonstrate a robust interference effect that is different from zero and from all the 'memorisation' control groups (who explicitly memorised the repeating sequence but only practised it for a short period), but is not different to the CON group. The purpose of the deliberate memorisation of the repeating sequence in the 'memorisation' groups was to examine the ability of subjects to employ explicit knowledge of the sequence during SRT performance. The obvious conclusion from these results is that explicitly memorising the sequence alone (i.e. in the absence of reasonable period of SRT performance with the repeating sequence) does not produce a behavioural advantage.

Irrespective of the sequence memorisation issue it is clear that amnesiac subjects can perform the SRT in a manner very similar to that of control subjects who experience identical experimental conditions. However in order to demonstrate a crossover effect Reber and Squire must also demonstrate the opposite dissociation

between explicit sequence knowledge for the amnesiac and CON groups.

However, Reber & Squire report that in the first test of explicit sequence knowledge, a verbal report procedure, the amnesiac subjects demonstrated reasonably good knowledge of the repeating sequence. This is surprising as it is typically expected that amnesic subjects will have poor explicit knowledge of the repeating sequence (indeed this is the point of using amnesic subjects). However, given that verbal report is supposedly a measure of explicit sequence knowledge the author's assertion that the ability of the amnesiac subjects to report a "fair portion" of the sequence is due to the influence of non-declarative knowledge is somewhat surprising. This becomes clearer when Reber and Squire acknowledge that during the verbal report amnesiac subjects received "repeated encouragement to guess" and it was at this point that they generated reasonably long sequence strings (up to 8 of 12 positions). It is readily apparent that such encouragement fundamentally changes the task requirements and shifts the focus of the verbal report procedure from an explicit to a more non-declarative test of sequence awareness but forcing the amnesiac subjects to rely on (necessarily implicitly informed) guesses. This point is supported by the finding that an amnesiac subject who scored moderately well on the (supposedly explicit) verbal report score "had no detectable declarative memory capacity" and as such his performance must reflect non-declarative knowledge. Therefore, as the verbal report test was initially intended to ascertain the amnesiac subject's level of explicit sequence knowledge, this raises the question of what possible utility the authors saw in deliberately forcing subjects to continue the task beyond the ability of their, impaired, explicit memory.

However, in a second test of explicit knowledge of the repeating sequence subjects performed a recognition test and while all control groups scored at above chance the amnesiac group who also recognised the sequence significantly more poorly than any of the other groups exposed to it. This test therefore produces results consistent with expectations and provides evidence that any capacity of amnesiac subjects to learn serial-order information can be exercised in the absence of explicit knowledge of the sequence.

Thus, because the amnesiac subjects demonstrated good SRT performance in the absence of any explicit memory for the repeating sequence it is reasonable to conclude that this study has succeeded in demonstrating a performance crossover

between explicit and non-declarative memory. Both the amnesiac and CON groups show a dissociation between explicit and non-declarative knowledge unlike the various control conditions who do.

Reber and Squire go on to note that their CON group received extensive SRT practise and also acquired some explicit knowledge of the sequence displayed a (nonsignificant) numerically larger reaction time slowdown than the amnesiac subjects, suggesting the CON group was able to apply some explicit knowledge two the non-declarative test. This is consistent with findings of the studies mentioned above that found an advantage of explicit sequence knowledge and suggests that it is the conjunction of both explicit and non-declarative sequence knowledge that enables non-neurologically impaired subjects to produce very short reaction time's during the SRT.

Analysis of a second SRT session by Reber & Squire (performed by amnesiacs and the primary control group only) some three to 12 months after the first session revealed no difference in reaction times between the amnesic and the CON group (the interference effect is not reported for the second session), and both groups exhibited a significant reduction in reaction times across blocks. Interestingly, while no mention is made of it there is a strong suggestion in the graph that neither group exhibited the reaction time saving between sessions that both the AD and control subjects displayed in Nissen, Willingham and Hartman, 1989 (below), and in Knopman 1991a (above). However in these cases the delay was only 1 or 2 weeks, whereas in Reber and Squire the delay between sessions was 3 to 12 months, suggesting sequence knowledge decays over extended periods.

Reber and Squire's conclude that they have demonstrated a crossover interaction between non-declarative and explicit knowledge, and that this result, in conjunction with findings of selective impairment in amnesiacs subjects, provides strong evidence "that non-declarative and explicit sequence learning must depend on separate brain systems supporting separate representations of sequence knowledge". This finding is of especial interest in light of Shanks and St. John's comments reported in Chapter 1, and these results would seem to be precisely those that Shanks and St. John describe as being "much more persuasive".

Korsakoff's Syndrome

In the final experiment in their 1987 paper Nissen and Bullemer examined the ability of Korsakoff's syndrome (KS) subjects to display sequence learning (having previously shown that attention was necessary for sequence-learning in normal subjects) in order to determine wether-or-not KS induced amnesia disrupts attention. The ability of KS sufferers to perform the SRT was also of interest as the SRT is a novel non-verbal association learning task and the authors were interested in light of the (then) recent evidence that some amnesiac patients were capable of learning novel associations (Graf and Schacter, 1985). Similar to the results found with AD subjects above the KS subjects responded significantly slower than age-matched controls but they also displayed a very similar pattern of behaviour to that of the controls. There was no difference between the slopes of the Korsakoff or control subjects indicating the two groups learnt the sequence at similar rates. Unfortunately no analysis of the increase in reaction time when switched from the repeating to the random sequence was reported. However, it is clear from the data Nissen and Bullemer presented in the study that while the KS subjects displayed an interference effect it was smaller than that shown by control subjects, but it is not known if the difference was significant. What is especially interesting in this study is that, when asked, all of the control subjects reported becoming aware of a repeating sequence (verbal report only) whereas none of the Korsakoff subjects became aware of it. So it is apparent therefore that the amnesia produced by Korsakoff's syndrome does not interfere with non-declarative memory as sufferers demonstrate a spared memory ability for sequence learning in the absence of conscious awareness of the sequence. Thus Nissen and Bullemer conclude that patients with Korsakoff's syndrome could learn the SRT despite severe impairments in consciously recalling verbal and non-verbal information related to it, but this conclusion must be viewed with some caution as no attempt to quantify the difference between interference effects for control and Korsakoff's subjects was made.

In another study with Korsakoff subjects Nissen, Willingham and Hartman (1989) compared them to age-matched controls and an alcoholic control group. All groups received two sessions of a typical SRT task separated by one week. Although significantly slower than both the age-matched and alcoholic control groups (who were not different from each other) the Korsakoff's group once again displayed the same

pattern of behaviour as the two control groups (in both sessions): reduction in reaction time over repeated sequence blocks and an increase in reaction time when switched to the random-sequence block. Furthermore all subjects displayed retention of the sequence between sessions as their reaction times in the first block of the 2nd session were very similar, or slightly faster, than that of the last repeating-sequence block in session one. However, when questioned about the presence of a repeating sequence at the end of the 2nd session five of seven age-matched controls, and six of eight alcoholic controls reported noticing it, whereas none of the KS subjects did. Suggesting once again that KS subjects could perform the SRT in the absence of any explicit knowledge of the repeating sequence, or that there even was a repeating sequence.

Other Limbic System Neuropathology

In a very recent study Stefanova *et al* (2000) compared their PD subjects to both healthy controls and subjects with anterior communicating artery aneurysms (ACoA). The ACoA group, who suffer a declarative memory impairment, were included to provide a neuropathological control for the PD group. Although the ACoA subjects displayed both a learning and an interference effect they were significantly slower overall compared to controls which is consistent with most limbic systems amnesiacs. However, while they demonstrated a significant interference effect it was significantly weaker than that of the healthy controls (while being significantly stronger than that of the PD subjects) and thus they do not perform the SRT in a control-like manner. Nevertheless, while capable of demonstrating a degree of spared sequence learning they also demonstrated a significant explicit memory impairment for the repeating sequence. Thus providing further evidence for the independence of explicit and non-declarative memory, and the utility of the SRT for demonstrating such a dissociation.

Conclusions on Limbic System neuropathology and the SRT

In conclusion there is considerable evidence that subjects with limbic systems amnesia (LSA) can perform the SRT in a manner similar (but not identical to) to non-neurologically impaired subjects. While the fact that LSA subjects are often slower than control subjects and / or have a somewhat weaker interference effect is of concern the fact that LSA subjects display both a learning and interference effect irrespective of their overall slower reaction times is telling. However, the fact that most

SRT studies may not have sufficient statistical power (due to small sample sizes) to detect a small difference between LSA and control groups is worrying (see Chapter 3). However, the (in)equivalence with controls subjects is not the definitive measure of LSA subjects' ability to perform the SRT. It is enough currently to demonstrate that LSA subjects enjoy some spared non-declarative memory function in contrast to their often seriously impaired explicit memory. Given that such subjects often suffer a relatively broad neurological impairment (i.e. not simply an explicit memory impairment, e.g. dementia) it should perhaps come as no surprise that such subjects don't perform identically to controls and what perhaps is more interesting is ascertaining any discernable differences in the ability of the different LSA etiologies (AD, KS, non-specific amnesia) to perform the SRT. This is not to dismiss comparisons with controls groups completely but rather to acknowledge that such comparisons are only one part of the overall picture of any neurologically impaired groups' ability to perform the SRT.

2.2.2 Basal Ganglia Syndromes and the SRT

Given that limbic systems amnesiacs demonstrate a reasonably good ability to perform the SRT the next question is how subjects with basal ganglion (BG) disorders, (traditionally the neural substrate for non-declarative / procedural memory), perform. Caudate nucleus involvement in non-declarative learning and memory has been proposed by a number of authors (see Squire, 1992; and Cohen and Eichenbaum, 1993, for reviews) and is known to be responsible for a habit system in primates, which may be analogous to procedural learning in humans (but see Wise, 1996, for a critique of this view). The expectation is that such subjects will, in diametric contrast to LSA subjects, demonstrate an impairment on the SRT in the absence of any explicit memory deficit. Such a pattern of results would further strengthen the case for multiple memory systems in general and the explicit / non-declarative distinction in particular.

Parkinson's Disease

As discussed above Ferraro, Balota and Connor (1993) compared PD suffers with AD sufferers and controls and found that although the PD subjects demonstrated a good learning effect their interference effect was significantly weaker than either the control or very mildly dementing AD groups. Unfortunately the authors did not test the PD subject's explicit memory for the repeating sequence, however, the PD subjects

did perform a battery of psychometric tests which suggest they did not have an explicit memory deficit. Thus supporting the hypothesis that BG dependent neuropathologies produce the opposite pattern of results (spared explicit memory, impaired non-declarative memory) to that of LSN subjects.

In another study with Parkinson's disease subjects Pascual-Leone *et al*, (1993) compared normal-controls, subjects with cerebellar damage and Parkinsonian subjects while on and off their medication. Unlike previous studies Pascual-Leone *et al* defined 'response time' as the interval between the onset of the stimulus and the depression of *any* response key, regardless of if it was the correct one or not. They also did not discard the reaction time's from error trials (initially pressing an incorrect button) as is typically done in other studies. Nevertheless, although the Parkinsonian subjects did display a decrease in reaction time across repeating sequence blocks it was significantly shallower than that shown by the controls (the number of errors made per block showed a similar pattern). The authors cite this as evidence of a lesser degree of procedural learning in the PD subjects. While the authors do not report the results of any analysis on the interference effect the graphs provided in the article suggest a very similar degree of interference in both reaction times and error-rates for control and PD subjects. What will also be evident from the figures is that the cerebellar group display neither a learning-effect (reduction reaction time over repeating sequence blocks) nor any interference-effect for either measure.

In the 2nd experiment in their study Pascual-Leone *et al* varied the length of the repeating-sequence from 8, to 10, to 12 trials and found that the degree of procedural learning for both the control and PD subjects was inversely related to the length of the sequence. However, at longer sequence lengths PD subjects showed significantly less learning over blocks than did the control subjects, and this was especially evident in the PD subjects while in their unmedicated state. While the authors again fail to mention any analysis of an interference effect it is clear that the control subjects display a robust interference effect at all sequence lengths, whereas the on-medication PD subjects show an inverse relationship between sequence-length and strength of the interference effect. Furthermore, it is clear that the on-medication PD subjects have only a very modest interference effect while performing with the 12-trial sequence, especially in comparison with the very robust effect seen from the control

subjects. Although the unmediated-PD subjects show a similar interference effect for both the 8- and 10-trial sequences it entirely absent for the 12-trial sequence suggesting a much reduced ability to learn the 12-trial sequence. Furthermore, although general explicit memory ability did not differ between PD and control groups significantly fewer of the PD subjects developed explicit knowledge of the repeating sequence. This pattern of results clearly suggests a substantial non-declarative learning impairment for PD subjects at the longer sequence-length but this conclusion must be viewed with some caution in the absence of any actual analysis of the data.

In the second experiment of their study Jackson *et al* (1995) examined the ability of Parkinson's subjects to perform an 11-trial SRT task (the first experiment is discussed separately below). Although statistically equivalent after two 'practise' blocks (pseudo-random sequences) once the repeating-sequence blocks began the PD subject's reaction times only improved for the first two blocks (of six) and thereafter showed no further reduction. Furthermore, while the control subjects show a marked slowing of reaction time when switched to the random-sequences the PD subjects show only a very slight slowing, which is most likely simply inherent behavioural variation rather than a meaningful response to the alteration in sequence information, and statistical analysis supports this conclusion. Furthermore, the PD subjects demonstrated no explicit knowledge of the repeating sequence.

Jackson *et al*, in light of the (then) recent speculation that "the integrity of the frontal lobes may be a key in accounting for many of the cognitive deficits associated with PD", went on to sort their PD subjects *post-hoc* into having either a 'frontal' or 'non-frontal' impairment (via scores on the Wisconsin card-sort test). Evaluation of performance on the SRT task between PD conditions produces a marked difference. Due to the small sample sizes resulting from splitting the PD group no statistical analysis was undertaken but it is clear that the frontal-PD group display neither a learning, nor an interference effect. While the non-frontal-PD group initially respond faster than the controls it should be noted that the marked improvement only occurs within the first two practise (random sequence) blocks and once the non-frontal PD subjects begin the repeating-sequence portion of the study their reaction time showed only a minor decrease across blocks, suggesting they failed to learn the repeating sequence. This conclusion is borne out by the lack of a meaningful slowing in reaction

time when the non-frontal PD subjects are switched the random-sequence condition. The authors go on to note that the Parkinson's subjects in a previous study by Ferraro, Balota & Connor (discussed above) have psychometric test scores that suggests they (the PD subjects) would be categorised into the non-frontal group, which would explain why the PD subjects in that study appear to learn at near control levels of performance.

Westwater *et al* (1998) demonstrated that PD subjects who were not different to controls on several psychometric tests (e.g. the National Adult Reading Test) demonstrated a significantly weaker interference effect when switched from a repeating to a random sequence (control reaction time increase = 109ms, PD reaction time increase = 47ms). Although no test of subject's explicit sequence knowledge is reported it is enough currently to show that PD subjects demonstrate a SRT impairment without any explicit memory deficit.

Sommer *et al* (1999) show a stronger interference effect for control subjects than PD subjects (104 & 78ms respectively). Furthermore, PD subjects performed only "marginally worse" than control subjects on a declarative learning task (the California Verbal learning Task). Thus these PD subjects also demonstrate a SRT impairment in the absence of an explicit memory impairment (or very weak impairment).

Finally Stefanova *et al* (2000) report that PD subjects are substantially impaired on the SRT task in comparison to healthy controls and ACoA subjects. Somewhat atypically PD subjects in this study demonstrate no learning effect of any kind and virtually no interference effect (see Fig. 2.X). Such a comprehensive SRT impairment is unusual and there is nothing in the particular methodology of this study or subject characteristics to suggest why the PD subjects were so completely impaired on the SRT. What is also unusual about the results of this study is that all subjects developed good explicit knowledge of the repeating sequence, even PD subjects, over the course of the test. But yet the PD subjects were not able to employ this explicit knowledge to their advantage. Furthermore the PD subjects did not suffer an explicit memory impairment (Wechsler Memory Scale-Revised) relative to healthy controls, unlike the ACoA subjects who were impaired relative to both PD and control groups. Thus this pattern off results perfectly matches that predicted by the double-dissociation

hypothesis. PD subjects are grossly impaired on the SRT but have normal explicit memory abilities, whereas ACoA subjects demonstrate good SRT performance (albeit not quite as good as controls) while suffering an explicit memory deficit.

Huntington's Disease

Having shown that SRT performance was not impaired in a number of limbic system dependent amnesic syndromes Knopman and Nissen (1991) then turned their attention to the basal-ganglion dependent syndrome Huntington's disease (HD) in the expectation that this would impair SRT performance. In this paper Knopman and Nissen report that HD subjects were impaired on "sequence-specific learning" (i.e. they had a weaker interference effect) and performed at chance level on a test of explicit sequence knowledge. There is something of a confound here in that the HD subjects also demonstrate impaired general explicit memory. It is possible therefore that this explicit memory deficit contributed to their impaired SRT performance. However, the point that PD subjects (with similar BG damage) have an impaired ability to perform the SRT but no explicit memory impairment and no dementia (all PD subjects discussed in this chapter are non-dementing) suggests that the HD subject's explicit memory impairment is more a consequence of their dementia rather than a causative agent of SRT impairment.

It is interesting to note that although the authors cite that HD subjects were substantially slower than the control subjects as evidence of a procedural learning impairment this is an identical pattern of behaviour to the Alzheimer's subjects in their own 1987 study in which they concluded the overall slower reaction times were not evidence of an non-declarative memory impairment in the AD subjects. It would appear, therefore, that they consider the interference effect a more sensitive measure of sequence learning than the learning effect.

Willingham and Korroshetz (1993) tested Knopman and Nissen's 1991 finding that HD subjects were impaired on the SRT and included a methodological variant in that makes the study of particular interest. Rather than simply comparing raw pre- and post-interference reaction times Willingham and Korroshetz calculated a 'predicted reaction time' score for each subject for the random sequence block based on a regression line calculated from the first nine (repeating sequence) blocks, and

compared this figure with the observed post-interference reaction time, and used the difference scores as a measure of Knopman and Nissen's sequence-specific learning (interference effect). Otherwise the methodology was identical to the standard SRT.

Analysis revealed that the slope of the curve for the HD subjects was significantly shallower than that of the controls suggesting the controls learned faster than the HD subjects. Furthermore the predicted vs. observed difference scores for the HD subjects were significantly smaller than for the control subjects suggesting the former had substantially less sequence-specific knowledge. Nevertheless a post SRT interview revealed that only one of the control subjects claimed knowledge of a sequence, and the subject could not accurately describe any portion of it, and none of the HD subjects claimed any awareness of a sequence. Furthermore, although all HD subjects were dementing their explicit memory ability was only mildly impaired relative to control subjects and therefore an unlikely cause of any SRT impairment.

Having demonstrated that HD subjects were impaired on the SRT Willingham and Korroshetz then went on to test their ability to learn a motor skill that did not contain a repeating sequence. In a modified SRT they label the 'Incompatible SRT task' subjects are required to press the button to the *right* of the stimulus position and only ever saw random sequences. The prime measure of behaviour in this task therefore is the reduction in reaction time over blocks. Although HD subjects had slower reaction times there rate of improvement over blocks was not different to that of control subjects, nor was their accuracy (suggesting HD subjects were not trading accuracy for faster reaction times). The conclusions that can be drawn from this study is that HD subjects display a sequence-learning impairment that is not attributable to a simple motor-skill deficit.

In conclusion subjects with BG disorders demonstrate an impaired ability on the SRT while retaining (near) normal explicit memory function. This diametrically contrasts with the behaviour of amnesic subjects and provides strong evidence that the two neural substrates are responsible for different and dissociable memory systems.

2.2.3 The SRT And Other Forms Of Neural Impairment.

Although the SRT literature is primarily concerned with standard memory disorders a number of studies have examined the ability of subjects to perform the SRT after various other neurological events (e.g. traumatic head injury, drug administration etc.). These studies provide parallel strands of evidence for the possibility of multiple memory systems while also adding to our understanding of the processes involved in SRT performance.

Neuropharmacological Studies

In an experimental manipulation of neurologically normal subjects Nissen, Knopman and Schacter (1987) administered the anticholinergic drug scopolamine and then had subjects complete several tests of memory and cognition (e.g. the Boston Naming Test) before performing the SRT task. Although the scopolamine group was impaired on several of the declarative memory tests in comparison to the saline-control group they were not impaired on the SRT as measured by the reduction in reaction time over repeating-sequence blocks and the increase in reaction time when switched to a random-sequence. All subjects went on to complete a generate task (for explicit knowledge of the repeating sequence) and although the scopolamine subjects responded significantly less-accurately than the control group both groups displayed worryingly high levels of accuracy (greater than 70%). In a later article one of the authors (Knopman, 1991a) noted that chance level of generate-task accuracy is 33% and as a result described generate scores of ~60% as reflecting “partial awareness of the repeating sequence” and scores ≥70% as “a marker for the presence of complete explicit knowledge of the repeating sequence”.

In his 1991 study Knopman (1991b) examined neuropharmacological manipulations of the cholinergic and GABAnergic systems with administration of lorazepam (all experiments) and scopolamine (3rd experiment only) respectively. In the first experiment lorazepam was found to disrupt free-recall of word lists (considered an explicit memory task) but did not significantly disrupt SRT performance (although scopolamine subjects had generally slower reaction times and a slightly weaker interference effect). All subjects completed a generate task and both the lorazepam and control groups showed very similar degrees of explicit awareness of the repeating sequence (62.6% for the lorazepam group, and 64.2% for the control group). As noted

above Knopman considers this to reflect partial awareness of the sequence.

In the second experiment in the study Knopman (1991b) replaced the (typical) motor-SRT task with a verbal-SRT task. Identical to the motor-SRT except that subjects responded verbally to the location of the stimuli (presented in one of the four cardinal compass points), Knopman reports verbal-SRT learning is associated with a smaller proportion of subjects gaining awareness of the repeating sequence than in the motor-SRT task. Once again lorazepam subjects were impaired on delayed free-recall compared to the control group but none of the other explicit-memory psychometric test. However in contrast to findings from the first experiment lorazepam subjects displayed a significant impairment on the verbal-SRT task, displaying a minimal reduction in reaction time over the repeating-sequence blocks and no increase in reaction time when switched to a random-sequence. Furthermore the generate task showed than only half of the control subjects had acquired explicit knowledge of the sequence while none of the lorazepam subjects did. The verbal-SRT therefore enjoyed several advantages compared to the usual motor-SRT in that it produced a lesser degree of explicit awareness for the sequence and demonstrated a clear impairment of sequence learning in subjects with GABAnergic blockades.

In the final study of the same paper, which again used the verbal-SRT, Knopman compared scopolamine with a lower dose of lorazepam than in the previous study (1.5mg compared to 2.5mg). Subjects in this study only every performed repeating-sequences and thus no cannot display an interference effect. Unlike the previous experiment the low-dose lorazepam subjects displayed reduced reaction times over the course of repeated-sequence blocks, and their reaction times were not different from the placebo group. In contrast the scopolamine group did not display reduced reaction times over blocks and had significantly slower reaction times than the placebo group. Furthermore, the low-dose lorazepam and control groups were not impaired on free recall, but the scopolamine group was. Although stem-completion was reduced in the lorazepam group this phenomena is clearly dose dependent as the difference was not statistically significant at this, lower, dose. Similarly there was no difference between the placebo and scopolamine group on the stem-completion test, whereas scopolamine did result in impaired free-recall. Interestingly the generate task revealed that all three groups had very similar generate accuracies (~50%) and, more

importantly, when the those subjects with a high degree of awareness of the sequence (≥70%) were removed from the analysis the results did not change, providing some of the strongest evidence that explicit awareness is not necessary for sequence learning.

Traumatic Brain Injury

In a study examining the ability of subjects with traumatic brain injury to perform the SRT Mutter, Howard and Howard (1994) found that subjects with mild (Glasgow Coma Scale (GCS) scores from 13-15) traumatic brain injury (TBi) had no SRT impairment in comparison with either case-matched controls or college student controls, even though the TBi subjects did have lower verbal memory test scores than both control groups. However all groups showed very similar evidence of a high degree of explicit awareness for the, 10-trial, sequence (~80%).

In the second experiment of the study the same authors examined subjects with moderate-to-severe TBi (GCS <13) and found that this group had significantly slower reaction times than the uninjured control group but not when compared to a moderately-injured (GCS>13) group. The more severely injured group also displayed less reduction in reaction times over repeating-sequence blocks and a weaker interference effect, although neither of these results were significant. Thus moderate, or greater, head injury results in impaired SRT behaviour concurrent with reduced explicit memory abilities, however, given the variation in injury locus, little can be concluded as to why.

Beldarrain *et al*, (1999) tested the ability of subjects with unilateral prefrontal cortex lesions (pFC) to perform two SRT tests, one with a 10-trial repeating sequence and one with a 4-trial repeating sequence. Psychometric testing revealed that the pFC subjects were mildly impaired on tests of explicit memory relative to controls. Furthermore, pFC subjects demonstrated both weaker learning and interference effects than control subjects on the 10-trial sequence. Although pFC subjects had a similar learning effect to controls for the 4-trial repeating sequence their interference effect at this trial length was substantially weaker. All subjects, including controls, had poor explicit knowledge of the 10-trial repeating sequence and this was also true of pFC subjects for the 4-trial repeating sequence whereas controls demonstrated reasonable explicit sequence knowledge for this trial length. Thus, although pFC

subjects had only a very mild explicit memory deficit their non-declarative memory performance was substantially impaired.

Transcranial Magnetic Stimulation

Given the rich afferents from the basal ganglia to the dorsolateral prefrontal cortex (dl-PfC) Pascual-Leone *et al*, (1996) hypothesised that the dl-PfC might be an essential component of the neural system responsible for procedural learning. To test this they disrupted dl-PfC activity during SRT performance with transcranial magnetic stimulation (TMS). TMS had no effect on SRT performance during stimulation of either the supplementary motor cortex or the ipsilateral dl-PfC, but produced a marked impairment with stimulation of the contralateral (to the hand used to perform the SRT) dl-PfC. In this condition subjects displayed neither a reduction in reaction time across repeating-sequence blocks, nor an increase in reaction time when switched to a random-sequence block and as such there SRT performance was considered severely impaired. Unfortunately no measure of explicit awareness of the sequence is reported.

Cerebellar Injury

As discussed above Pascual-Leone's 1993 study compared subjects with cerebellar lesions to control and PD subjects. Cerebellar subjects were completely incapable of demonstrating either a learning or interference effect, irrespective of sequence length (4, 8 or 10-trial repeating sequences). Furthermore they were demonstrated very poor explicit sequence knowledge although no mention is made of their general explicit memory ability.

Down Syndrome

Vicari, Belluccia and Carlesimo (2000) examine the ability of subjects with Down syndrome (DS) to perform the SRT. They note that while subjects with mental retardation usually have a declarative memory impairment there is some evidence that subjects with mental retardation show a relative preservation of on-declarative memory (see article for review). For this reason they posit that subjects with DS would not be impaired in a variety of non-declarative memory test including the SRT. Although subjects with DS were impaired relative to controls on explicit memory tests (immediate recall, word recognition, picture recognition and visuo-spatial sequence

learning) both groups demonstrated very similar interaction effects when switched from a repeating to a random sequence in the SRT. However, much as in AD subjects, DS subjects always reacted more slowly than control subjects. Furthermore the interference effect demonstrated by DS subjects was numerically weaker than control subjects. These points suggest DS subjects do not perform the SRT to exactly the same degree as that shown by neurologically intact subjects and that DS subjects may also suffer a mild SRT impairment that was not discovered in the study due to the test between groups having insufficient power (see Chapter 3 for a discussion of power and effect-size issues with SRT studies). Note: Evidence of this study appeared only very shortly before completion of this Ph.D. thesis. This DS study was not included in the meta-analysis presented in Chapter 3 for this reason, but the nature of DS is problematic and its inclusion in the meta-analysis would probably be inappropriate.

2.2.4 Conclusions on Neuropathology and the SRT

A clear pattern emerges when considering various amnesic etiologies and SRT performance. Those subjects with some form of limbic system insult demonstrate a reasonable sparing of SRT performance, albeit not strictly identical to controls, even with a substantial comorbid explicit memory impairment. In contrast those subjects with basal ganglia injury show an opposing pattern of behaviour; little, if any, explicit memory impairment, but some form of SRT impairment (typically a weaker interference effect). Whereas subjects with neither limbic system or basal ganglion injury are often substantially impaired in both the SRT and explicit memory tests. This is precisely the pattern of results predicted by the multiple-memory systems model. Furthermore, *all* the studies produce consistent results and thus provide a good degree of convergent validity, and the fact that Reber and Squire (1998) successfully managed to demonstrate a crossover interaction in a study with good methodological rigour lends substantial credence to both the validity of the SRT as a test of non-declarative memory and the likelihood of multiple memory systems.

2.3 Methodological Variations and SRT Performance

If forced to rely on the evidence from neuropathological studies alone it could reasonably be concluded that the two memory systems were stochastically independence. Fortunately there is also a similarly compelling set of evidence

provided by studies that attempt to dissociate memory performance experimentally in neurologically normal subjects. The following section will discuss a number of experimental manipulations that demonstrate the potential for dissociating between the different memory types via the SRT and in doing so present evidence that the SRT is *primarily* a non-declarative memory task, and explicit and non-declarative memory are independent and dissociable systems.

2.3.1 The Role Of Attention In The SRT.

Nissen and Bullemer (1987) tested subjects in the SRT under dual-task (tone counting) conditions in order to examine the contribution of attention to sequence learning. They found that subjects who experienced dual-task conditions during SRT performance responded more slowly than those subjects who performed the SRT alone. Furthermore, subjects in a dual-task condition who experienced a repeating-sequence in the SRT did not differ statistically from those dual-task subjects that only ever saw a random-sequence, suggesting that the reaction time decrease shown by the dual-task repeating-sequence subjects was simply due to motor learning and did not reflect acquisition (implicit or otherwise) of the repeating sequence In a subjective report measure nine subjects (of 12) in the single- task repeating-sequence condition reported becoming aware of a sequence, whereas only one subject (of 12) in the dual-task repeating-sequence condition became aware of the sequence, and then only in the last block. Therefore while a distracter task severely inhibits developing awareness of a sequence it also inhibits learning sequence specific information (but see Stadler, and Reed and Johnson below), suggesting that some form of conscious awareness is necessary for sequence learning.

In an extension of this study Nissen and Bullemer went on to employ two groups which underwent a series of dual-task repeating-sequence SRT blocks *before* being switched to either single-task repeating-sequence or single-task random-sequence conditions. The expectation was that if something was learnt about the sequence during the dual-task portion of the experiment the single-task repeating-sequence group should display some advantage over the single-task random-sequence group. While analysis revealed no difference between the groups during the dual-task portion of the experiment (as expected) once switched to the single-task condition the repeating sequence group soon showed a decrease in reaction time

compared to the random-sequence group (as would be expected from the previous experiment above). What is of prime interest in this study is the block of trials immediately after the switch from dual to single task, if any learning of the sequence had taken place during dual-task performance it should produce an immediate advantage for those who continued to experience the repeating sequence in the single-task condition in comparison to those who, at this point, switch to a random-sequence. However there was no difference between the two sequence condition groups in this block, suggesting that the subjects in the single-task repeating-sequence condition had not learnt the sequence during dual-task performance. These results clearly demonstrate that prior-practise in the dual-task condition does not produce savings in sequence learning.

In response to Nissen and Bullemer's finding that dual-task performance disrupted serial-order learning Curran and Keele (1993) set out to examine whether variation in attentional availability qualitatively altered SRT results (in order to test their hypothesis that serial-order information can be learnt by two independent mechanisms). The first mechanism they propose requires attention to the relation between successive events while the second operates independently of such attention. Consequentially they predict that dual-task performance will disrupt serial-order learning by degrading attention to the relation between successive events.

This hypothesis was tested by varying the presence of a dual-task and comparing subjects in an 'incidental' learning condition who were either "more aware" of the sequences to those in the same condition who were "less aware" (group assignment via *post hoc* analysis of questionnaire reports) and subjects in an 'intentional' learning condition who had been provided with the actual sequence prior to SRT performance. In the first, single-task, phase of the experiment subjects in either the 'intentional' or 'more-aware' conditions displayed similar amounts of serial-learning (both learning- and interference-effects) consistent with previous studies. Although the 'less-aware' group also showed learning- and interference effects both were significantly weaker than either the other two groups. However, once the dual-task was imposed all groups demonstrated a far weaker interference effect (although still significant) but unlike in the single-task conditions there was now no between group differences. Thus Curran and Keele conclude that "variations in single-task

learning, caused by awareness differences, was not transferred to dual-task conditions".

In their second experiment Curran and Keele covaried the presence of dual-task and awareness of the sequence such that one group was informed of the sequence prior to the task and practised the SRT task for eight blocks under single-task conditions, before switching to a dual-task condition for the test of sequence learning. In contrast the comparison group was not informed of the presence of a sequence and practised the SRT, and was tested for sequence learning, under dual-task conditions. Although, as expected, the 'dual-task-unaware' group responded far more slowly than the 'single-task-aware' group during SRT practise there was no difference in the strength of the interference effect during the, latter, phase of the study when both groups were performing under dual-task conditions. Thus any explicit knowledge gained under single-task intentional conditions is not expressed in the dual-task condition.

Curran and Keele argue that the asymmetry in abilities of differing levels of either awareness (experiment one) or attention (experiment two) to effect initial sequence learning when expressed during dual-task performance is due to "the parallel acquisition of two forms of sequential knowledge – attention and nonattentional – under single-task conditions". Thus, because dual-task performance disrupts attentional learning (by whatever mechanism), only non-attentional learning is expressed during dual-task performance. To further demonstrate this point Curran and Keele had a single group of subjects perform the SRT under (incidental learning) dual-task conditions before switching them to single-task SRT performance in the expectation that only nonattentional learning can occur during dual-task SRT performance. Subjects should display equivalent levels of sequence-learning during single-task performance as during dual-task performance in the absence of sufficient practise to allow attentional learning in the single-task condition. If however they do not display equivalent levels of sequence-learning in the dual-task and single-task conditions this would suggest something other than either attentional or nonattentional learning is influencing reaction time differences. Fortunately the level of sequence-learning was very similar in both test conditions, consistent with the view that only nonattentional learning was exhibited in both conditions.

In light of these findings Stadler (1995) examined the roles of attention in non-declarative learning in order to reconcile Nissen and Bullemer's findings with those of later authors (Cohen, Ivry and Keele, 1990, and Curran and Keele, 1993) whose subjects demonstrated some ability to learn serial order information while performing dual tasks. Stadler noted that the typical task used to interfere with attention while performing the SRT, a tone-counting task, may disrupt learning either by requiring subjects to withhold needed attention from the SRT task (as assumed by most experimenters employing it) or because it disrupts a subjects ability to meaningfully parse the sequence. A number of studies have shown that sequence learning is sensitive to organisational variables (Frensch, Buchner and Lin 1994; Jacoby *et al*, 1989; and Stadler, 1993). Previously Stadler (1993) had demonstrated that "consistent organisation produced greater learning than…. no organisation, and inconsistent organisation produced less learning than….. no organisation". As Stadler notes tone-counting typically requires subjects to maintain a running count of a particular type of tone (typically high tones), thus subjects must assess the tone heard in all trials but on some trials (usually 50%) subjects must then go on and update their count. Stadler suggests that this additional requirement may be sufficient to disrupt sequence organisation instead of attention.

Stadler manipulated dual-task conditions during SRT performance such that one condition imposed additional attentional demand but did not affect sequential organisation and another condition disrupted sequence organisation but did not impose additional attention demands. Although all dual-task conditions ('additional-attentional demands', 'disrupted-sequence', and 'normal' dual-task tone-counting) disrupted SRT performance (in comparison to an identical SRT task in which subjects were instructed to ignore the tones and concentrate on the their reaction time's) there was still good evidence of sequence learning in all dual-task conditions. However, while the disrupted-sequence-organisation condition produced very similar levels of sequence-learning (interference effect) to the standard tone-counting task the additional-attentional-demands condition produced a larger effect (stronger interference-effect) than either the tone-counting or pause conditions, which is consistent with the contention that dual-task tone-counting affects learning because it disrupts sequence organisation.

Stadler repeated the experiment with longer sequence lengths (10-trial instead of 6-trial) in order to control for sequence structure in light of several findings that sequence structure interacts with secondary task such that learning is impoverished under dual-task conditions for some sequences and not others (Cohen *et al*, 1990; and Curran and Keele, 1993). Although Stadler notes that "in Cohen *et al*'s terms, the sequence had no unique associations" any 10-trial sequence has an inherent sequence structure independent of its serial order information which can produce SRT-like learning in the absence of learning any serial-order information (see below), and this must kept in mind when considering Stadler's findings. Of course this critique is even more troubling when applied to his earlier use of 6-trial sequences which not only contain very high levels of sequence structure (unique associations etc.) but also, being so short, raises the strong possibility of subjects becoming explicitly aware of it. However, these concerns notwithstanding, Stadler's second experiment produced the same pattern of results as the first providing further support for the hypothesis that the tone-counting task disrupts sequence organisation rather than attention. What is especially interesting is that in this longer sequence condition both tone-counting and sequence-disruption virtually eliminate any evidence of serial-order learning and manipulating attention demands resulted in only modest evidence of learning. However, those subjects who essentially performed the task under single-task conditions still showed reasonable amounts of non-declarative learning. This suggests that non-declarative learning is capacity limited especially when employed in conjunction with a dual-task, but not as a function of attentional load,

Almost irrespective of how he arrives at his conclusions Stadler (1989, 1992, 1995) is right to point out that one thing attention does is to produce organisation, as attention shifts it parses experience into meaningful chunks. An interruption in a sequence of events creates two sequences, one just ending and one just beginning. Thus attention inevitably produces organisation by determining which parts of the stimulus environment are associated together. For this reason pauses due to dual-task requirements disrupt the organisation of repeating sequence as a complete unit forcing the subject to learn the sequence in a series of variable subunits (due to the variable occurrence of dual-task producing pauses), which is a less efficient method for learning the sequences than whole-sequence parsing. Stadler also concludes that

attention depends on organisation especially when “previously acquired associations…. influence processing”.

Stadler’s conclusions are extended by Rah, Reber and Hsaio (2000) who found that instead of being an invasive additional task subjects treated the tone-counting as if it were “an integral component of a complex stimulus environment”. Subjects treated the tone-counting and SRT as two parts of a single task, and in doing so scan the environment for potential patterns of covariance which is necessarily more cognitively demanding than performing either task alone. Thus rather than being a distracter in the sense of competing for cognitive resources the dual-task SRT compromises performance in a manner more akin to a highly complex single task. Specifically, the non-contingent nature of the two tasks acts as an interferer, subjects attempt to establish covariant relationships which do not actually exist and therefore ‘waste’ the cognitive resources employed in the effort. Secondly, by treating the dural-task SRT as a more complex task than either of the two tasks alone performance is impaired by the fact that the task now becomes ‘too’ complex to perform properly.

Thus while earlier studies of attention and the SRT seem to implicate attention as a necessary component of sequence learning it is now apparent that in fact the role of attention in the SRT is somewhat more subtle. Specifically that attention allows the efficient organisation of stimuli into meaningful units, and thus anything that disrupts attention disrupts this organisation which is what results in weaker sequence learning rather than attentional disruption *per se’.* Furthermore, that subjects treat the dual-task SRT as a highly complex multivariant task rather than two separate and competing tasks. Therefore, performance, and acquisition of explicit sequence learning in particular, is degraded by providing examples of spurious covariance between the two tasks. Thus the tone-counting dual-task SRT would appear to be an effective and conceptually sound method for limiting explicit sequence knowledge and inhibiting SRT performance in neurologically intact subjects.

2.3.2 Neuroimaging and the SRT

Neuroimaging studies provide evidence of the basal ganglia hypothesis for non-declarative sequence learning and are consistent with the multiple-memory hypothesis. Specifically, that discrete and independent neural substrates are

responsible for non-declarative and explicit sequence learning. While the critical regions responsible for different forms of learning are difficult to enumerate there is a clear dissociation in terms of explicit learning involving more cortical structures whereas non-declarative learning involves more sub-cortical structures, including the motor areas of basal ganglia (putamen). This cortical / sub-cortical dissociation is consistent with the assumptions of the limbic system / basal ganglia dissociation.

Grafton, Hazeltine and Ivry (1995) used PET scanning in conjunction with a dual-task SRT procedure (SRT and tone-counting) and found learning related increases in regional cerebral blood flow (rCBF) in left hemisphere motor effector areas (all subjects were right-hand dominant). In particular the motor cortex, the supplementary motor area and the putamen. What is especially compelling about this result is that dual-task performance prevented the acquisition of *any* explicit sequence knowledge and thus the activation of these neural regions is specific to non-declarative sequence learning. Furthermore, subjects in this study immediately went on to do a single-task SRT study with PET scanning during which most subjects acquired good explicit sequence knowledge. However, during single-task performance different neural regions were activated (the right-hand: dorsolateral pre-frontal cortex, pre-motor cortex, and ventral putamen, and the biparieto-occipital cortex). Furthermore, there was no difference in the pattern of neural activation between subjects in the single-task SRT that did or did not acquire explicit sequence knowledge. Therefore, non-declarative learning involves activation of motor effector areas and the motor region of the basal ganglia in the left hemisphere, undoubtedly because these areas control the hand subjects respond with. However, once learning becomes explicit activation shifts to the right hemisphere. Most likely because task focus then shifts to requiring subjects to learn about the sequential nature of spatial events.

In a follow-up study Hazeltine, Grafton and Ivry (1997) performed a similar experiment in which they substituted a ‘perceptual’ SRT task for the usual spatial SRT task. The perceptual SRT task was identical to the spatial task except for all the stimuli appearing a central location and varied by colour rather than position. The results of the perceptual SRT PET study supported that of the earlier spatial SRT PET study. During dual-task performance no subjects developed explicit sequence knowledge

and rCBF increased in the contralateral frontal and parietal areas. In particular the supplementary motor area, the motor cortex and the (subcortical) putamen / thalamus. In contrast rCBF decreased in the bilateral middle temporal cortex, the inferior occipital areas and the cerebellum. Once again neural activation changed when subjects were switched to the single-task perceptual SRT in which some subjects developed explicit sequence knowledge. Specifically rCBF increased in the right-hand inferior temporal and frontal areas and bilaterally in the anterior cingulate. Similar to the previous study there was no difference in neural activation for subjects with or without explicit sequence knowledge during single-task SRT performance.

Two particular patterns of results are evident in these studies. Firstly, perceptual SRT performance produces more ventral activation than spatial SRT performance, and spatial SRT performance produces activation in the dorso-lateral pFC while perceptual SRT performance does not. Furthermore, the pattern of neural activation between the two tasks is exclusive as there is no foci of activation common to both tasks. Secondly, although not all subjects acquired explicit sequence knowledge during the single-task condition in either study there was absolutely no difference in the pattern of rCBF activation between 'aware' and 'unaware' subjects. It is also worth noting that these results support Curran and Keele's (1993) finding that different neural systems are responsible for sequence learning under distracted and non-distracted conditions.

Hazeltine, Grafton and Ivry note that while the spatial SRT removes the spatial component of the stimuli the spatial component of the *response* remains the same as in the spatial-SRT (subjects physically respond in the same way). However, removing the spatial component of the stimuli helps to determine whether sequence learning is more dependent on perceptual or motor events (Willingham, Nissen and Bullemer, 1989; Keele *et al*, 1995). Hazeltine *et al* note that if sequence learning is more perceptual than motor orientated then 'we would expect to find the change in stimulus properties to lie outside areas associated with motor control'. In contrast if sequence learning is more motor orientated then blood flow changes should be relatively restricted to motor areas. As blood flow changes do occur outside motor areas Hazeltine *et al* conclude sequence learning does not simply depend on learning a series of stimulus locations but rather "occurs at a more abstract or response-related

level".

In the latest study in the series Grafton, Hazeltine and Ivry (1998) examined the question of why behavioural evidence shows near perfect transfer of knowledge to novel sets of effectors when non-declarative sequence learning is predominantly (but not exclusively) supported by motor regions. The authors note that SRT learning can consist of any combination of three functional attributes: motor knowledge, perceptual knowledge, and abstract (goal) knowledge. Assuming that SRT performance relies primarily on abstract sequence representations (per their findings above) rather than motor knowledge helps explain the flexible nature of the knowledge. In order to test this assumption the authors employed the perceptual SRT from their previous study (including the distracter task) and varied the presence or absence of a repeating sequence and the form of motor responses to stimuli (either the traditional SRT 4-finger response panel or a much larger keyboard requiring complete arm movements). Unlike the previous two studies sequence awareness was not varied in that subjects always performed under dual-task conditions and never acquired explicit sequence knowledge. They found that, irrespective of response style, during repeating sequences rCBF increased in the contralateral (left-hand) sensorimotor cortex, supplementary motor area and rostral inferior parietal cortex. Furthermore, rCBF activity peaked in the cingulate motor area during transfer between response styles, suggesting a role linking abstract sequence information and effector systems. After transfer activity in the inferior parietal cortex remained high whereas sensorimotor cortex activity shifted to more dorsal loci. From these results they concluded that abstract sequence information resides in the inferior parietal cortex and is channelled through the cingulate motor area (Brodman's area 24) to particular effector systems.

Pascual-Leone *et al* (1999; see also Pascual-Leone, Grafman, and Hallet, 1994) report conceptually similar results from a transcranial magnetic stimulation SRT study. During early single-task SRT performance, during which the authors assume all learning is implicit, the contralateral (to the hand in use) motor cortex output map significantly increases in size. This increase plateaus during the subsequent phase of performance, which the authors assume consists of a mixture of non-declarative and explicit learning, before rapidly decreasing in the final phase which is assumed to consist entirely of explicit learning. While the assumptions as to when the shifts in

learning style occur during the various phases of SRT performance are open to question the clear fact is that there are two very distinct patterns of motor cortex output which, at the very least, suggest two distinct forms of learning are taking place.

Finally, Rauch *et al* (1995) report a PET scan study of subjects performing the SRT and found that the right ventral premotor area, the right ventral caudate / nucleus acumbens, and the right thalamus were activated during the implicit condition (uncued repeating sequence information). Whereas activation in the explicit condition (after explicit training with the repeating sequence) occurred in the primary visual cortex, pre-sylvian cortex and cerebellar vermis. As a result the authors concluded that "implicit sequence learning is mediated by cortico-striatal pathways".

In summary neuroimaging SRT studies produce good, but not totally unequivocal, evidence that non-declarative sequence learning generally involves subcortical / basal ganglia structures. The biggest limitation with these studies is that they can (currently) only generate relatively low resolution indexes of neural activation. Although the general pattern of neural activation in these studies is consistent with the multiple memory systems theory (i.e. non-declarative memory relies on the basal ganglia whereas declarative memory relies on the limbic system) the scanning techniques employed so far cannot reveal the degree of neural specificity necessary to conclusively demonstrate the neuroanatomical independence of the memory systems that declarative and non-declarative memory are dissociable and independent. However, when the neuroimaging evidence is combined with clinical studies with neuropathological patients the weight of evidence in favour of the multiple memory systems theory is compelling.

2.3.3 The Effect Of Sequence Structure On SRT Performance

It well known that the longer sequence is the more difficult it is to deliberately remember, which can only be a good thing in any test of non-declarative memory. However, the more important point is that sequence length, in conjunction with the number of stimulus locations, imposes constraints on what is commonly termed the 'sequence structure' or the 'statistical structure' of the sequence. An example drawn from the SRT literature will readily illustrate the concept. When Knopman and Nissen (1987) first introduced the SRT they employed the following 10-trial sequence (where

‘A’ is the left most stimuli position and ‘D’ the right most) DBCACBDCBA. However, given that there are only four stimulus positions in the SRT task there must necessarily be an inequality in the number of locations presented to a subject within a single repetition of the sequence. Thus the two ‘outer’ stimulus positions (‘A’ and ‘D’) are presented twice during one repetition whereas the two ‘inner’ positions (‘B’ and ‘C’) are presented three times. Therefore, there is unequal stimulus-frequency within the sequence and a subject could facilitate their performance simply by learning (explicitly or implicitly) that two of the locations have a higher probability of appearing next and thus predisposing themselves to respond to those positions, which would ‘pay off’ half of the time. However, the random-sequence used by Knopman and Nissen was not similarly biased, all stimulus locations other than the current location had an equal probability of appearing next, therefore a subject could not facilitate performance via simple stimulus-frequency information. The critical point is that a subject could respond faster in the repeating sequence condition without actually learning the repeating sequence, but instead by learning simple frequency information. However, when compared to reaction times in the random-sequence condition, a subject would fulfil all the criterion of having learnt the sequence (faster reaction times during the repeating-sequence blocks and a slowing in reaction times when switched to the random-sequence block), when this was not necessarily the case. Thus it is necessary to ensure two things: that a repeating sequence be consistent within itself (e.g. have equal stimulus frequency etc.) and that it be consistent with a random sequence such that only the actual sequence information varies between the two types of sequence. As will be seen in the following discussion subjects will readily seize on variations in simple frequency information in order to facilitate their performance if such information is available to them, rendering conclusions about sequence learning in such situations problematic. However, it will also been shown that when sequence structure is strictly controlled such that subjects can only improve their performance by learning the repeating sequence learning they do learn it, and thus, under such rigorous methodological conditions, the SRT is a valid and reliable test of sequence-learning.

In a study designed to elucidate exactly what is learned during sequence learning Reed and Johnson (1994) first discuss a number of different types of simple frequency information contained within a sequence that may provide subjects with an

advantage when performing the task, and hence give the appearance of actually learning the sequence. They identify 5 types of simple frequency information inherent in sequences:

1) Location Frequency: The frequency with which each target location is presented within a sequence.
2) Transition Frequency: The frequency with which each possible transition (from one stimulus to another, e.g. A→C, or B→C or D→A etc.) occurs within a sequence.
3) Reversal Frequency: The frequency with which back-and-forth movements occur, e.g. A→B→A
4) Rate of Full Coverage: The average number of stimuli encountered so that each possible location has been presented at least once.
5) Rate of Complete Transition Usage: The average number of stimuli encountered so that each possible transition has been presented at least once.

Reed and Johnson cite a study by Cohen, Ivry and Keele (1990) in which subjects were first trained with Second Order Conditional sequences (SOC: A SOC exists when a stimulus location is entirely predicted by the previous two locations, whereas knowledge of the immediately prior position provides no information regarding the next) and then switched to random sequences, displaying an increased reaction time after the switch. When comparing the structures of the repeating sequences and random sequences Cohen *et al* used it was found that, while the location and transition frequencies were very similar, other frequency information associated with the repeating sequence was very different to that of the random sequences. In comparison with the SOC sequence the random sequences contained higher reversal frequencies, rates of full coverage and rates of full transition usage. Therefore it is possible the increased reaction time's demonstrated by these subjects reflects the learning of any-or-all of the frequency information that changes during transfer trials rather than sequence learning as originally assumed.

Reed and Johnson went on to conduct several experiments designed to determine if this sort of variation in simple frequency information could produce SRT-

like learning. In the first they trained subjects on a series on non-repeating trials that were characterised by “nearly identical” event frequency properties as a SOC sequence. After training subjects were transferred to either a truly random sequence or a SOC sequence, in the expectation that if subjects had learned the structural frequencies during training their performance would no deteriorate when switched to the SOC sequence, but would when switched to truly random sequences. (Note: All subjects completed this experiment under dual-task, tone-counting, conditions). Although there was no actual sequence information to be learnt during the training trials those subjects that were switched to random sequences with different structural frequency information than the training trials displayed a significant interference effect. Whereas those subjects that were switched to a SOC sequence with similar event frequency information to the training trials did not. Thus those subjects that were transferred to one random sequence condition from another demonstrated an SRT-like interference effect which can only be attributed to the variation in the structural information contained in the different conditions. This raises the possibility that what is considered evidence of sequence learning in most SRT studies may in fact actually be due to variations in structural information rather than sequence information. As a result Reed and Johnson conclude “transfer sequences should match training sequences on all properties other than the specific structural characteristic being investigates”.

In their second experiment Reed and Johnson examined the ability of subjects to learn a SOC sequence when tested with a random sequence that was identical with regard to simple frequency information but varied completely in regard to SOC (sequence) information. The experimental group underwent blocks of one particular SOC sequence before being switched to a single block of a novel SOC sequence that was structurally identical to the first SOC sequence.. Analysis showed that all groups became significantly faster over blocks but that there was no difference between groups, thus learning an actual sequence gave no advantage over learning simple frequency information. However, the analysis of the experimental group’s transfer block revealed a significant slowing in reaction times which can only be attributed to the variation in sequence information, and further analysis showed that subjects had not simply learned a subset of the SOC sequence, strongly suggesting that subjects are capable of learning complex sequence information. The performance of repeating-only control subjects (who performed the repeating-sequence portion of the SRT but

were not switched to a random sequence of any kind) in a subjective (verbal-report) test revealed between 15-20% of subjects became consciously aware of a sequence during training, but none were able to actually specify it. Furthermore, a correlation between the slope of subjects learning curves and their performance on the tests for explicit sequence knowledge was not significant suggesting the independence of non-declarative and explicit sequence knowledge. Thus while subjects will use simple frequency information to facilitate performance if they can they will also learn complex sequential information if that is the only way they can facilitate their performance. This conclusion is further supported by studies that find simpler sequences (those that include both SOCs and First Order Conditionals (FOCs)) are learnt more readily than more complex (SOC only) sequences (Cohen at al, 1990, and Keele and Jennings, 1992). Therefore attribution of learning to acquisition of complex sequence information is problematic when simpler information is also allowed to vary between training and test conditions.

Jackson and Jackson (1995) argue that SRT performance could be due to learning simple probabilities present within the repeating sequence, rather than a more complex representation of serial-order information. Similar to Johnson and Reed's argument Jackson and Jackson also note that the structure of a sequence (less than 12-trials long) when performed on a 4-reponse apparatus (as per the normal SRT procedure) necessarily produces a sequence 'grammar' in that some transitions (the relationship between two sequentially adjacent sequence elements as measured by the probability that one stimulus location will be followed by another) occur less often than others (or even not at all). An examination of the 10-trial sequence first introduced by Knopman and Nissen in 1987 (see above), and widely used thereafter, reveals a very uneven transition grammar (see Table 1). Jackson and Jackson argue that simply learning unequal transition probabilities within a sequence could produce SRT-like performance in the absence of any serial-order learning, and note that it is possible to have knowledge of transition probabilities in the absence of knowledge of serial-order information. Furthermore they claim that common methods of explicit-awareness analysis do not asses the former kind of knowledge, therefore common methods of testing for explicit sequence awareness fail to meet the conditions for Shanks and St. John's information criterion because they do not test for the information that actually produces the behaviour (assuming simple probabilistic

learning is sufficient to produce a behavioural advantage during SRT performance).

When Jackson and Jackson reanalysed generate-task data from their own studies they found a positive relationship between generate accuracy and the correlation of subject's responses and the transition table for each of the different sequences examined. Further examination of these results revealed a small number of subjects whose responses correlated highly with the transition structure of the relevant sequence while their accuracy performance was at chance levels. Strongly suggesting these subjects had learned the transition structure of the sequence rather than the repeating sequence.

Table 2.1, A Table of Transition Probabilities for Knopman and Nissen's 10-trial Sequence: DBCACBDCBA

		2nd Element			
		A	**B**	**C**	**D**
1ST Element	**A**	-	0.00	0.5	0.5
	B	0.33	-	0.33	0.33
	C	0.33	0.66	-	0.00
	D	0.00	0.5	0.5	-

In a study also concerned with sequence structure Stadler and Neely (1997) explore a possible confound between sequence length and sequence structure. A number of studies (Howard and Howard, 1992, and Pascual-Leone *et al*, 1993) concluded non-declarative memory is capacity limited as non-declarative learning diminishes when sequence length increases and Stadler and Neely's experiments also reveal some very interesting consequences of various sequence-structure variations. As they note sequence structure can be coded in many ways and they choose to employ an information metric approach (per Attneave, 1959), and specifically focus on per-cent redundancy within a sequence. As a sequence departs from complete randomness, it increases in redundancy. A completely random sequence has 0% redundancy; a completely predictable sequence has 100% redundancy (see the article for a discussion of how redundancy is calculated). Stadler and Neely go on to note that the sequences used by both Howard and Howard, and Pascual-Leone not only varied by length but also by redundancy, such their longer sequences had less redundancy than the shorter sequences. Therefore, rather than

the variation in sequence lengths, it may have been the variation in sequence structure / redundancy that produced learning differences in these studies.

To address this concern Stadler and Neely compared sequences of different lengths with sequences of varied structures. Specifically they compared the two 10-trial and two 16-trial sequences used by Howard and Howard (1989) with two additional 16-trial sequences of their own devising which “matched the 10-trial sequences in statistical structure”. Therefore, if sequence length is an important factor in serial-order learning the difference between 10- and 16-trial sequences found in the Howard and Howard study should be replicated here, regardless of statistical structure. On the other hand if sequence structure is important there should be no difference between Howard and Howard’s 10-trial and Stadler and Neely’s 16-trial sequences. Analysis of the slowdown in reaction time after switching to random sequences showed that the 16-trial sequences generated by Stadler and Neely produced as much interference as Howard and Howard’s 10-trial sequences allowing Stadler and Neely to conclude “Thus, when sequence structure is equated, length has little effect on learning” with the obvious consequence that sequence structure does. Furthermore, although the difference was not significant the mean interference score for the 10-trial sequences was actually less than that for the 16-trial sequences, which is in the opposite direction to that predicted by the sequence length hypothesis.

Stadler and Neely next manipulated both sequence length (with 8-, 12- and 16-trial sequences) and sequence structure (high and low) and again found that structure was the main determinant of learning (highly structured sequences resulting in greater learning than less structured sequences), and if anything learning actually increased with trial-length.

Finally Stadler and Neely manipulated sequence length (8- and 12-trial sequences), by co-varying it with number of stimulus locations, (four and six respectively) and sequence structure (high and low). Their results were consistent with their previous experiments, finding a significant effect of structure (higher structure producing more learning) regardless of sequence length. Examination of a sequence recognition test showed that approximately half the subjects recognised the sequence,

however[2], as a general rule, these subjects actually showed less learning (a lower reaction time slowdown) than those subjects who did not correctly recognise it allowing Stadler and Neely to conclude that "subject's awareness…. appears not to affect the pattern of results". However, it is worth noting that Stadler and Neely's recognition test explicitly informed subjects that a repeating sequence had been present before forcing them to choose between one of four sequences, where perhaps a more graduated procedure (e.g. foils) might have been more appropriate. Furthermore, the actual proportion of correct judgements is not reported which is unfortunate, especially as they also asked subjects to rate how confident of their judgement they were which, when combined with raw accuracy, is likely to be a sensitive test of conscious awareness.

Finally, Curran (1997) manipulated the presence of FOCs and SOCs in sequences in order to re-evaluate higher-order association learning in light of Reber and Squire's (1994) finding that amnesic and control subjects showed similar levels of non-declarative sequence learning when the repeating and random sequences contained different serial-order information but identical stimulus (simple-) frequency information.[3] Curran used two repeating sequences with different degrees of pairwise-association predictiveness, the first sequence contained elements that were probabilistically predictive (i.e. FOCs) whereas the second repeating sequence contained SOCs which were not probabilistically predictive, so simply learning pairwise information is an inadequate technique for learning the SOC sequence.

In a very interesting departure from normal SRT methodology Curran, instead of having subjects perform several blocks of a repeating sequence and then switch to a block of random sequences, intermixed the repeating and random sequences in the same block. Thus (using 'R' to denote 12 random trials and 'S' to denote one cycle of the 12-trial repeating sequence, either the FOC or the SOC sequence) each block of 120 trials was arranged as: R-S-S-R-S-S-R-S-S-R. Curran notes two main advantages of this methodology; it helps obscure the presence of a repeating sequence thus

[2] On a methodologically similar note Pascual-Leone *et al* (1993) reported that while 80% of their control subjects developed declarative knowledge of the 8-trial sequence ('declarative knowledge' was defined being able to consciously reproduce 50% of the sequence) only 10% developed it for the 12-trial sequence.

[3] Curran uses the terms first-, and second-, order *predictive*, i.e. FOP and SOP, rather than first-,and second-, order *conditional* as used by Reber and Squire, but the terms are synonymous and this thesis will continue to use FOC and SOC to maintain consistency with the discussions above.

reducing explicit awareness of it, and allows a within-subject measure of learning to be obtained in each block rather than at a single point towards the end of the experiment.

Analysis revealed that although subjects learned more while performing the SOC rather than the FOC sequence there was no difference for either sequence between groups, thus amnesiac subjects displayed similar levels of learning compared to control subjects for both sequence types. The only difference between the amnesiac and control groups was that while the amnesiacs showed significant learning of both sequences when analysed separately from the control group they did not show a greater learning effect for the SOC sequence as compared to the FOC sequence while, when analysed separately, the control group did.

As mentioned the intermixed design allows an analysis of pairwise learning, such that it was possible to compare reaction times for identical stimulus transitions, e.g. 1-2, in both the random and repeating conditions. Not only was there no effect of group when analysing the FOC sequence in this way but the analysis also suggested that, when learning the FOC sequence, there was little higher-order information learnt by either group. This is consistent with the studies presented above that suggest subjects will use simple frequency (FOC) information to perform the task if they can. In contrast a pairwise-analysis of the SOC sequence learning revealed a significant difference in the amount of higher-order learning between the random and repeating sequences. Thus, on average, there was significant learning of higher-order (SOC) information. While there was no main effect of group in this analysis, examination of group results independently of each other revealed that while the control group showed a significant effect of sequence type this only approached significance ($p=0.07$) in the amnesiac subjects, suggesting that control subjects learnt more than mere pairwise information in most sequence positions whereas amnesiac subjects also learnt this but in fewer positions (but see Chapter 3).

When explicit sequence knowledge was assessed (using recognition tests) it was found that neither group displayed above-chance levels of recognition on any test. Curran notes that the particular recognition tests employed had previously (Curran, 1997) been confirmed as valid and sensitive and suggest that the low levels of explicit sequence knowledge are likely due to the intermixing of random and

sequence trials.

Thus Curran concludes that while amnesiac subjects demonstrated some higher-order learning they displayed less than control subjects and are therefore somewhat impaired in comparison, and this may explain why many SRT studies find amnesiac subjects typically react more slowly than control groups, even though the difference is typically not significant. However, it is also worth bearing in mind that the presence of intermixed sequence types may have instead produced a disproportionate degree of disruption in amnesic subjects as it has been suggested amnesic subjects show greater susceptibility to such interference (Shapiro and Olton, 1994). Regardless, this study also produces good evidence of: the ability of both amnesic and neurologically-normal subjects to demonstrate sequence-learning in the absence of any conscious knowledge of the critical (SOC) information contained in the sequence and the importance of ensuring that only intended sequential information is allowed to vary between different sequence types.

It will be apparent from the forgoing discussion that considerable methodological rigour is required in order to ensure that what appears to be sequence-learning is in fact just that. Nonetheless, it is also clear that with careful consideration of these issues it is still possible to demonstrate an SRT phenomena in both intact control and neurologically impaired subjects.

2.4 Methodological Concerns; Measuring Explicit Sequence Knowledge in the SRT

As has become apparent in the forgoing discussion there are a number of methodological and conceptual / theoretical concerns with the SRT. Although the previous section dealt with concerns relating to sequence structure there remains the problem associated with accurate and valid tests of a subjects explicit knowledge of the sequence. This is one of Shanks and St. John's primary concerns (and led to the development of their sensitivity criterion) and the following section will discuss a number of different procedures used to this end, and the problems and advantages associated with each.

The contamination of SRT performance by explicit awareness of the repeating-sequence (either in part or in its entirety) is, given the SRT is a test of non-declarative

memory, of concern. The least empirically rigorous technique for assessing explicit sequence knowledge is a simple verbal report. Subjects are simply asked, at the completion of the SRT, if they became aware of a repeating sequence. A number of concerns have been mooted with this approach, ranging from issues of how confident of their opinion the subjects are to concerns with the poor fit between free-recall and SRT task characteristics (Shanks and St. John, 1994). Prefacing the definition of their criterion for non-declarative learning Shanks and St. John comment that many studies have reported subjects can acquire information without being able to verbalise it later on, and note that these findings are often used to support the claim of unconscious learning. They then go on to propose that "if the aim is to establish what the subjects' state of awareness was at t_1 [time 1], examining the content of their verbal reports at t_2 is certainly not the only way to do this and may not be the best one".

> In an extension of the free-report technique some authors instead adopt a structured-interview approach. Rather than simply being asked if they noticed a sequence, subjects are asked a series of progressively more focused questions, ranging from "Did you notice anything about the task" to asking them to reproduce the actual sequence. Although Willingham *et al* (1989) found a reliable SRT learning effect even after removing subjects who appeared to have explicit knowledge of the sequence after such an interview this approach is also open to many of the concerns with free-recall.

In conjunction with the introduction of the SRT task itself Nissen and Bullemer introduced the generate-task (AKA prediction task) whereby subjects are typically required to identify the stimulus position coming next (a cued-recall task). While this test is undoubtedly a more sensitive / rigorous test of conscious knowledge (see Shanks and St. John, 1994). However, Jackson and Jackson (1995), in their critique of methods for assessing explicit awareness, note their own demonstration (Jackson and Jackson, 1992) that estimates of explicit knowledge identify only partially overlapping sub-populations of subjects. Furthermore they also note that Shanks (1993) "showed that subjects classified as unaware… were significantly above chance on a cued recall task".

Reber and Squire (1998, above) and Perruchet and Amorim (1992) employed a recognition test to examine subject's explicit knowledge of a repeating sequence. Typically this requires subjects to rate various sequence elements (e.g. 4-trial subsets of a 10-trial sequence) as having been present (or not) in the previously performed

SRT task. Elements of the actual sequence are juxtaposed with foils and recognition accuracy is correlated with reaction time performance. In the Perruchet and Amorim study this produced a correlation of 0.8, which the authors decided was evidence that SRT performance was a consequence of explicit knowledge of sequence fragments. Shanks and St. John also conclude that a recognition test is a valid measure of explicit knowledge. They come to this conclusion because this task (as well as the generate task) reproduce the stimulus context, and can be performed at above-chance levels regardless of whether the subjects knowledge is of fragments or of the entire sequence, hence fulfilling both their sensitivity and information criteria.

Therefore recognition tests would appear to be valid and appropriate method for testing subject's explicit sequence knowledge. As a result more recent studies often employ this procedure (typically in conjunction with other tests of explicit sequence knowledge, e.g. generate tests) and report evidence of non-declarative sequence learning in the absence of explicit sequence knowledge (Perruchet and Amorim, 1992; Reber and Squire, 1998).

2.5 Other Methodological Factors.

In one of the first explorations of precisely what is learned during a SRT task Willingham, Nissen and Bullemer (1989), in the first part of their study, demonstrated that some subjects were capable of significant serial-order learning in the absence of any explicit knowledge for the sequence (as assessed by a standard generate task). As later authors would also do they removed those subjects from the analysis who they classified as making anticipatory responses (those subjects that initiated a motor-response prior to, or extremely quickly (<100ms) after, stimulus onset). Willingham et. al. quantified anticipation as any response quicker than 100ms in light of Wood's (1997) finding that the response portion of a simple reaction time is approximately 100ms, and go on to note that while this type of response was initially rare by the end of the experiment those subjects with a high degree of declarative knowledge of the sequence were making anticipatory responses 50% of the time. The overall pattern of results was similar with-or-without the anticipatory responses removed except and, as would be expected, the 'full explicit knowledge' group did not reach the same extremely fast reaction times by the end of the experiment, when anticipatory responses were removed, as they had with them included. What is especially

interesting is that with anticipatory responses removed none of the groups ('no explicit knowledge', 'some explicit knowledge' and 'full explicit knowledge') differed from each other, all showed a similar degree of reaction time reduction over blocks and similar levels of absolute reaction time, again suggesting that it is possible to perform the SRT without any declarative knowledge of the sequence, and that any degree of (anticipatory independent) declarative knowledge for the sequence confers no behavioural advantage on the SRT task.

Willingham et. al's second experiment was designed to "investigate the temporal relation between the emergence of procedural and declarative knowledge of the sequence". To this end they compared groups with varying lengths of exposure to the sequence (number of SRT blocks completed) and found that both non-declarative and explicit knowledge increased with performance. However, they went on to assess individual subjects degree of non-declarative and explicit knowledge and found that there were comparable proportions of subjects who developed; both non-declarative and explicit sequence knowledge, just non-declarative knowledge, and just explicit knowledge. Allowing the author's to conclude that "It is apparent that declarative learning can take place in the absence of procedural learning, just as procedural learning can take place in the absence of declarative learning".

2.6 Shanks and St. John (1994) Revisited

As promised in Chapter 1 the discussion will now return to a consideration of Shanks and St. John's conclusion (that non-declarative learning has not been adequately demonstrated) within the specific context of the SRT task. This section will first examine Shanks and St. John's treatment of SRT studies in their article before moving on to later studies that have a bearing on their conclusions. It will be shown that several studies unequivocally fulfil both their criteria and are hence compelling evidence for both the explicit / non-declarative dichotomy and multiple memory systems in general.

Like many authors at the time Shanks and St. John criticise the use of repeating sequences that contain probabilistic structures (e.g. stimulus frequency) other than mere serial order and also the use of supposed random sequences which vary in simple frequency information from that found in the repeating sequence. They

support these criticisms with the results of a study, Shanks, Green and Kolodny, (1994) based on Willingham, Nissen and Bullemer's, (1989) study in which both studies compared a 'normal [repeating] sequence'[4] group, with a 'pseudorandom' sequence group (who received a "series constrained to have the same number of each stimuli… per 10 trials as appear in the sequence proper") and a "truly random" group who's stimuli were only constrained by the injunction that they not immediately repeat (i.e. a 'classical' control group typical of early SRT studies). After classifying subjects in the normal sequence group via structured interview as having: no knowledge, some knowledge, or full knowledge of the repeating sequence they discovered that while reaction time did diminish significantly for all but the truly random group (indicating the other groups had learnt something) there was no significant difference between the normal-sequence / no-knowledge group and the pseudorandom group (but note they are solely concerned with the 'learning effect' here and make no comment about their subject's 'interference effect'). As a result they conclude this experiment, and therefore Willingham *et al*'s also, fails the information criterion. Given the discussion above the results will come as no surprise and is perfectly compatible with the contention that subjects will employ simple probabilistic information to improve performance where possible.

However, while Shanks and St. John's conclusion isn't disputed there are two points worth making. Firstly that, as discussed above, their own normal-sequence and pseudorandom conditions are vulnerable to criticism based on the variation of simple frequency information (other than simple stimulus frequency for which they did control) and one can't help but wonder what the result might have been if they used a repeating sequence and appropriate random sequences that varied no frequency information other than the actual repeating sequence (i.e. a 12-trial repeating sequence). Secondly, Shanks and St. John are open to the criticism that they have adopted a straw-man hypothesis in terms of what they decide constitutes non-declarative learning. In much the same way that Cleereman's (1994) criticises them for attacking a position that "I doubt anyone researching non-declarative learning would be willing to defend" their decision to confine their attention to reaction time reduction (the learning effect) as opposed to reaction time increase when switching from a repeating to random sequence (the interference effect) is indefensible. Even the early SRT studies recognised the interference effect is a more reliable measure of

[4] It is assumed, in light of other comments, that this is the usual 10-trial sequence introduced by Knopman and Nissen, 1987

non-declarative learning than the learning effect and this approach was well established in the literature by the time Shanks and St. John undertook their study. Furthermore, they *expressly* sought to replicate Willingham *et al* who most definitely included, and analysed, an interference condition and thus Shanks and St. John's omission is even more inexplicable. Although Shanks and St. John's findings are in line with others concerned with the effect of sequence structure the fact that they chose to test their hypothesis against the weaker of the two articles of proof for non-declarative learning, while not defeating it, reduces the value of their study.

A quick examination of Reed and Johnson's 1994 study (discussed above) reveals that it fulfils both Shanks and St. John's criterion. Reed and Johnson's random sequence was structural identical to the repeating sequence and thus fulfil the information criterion. Furthermore, in order to demonstrate an absence of conscious knowledge of the repeating sequence Reed and Johnson employed both a prediction and a recognition test, the latter of which Shanks and St. John conclude is "a genuine measure of awareness of the sequence". As discussed above Reed and Johnson's subjects performed the recognition task at chance levels while showing significant reaction time slowing when switched to interference trials in the SRT and, as Reed and Johnson's study meets both criterion, it is undisputable evidence for unconscious sequence learning during SRT performance. Reed and Johnson note three points to explain the difference in results between their study and Shanks and St. John. Firstly, as just noted, Shanks and St. John compared learning slopes between groups whereas Reed and Johnson compared disruption in reaction time (interference), Reed and Johnson note that the latter measure is more sensitive, especially in light of the fact that they found no correlation between slope and magnitude of reaction time disruption. Secondly Reed and Johnson's subjects performed under dual-task conditions and they suggest that the lack of any distraction in the Shanks and St. John study (both only employed neurologically intact subjects) may well account for the high degree of conscious sequence knowledge. Thirdly that the subjects in Shanks and St. John saw 40 repetitions of their 10-trial sequence whereas those in Reed and Johnson saw 136 repetitions of their 12-trial sequence, thus the Shanks and St. John subjects may not have had sufficient exposure to the sequence.

Nevertheless, Shanks and St. John criticise Reed and Johnson's conclusion on three grounds. Firstly they note that the subjects performed a "relatively small number

of (prediction and recognition) trials" and thus there may have been insufficient opportunity to "detect small savings from the training phase". This criticism is somewhat churlish given that Shanks and St. John have expressed concern on a number of occasions that a prediction test (with feedback) might well induce sequence learning within the prediction phase which could therefore erroneously inflate a subject's apparent conscious knowledge of the sequence. Thus it is obviously desirable to keep the prediction phase as short as possible and yet they criticise Reed and Johnson for doing so. Furthermore, irrespective of Shanks and St. John insistence that it is necessary for there to be absolutely no conscious knowledge of the sequence in order to definitively demonstrate non-declarative learning, the fact that they themselves describe any conscious knowledge that the subjects may still have had as "small" is telling. Shanks and St. John are troubled that Perruchet and Amorim's subjects in a similar task were able to perform at above chance in the prediction task whereas Reed and Johnson's were not. It seems likely that Perruchet and Amorim used a single-task rather than dual-task design and if so the same point Reed and Johnson made about the Shanks and St. John study above holds here also. On the face of it the most telling criticism Shanks and St. John make is that when they replicated Reed and Johnson's study their (Shanks and St. John's) subjects performed the prediction test at above chance, from which Shanks and St. John concluded their subjects "appear to have [conscious] access… to second-order sequence knowledge". Once again however Shanks and St. John are being somewhat disingenuous as although they have endorsed the recognition test as appropriate and valid and although Reed and Johnson use this as their primary measure of conscious knowledge Shanks and St. John either fail to use it in their 'replication' or at least fail to report it. In this sense Shanks and St. John are guilty of violating their own (information) criteria. Furthermore they note that their own subjects display significant evidence of conscious sequence knowledge within the first 12 trials of the 96 trial prediction task, yet they have just finished criticising Reed and Johnson for running too few prediction trials to demonstrate conscious knowledge, having themselves just reported that the very same task is sufficiently sensitive to show just that with only a 1/3 as many trials as Reed and Johnson use.

Shanks and St. John however are kinder to Stadler and Frensch who refute Shanks and St. John's contention that the failure of the prediction test to demonstrate conscious sequence knowledge in their (Stadler and Frensch's) 1989 study may be

due to interference and hence rapid forgetting during the prediction test (as it did not include feedback). Shanks and St. John acknowledge that Stadler and Frensch have satisfactorily addressed this point (by virtue of the fact that subject's performance was consistent throughout the prediction task) and go on to admit that "this study goes a long way to meeting the Sensitivity and Information Criteria…… [and thus these] results appear to provide good evidence of unconscious learning". This conclusion is rather surprising in light of Shanks and St. John's ultimate contention that there is no good evidence of non-declarative learning. However Shanks and St. John qualify their support for Stadler and Frensch by noting that the assessment of awareness in Stadler and Frensch was based on a fraction of the number of observations used to demonstrate reaction time reduction, and this may introduce problems with statistical power. It is strange that Shanks and St. John go on to comment that they are still concerned that withholding feedback during prediction testing "works against the possibility of finding significant savings" again having just condemned the practise of feedback in prediction tasks!. However, they do conclude that Stadler and Frensch's study merits replication and further exploration. Given that it has apparently fulfilled both their criterion this would seem to be a somewhat overly-conservative response, but their point is well taken, even though it does apparently provide good evidence of non-declarative learning it is (as far as Shanks and St. John are concerned) at odds with a number of quite similar studies and the reasons for this difference need to be determined before any firm conclusion can be reached. This notwithstanding, if it could be shown that other studies that were similarly rigorous produced evidence of non-declarative learning / memory then Shanks and St. John's conclusion would be seriously challenged, if not entirely invalidated.

As mentioned above this author feels that both Reed and Johnson and Stadler and Frensch have provided good evidence of non-declarative learning even though Shanks and St. John do not share this conclusion and a brief (re)visit to other studies that have been published since 1994 in light of Shanks and St. John's criterion illustrates the point.

In Jackson *et al*'s (1995) first experiment subjects completed a prediction test to measure explicit sequence knowledge and the authors found no difference between control and PD subjects. Furthermore Jackson *et al* used the same type of test in their second experiment but also went on and removed all subjects (control and PD) that

performed the prediction test at, or greater than, one standard deviation above chance performance, and found that doing so did not alter the results (PD subjects were still impaired on SRT performance in comparison with control subjects). Unfortunately Jackson *et al* are vulnerable to criticism on statistical-structure grounds (the statistical structure, e.g. frequency information, of their random sequences varied from that of their repeating sequence) and thus it cannot be concluded the study demonstrated non-declarative serial-order learning. This notwithstanding it can be concluded that *something* (probably simple frequency information) was learnt *implicitly* which is sufficient to support the global notion of non-declarative learning and thus multiple learning / memory systems.

As noted in the discussion above Reber and Squire's (1998) amnesic group produced recognition tests that were not significantly different from chance but y*et al*so demonstrated a strong interference effect when switched from a repeating to random sequence, on the face of it unequivocal evidence for non-declarative sequence learning (and of the utility of using amnesic subjects). Examination of the repeating and random sequences used shows Reber and Squire accounted for the possibility of simple probabilistic learning by employing sequences which were structurally identical except for the actual serial-order information (i.e. 'totally ambiguous' per Curran and Keele, 1993), thus any learning is highly likely to have been actual sequence-learning, satisfying Shanks and St. John's sensitivity criterion. Furthermore, the non-amnesic subjects who experienced the repeating sequence (the primary control group) displayed a greater degree of conscious knowledge of the repeating sequence than the amnesic subjects (although still far less than those who had explicitly memorised it) but also showed good evidence of non-declarative learning. The disparity in the degree of conscious knowledge of the sequence strongly suggests that while conscious knowledge is not *necessary* for sequence learning subjects take advantage of it to aid performance when available. This point highlights the value of using amnesic subjects and experimental conditions that inhibit the development of conscious sequence knowledge (e.g. dual-task conditions or mixed designs per Curran 1997, etc.). It also allows the re-examination of those amnesia studies that while failing the information criterion for sequence-learning may well demonstrate good evidence of non-declarative learning for something (again most likely simple frequency information) and thus provide evidence of independent learning and memory systems. Secondly, as mentioned in the earlier discussion of this

study, Reber and Squire produce good evidence of precisely the sort of crossover effect Shanks and St. John called for in their article to demonstrate non-declarative learning and the independence of the different learning / memory systems. As a result of demonstrating the crossover effect Reber and Squire conclude “that differential performance on non-declarative and explicit tests does not simply arise from sensitivity differences…. [but rather] indicates that non-declarative and explicit sequence learning must depend on separate brains systems”.

A similar, although not quite so unequivocal, result is evident when examining Curran, 1997. It will be recalled from the discussion above that Curran found that amnesic subjects learnt the SOC sequence as well as they learnt the FOC sequence, however while they could rely on simple frequency information to learn the latter they had to learn second-order probabilities in order to learn the former. Although amnesic subjects were mildly impaired in comparison with control subjects the main point currently is that they demonstrated learning in the absence of conscious knowledge of the information learnt (both amnesic and control subjects performed a recognition test at chance levels). The fact that amnesic subjects are mildly impaired compared to control subjects on an non-declarative memory test is an exciting result as it suggests the black-and-white distinction often made between spared and impaired abilities in amnesics is somewhat simplistic, and in doing so further refines our understanding of what amnesia is and suggests interesting avenues for future research. It does not however invalidate the conclusion that there are independent and dissociable forms of memory, but rather suggests the pattern of impairment suffered by amnesic subjects is more subtle and complex than hitherto thought.

The conclusions to be drawn from this section will be obvious and while Shanks and St. John have been criticised for their over stringent application of their criterion by providing such a strong test of the evidence they have ultimately done the field a great service as, once met, such robust standards-of-proof offer correspondingly strong evidence for the multiple-memory hypothesis.

Furthermore it is not expected that Shanks and St. John’s criterion will constitute the final-word in this matter but that refinements and criticisms of it (see Jackson and Jackson, 1995, for example) will continue to test the validity of the assumption of multiple-memory systems. However, in light of the evidence presented

and the criterion adopted this thesis is content to accept, *pro tem*, the hypothesis of multiple-memory systems as it currently stands and explore the contributions of different neural substrates within the framework of the SRT task.

2.7 General Conclusions

It is clear that the more recent SRT studies, which have addressed these serious methodological and conceptual issues, have managed to clearly demonstrate a capacity for non-declarative sequence learning in the absence of any (or severely reduced) explicit knowledge of the sequence. Moreover the fact that such a demonstration is possible when the only sequence information varied between the repeating- and random-sequences is the actual repeating sequence is especially encouraging as there seems little doubt that, in such situations, nothing other than sequence learning could produce the faster reaction times during learning, and the slowed reaction times after switching to a random sequence, that are seen in the SRT.

This notwithstanding is will be obviously from the preceding discussion that there are a number of contradictions and uncertainties within the SRT literature as regards the abilities of different neuropathological populations to perform the SRT. Of particular concern is the reliance on non-significant statistical test to conclude there is no difference between the various limbic systems amnesias and their control groups (see Chapter 3). Relying on a failure to reject the null hypothesis in order to demonstrate no difference between groups is a conceptually weak test of theory and particularly vulnerable to issues of insufficient statistical power. Specifically, if such a test has weak statistical power it will be insufficiently sensitive to small group differences and is therefore likely to make a type-II error (which, in this case, is incorrectly rejecting the null hypothesis of there being a group difference).

There is also some uncertainty about precisely which neurological aetiologies produce spared or impaired SRT and the degree to which they spare, or impair, SRT performance. For these reasons, and those given above, the SRT literature would greatly benefit from an analytical review (i.e. a meta-analysis) that will combine related studies to provide greater test accuracy / sensitivity.

As well as concerns with the precise aetiological consequence for SRT

performance there is a reasonable amount of uncertainty as to precisely which neural substrate are responsible for non-declarative sequence learning. The neuroimaging studies discussed above lend only secondary support to the notion that non-declarative sequence learning is dependent on basal ganglia structures. However, the general trend towards more ventral / sub-cortical structures in non-declarative sequence learning is consistent with the general thrust of the non-declarative / explicit memory hypothesis as regards the different substrates responsible for the two types of memory. Identification of the neural substrates responsible for non-declarative sequence learning, therefore, would benefit greatly for the an examination of the consequences for SRT performance of accurately sited and neuroanatomically restricted lesions.

The consequences of the concerns and criticisms discussed above suggest a very clear line of action. Firstly, a detailed examination of the human SRT literature in order to answer a number of questions raised by inconsistent results of various SRT studies. In particular the combination of SRT studies in order to allow the quantification of the literature, and in doing so increasing the sensitivity to group differences in SRT studies. This would allow the re-examination of various issues within the SRT literature as regards to the differing abilities of various aetiologies to perform the SRT, both in comparison with other neuropathological aetiologies and in comparison with healthy control subjects. The last point will allow a more sensitive test of the commonly held assumption that some limbic-system dependent amnesias spare SRT performance to the extent that subjects with these disorders perform the SRT in a control-like manner. As discussed above this assumption may be due more to the insensitivity of the methods used to test for a difference between limbic system amnesiacs and control subjects than anything else and a more sensitive test of the matter should help resolve the issue one way or the other. Furthermore, a quantification and the consequent re-analysis of the SRT literature should provide empirical support for the re-interpretation of existing SRT studies and for both the particular methodological approaches and theoretical directions of future research.

Secondly, having gained a more accurate and detailed understanding of the nature and utility of the SRT task an obvious next step is to improve our understanding of the neural substrates responsible for non-declarative sequence learning. As discussed above determining the precise nature and extent of neural injury in human

subjects is difficult and leads to much of the uncertainty that surrounds the precise location and function of the various neural substrates that are responsible for SRT performance. In order to provide the degree of certainty about neural injury necessary to be able to discover the sites and mechanisms responsible for non-declarative sequence learning we must be able to specify both the precise location and extent of that injury, and lesion studies with animal subjects are the only practical option for doing that. Therefore, after the empirical quantification of the human SRT literature we will turn to a brief discussion of the animal literature relevant to the multiple-memory hypothesis in general, and non-declarative sequence learning in particular. Thereafter a series of experiments will be presented that, initially, were designed to develop an animal analogue of the human SRT and thereafter tested the behavioural consequences of various targeted neural lesions on animal SRT performance.

Therefore, the next chapter will present a power and meta-analytic review of the SRT literature. It is hoped that this review will allow us to draw definitive conclusions about what aetiologies are, and are not, impaired in the SRT. In order to answer this question we will firstly examine the relative abilities of neuropathological subjects to demonstrate an interference effect when switching from a repeating to a random sequence. Having done that we will then examine the performance of those same subjects relative to their controls in order to determined if some aetiologies (the limbic systems amnesias in particular) are capable of demonstrating control-like SRT performance as is sometimes claimed.

Chapter 3

A Meta-Analysis of the Human SRT Literature

General Introduction

The previous chapter discussed the development of the SRT task and its utility as a measure of non-declarative memory, especially with respect to empirical attempts to dissociate different types of learning in neuropathological populations. A demonstration of the ability of the SRT to dissociate between patients with different neuropathologies in terms of spared / impaired memory is especially pertinent in light of the criticisms raised by Shanks and St. John about the use of amnesic subjects in memory research. While most of the criticisms raised by Shanks and St. John specific to the SRT task have since been addressed (e.g. Reber & Squire, 1998) these criticisms, and others, (see Curran, 1995; Jackson and Jackson, 1995,) illustrate the point that there are as yet unexplained variations and contradictions within the findings from the SRT literature. Not only do studies report different results between neuropathological aetiologies, as might be expected, but also within a given condition (e.g. Alzheimer's Disease: Knopman & Nissen, 1987; Knopman, 1991; and Ferraro, Balota & Connor, 1993*).*

Cursory examination of the detail of some of the human findings suggests that small to medium differences between patients and control groups may exist even when 'non-significant' differences are reported. Indeed there appears to be general concerns with respect to the statistical validity of many human SRT studies. For these reasons the current chapter provides a quantitative examination of the SRT literature. As detailed below, SRT studies often rely on failing to reject the null-hypothesis to

demonstrate the lack of a difference between groups under conditions of low statistical power. Incorrect use of the null-hypothesis, especially, in conditions of low power substantially increases the probability of making a type-II error. Thus individual studies in this field often provide a weak empirical basis for testing the abilities of neuropathological subjects to demonstrate an interference effect relative to their preceding repeating sequence behaviour and especially in comparison with control subjects. Given these concerns, a meta-analytic evaluation of the SRT literature is clearly warranted to complement the qualitative review provided in Chapter Two.

Although the SRT task generates two measures of non-declarative sequence learning, the 'learning' and 'interference' effects, only the latter measure was used in the meta-analysis presented here. The reason for selecting the interference effect is that there is a clear consensus within the literature is that interference is the more valid and reliable measure of non-declarative learning (see Knopman & Nissen, 1987; Nissen & Bullemer, 1987; Reed & Johnson, 1994; Jackson & Jackson, 1995; and Reber & Squire 1998). Due to the amount of motor repetition necessary to perform a repetitive reaction time task the learning effect is unreliable as a test of non-declarative sequence learning. During sequence learning it is usually impossible to separate mere motor skill improvement in reaction times due to practice effects from improvements in reaction times due to non-declarative sequence learning. The interference effect does not suffer from this problem. If a subject's learning effect is solely due to motor skill improvement then the switch to a random sequence should have no effect on reaction times. On the other hand if the decrease in reaction times across repeating-sequence blocks is at least partially due to non-declarative sequence learning then switching to a random sequence should disrupt a subject's ability to continue reacting at the same speed or accuracy as previously, the interference effect is hence a clear behavioural test of non-declarative sequence learning.

Statistical power and the SRT

There are two main concerns with SRT studies as regards statistical power. Firstly most SRT studies employ small sample sizes and therefore tend to have low power as a result. Secondly, many SRT studies rely on failing to reject the null hypothesis in order to demonstrate that the neuropathological patients are not impaired relative to control subjects. This is conceptually and empirically problematic

at the best of times, but in conditions of low power almost guarantees the commission of a type-II error (erroneously failing to reject the null-hypothesis).

Statistical power is the probability that a test will report a significant difference if there is a real difference between groups. Thus a study has 'high' power when it is likely to (correctly) report a difference, and 'low' power when it is unlikely to do so. The commonly accepted level for adequate power is 80% (Cohen, 1988). Statistical power (often shortened to just 'power') is reliant on three factors: effect size, alpha level and sample size. The concept of effect size (ES) was initially developed by Glass (1976) and, while the specific denominator may vary, is usually conceptualised as an average difference between groups expressed in terms of their common variance. Effect sizes are thus independent of the specifics of a dependent variable (i.e. it expresses group differences on a standardised metric).

The main use of estimating statistical power is firstly to help interpret non-significant results and secondly to provide the researcher with reliable estimates of minimally acceptable parameters (i.e. sample sizes) in order to maximise the likelihood of reaching the correct conclusion. As most researchers are aware, a non-significant result is more properly described as 'inconclusive' rather than 'non-significant' and this distinction is particularly pertinent when a study has low power. A study with low power and a lack of significance is ambiguous as it could be due to either no difference between groups or due to the test being insufficiently sensitive to detect any difference between groups. Obviously, it is desirable to have high power in order to allow the researcher to differentiate between these possibilities.

Given these concerns the meta-analysis includes a complementary power analysis.

Effect Size, Power Analysis and Meta-Analysis

Although Glass (1976) first developed the concept of an effect size the *de facto* standard for effect sizes are Cohen's (1962) 'small', 'medium' and 'large' conventions, which provide the context for a power analysis. It is widely accepted that the norm for effect sizes in the behavioural sciences is in the small to medium range (Rossi, 1990; Sedlemeier & Gigerenzer, 1989). As a consequence, researchers will often fail to identify conceptually and theoretically significant differences due to their statistical

tests being insufficiently sensitive to small-to-moderate differences between groups. Furthermore, researchers may readily accept that there is no difference between groups (the null hypothesis) when this fits a particular theoretical point of view, instead of rigorously testing the research hypothesis with designs that achieve low Type-II error rates (perhaps 95% power; Cohen, 1988).

The main advantage of a meta-analysis is the increase in statistical power as a result of the larger sample sizes gained by aggregating studies (Hedges and Olkin, 1984). It also provides greater precision for the effect sizes of the studies included by calculating confidence intervals for their effect sizes. Now widely accepted in the behavioural sciences a meta-analysis has the advantage of reducing the likelihood of making a type-II error, increasing the probability of detecting small but theoretically important group differences, and enabling the researcher to distinguish between genuine non-significance and an inconclusive test result.

A meta-analysis also enjoys other advantages, in particular it allows the researcher to explore the differences between effect sizes for meaningful causative factors. A researcher can test the validity of different moderators as reliable predictors of behaviour and estimate how much variance in the set of effect sizes can be attributed to sampling error. While small amounts of variance can safely be ignored (Hunter & Schmidt, 1990) larger amounts cannot, but instead allow for the possibility of identifying the causes of this variance. Identifying causes of variation within a set of effect sizes has two benefits: it removes the need for additional exploratory studies to test moderators identified as important (although confirmatory studies may well be warranted) and it provides strong empirical support for possible directions in future research.

This chapter will test the ability of subjects with a variety of neuropathologies to perform the SRT by using statistical power and meta-analyses. Two main questions addressed by these SRT studies will be analysed separately. Part 1 evaluates the degree to which patients with a given neuropathological condition demonstrate SRT learning in terms of the strength of their interference effect when switched from a repeating to a random sequence. Part 2 is perhaps more critical as it will examine the behaviour of neuropathological subjects relative to control subjects and address the

issue of the size of any difference in the interference effect between neuropathological and control groups. Both sections will initially present a statistical power analyses designed to determine the typical power associated with current SRT studies. Thereafter a meta-analysis of each set of data will be presented in order to provide a quantitative estimate of the magnitude of the interference effects. Each meta-analysis will also include moderator analyses designed to identify possible sources of variation between the studies.

In summary, neuropathological populations in general have larger effect sizes (i.e. greater deficits) than normal subjects, but researchers often use very small sample sizes (for perfectly understandable reasons, i.e. the relative lack of availability of aetiologically 'pure' subjects). Therefore neuropathological studies tend to have low statistical power. As a result clinically and / or theoretically significant differences may go unreported due to insufficiently sensitive tests. Furthermore, relying a failure to reject the null hypothesis is always of concern and this approach is particular troubling in the context of SRT studies for these reasons (i.e. because SRT studies typically employ small sample sizes, see Tables 3A.3, and 3A. 4 in the appendix to this chapter). Increasing the power the between-group tests of by combining SRT studies in a meta-analysis addresses these issues by increasing the sensitivity and reliability of the tests and therefore increasing the likelihood of reaching correct conclusions.

Aggregating SRT Studies

As well as studies being grouped by individual aetiology in the meta-analysis (i.e. all AD studies are grouped together) they were also grouped according meta-aetiological category. As discussed in Chapters 1 and 2 damage to the limbic system (i.e. the extended hippocampal formation and the temporal lobes) results in an impairment on tasks that primarily measure declarative memory, but not on tasks that primarily measure non-declarative memory (i.e. subjects with limbic system amnesia should not be impaired on the SRT). Conversely damage to the basal ganglia (in particular the caudate nucleus) produces an impairment on some non-declarative memory tasks (i.e. the SRT task), but not an declarative memory tasks. For this reason studies were grouped into meta-aetiological categories according to their limbic system neuropathological or basal ganglia neuropathological (LSN or BGN, respectively) status. Furthermore, while the main interest was the BGN / LSN

distinction a third meta-aetiological category 'other neuropathologies' (ON) was included for comparison with the BGN and LSN meta-categories. The ON group consisted of SRT studies with any neuropathological populations other than those with LSN or BGN dependent disorders, and included subjects with generalised closed-head traumatic brain injury (TBI), pre-Frontal Cortex (pFC) injury due to diffuse phenomena (ischemic episodes, tumours etc.) and cerebellar lesions (Cb).

As the multiple-memory literature typically focuses on LSN and / or BGN injury in order to demonstrate a double dissociation between neural substrate and memory task it therefore makes few predictions about the abilities of ON subjects to perform the SRT. However, given that some authors have suggested a role for the cerebellar in (non-declarative memory dependent) motor skill learning (Layforce and Doyon, 2001; Doyon *et al,* 1998; Wickelgren, 1998; Salmon and Butter, 1995) and that SRT performance may require other cognitive abilities than just non-declarative memory (e.g. attention, see Jackson & Jackson, 1995; and Pascual-Leone *et al* 1995, in Grafman, Holyoak, and Boller, 1995) which are often disturbed by head injury, one might expect that ON subjects will display at least a mild SRT impairment.

While the meta-aetiological analysis will provide the opportunity to test the more general neurological hypothesis that LSN, BGN and ON subjects will demonstrate differing abilities to perform the SRT, it is also possible to perform an analysis of individual aetiologies. This will enable us to examine the possibilities that individual aetiologies (e.g. Alzheimer's disease) may not produce consistent results within a meta-aetiological category, and individual aetiologies may produce different results between meta-aetiological categories even if the meta-aetiological categories themselves do not differ (i.e. while the LSN and BGN meta-aetiological groups, for example, may not differ, patients with AD and PD may yet differ). Fortunately the SRT literature falls fairly easily into five primary individual aetiologies and (less easily) into three secondary / composite aetiologies. The five primary aetiologies are: Alzheimer's disease (AD), Mixed Amnesia (MA), Korsakoff's Syndrome (KS), Parkinson's disease (PD) and Huntington's disease (HD). The first three of these neurological disorders make up the LSN meta-aetiological category, and the last two the BGN meta-aetiological category. The three additional aetiologies (which make up the 'ON' meta-aetiological category) are: pre-Frontal cortex injury, cerebellar lesions and generalised

traumatic brain injury. Although there is only one cerebellar and one pFC SRT study currently published it was felt that these aetiologies are sufficiently different to warrant their inclusion as separate entities within the ON meta-category.

Method

Selection of Studies

A literature search was conducted using two on-line databases: PubMed (previously MEDLINE) and PsycINFO (previously PsycLIT; unpublished studies were not included in the meta-analysis). The keywords and key-phrases used in the search were: serial, reaction, SRT and serial reaction time. All articles written in English and published from 1987 were considered, excluding any animal studies (largely comprising the rat 5-choice SRT in use during this period). Reference lists of obtained articles were also examined for relevant studies. Importantly, selected studies all had to include the use of a repeating sequence which was replaced by a random sequence at some point. In total 25 articles were located. Two studies were removed for failing to include a switch from a repeating to a random sequence (Doyon, *et al*, 1997; and Doyon, *et al*, 1998). Three studies were removed as they were purely methodological manipulations (e.g. sequence length variations, dual-task conditions etc.) and did not include any neuropathological group (Hoffman and Koch, 1997; Stadler and Neely, 1997; Zhuang, Warzeri, Gerloff and Hallett, 1998). Finally three studies were removed due to the neurological condition being a temporary product of experimental manipulation (Knopman, 1991b; Nissen, Knopman and Schacter, 1987; Pascual-Leone *et al*, 1996). Thus 17 articles were retained for analysis, from which 22 effect sizes were generated.

Calculation of Effect Sizes

Effect sizes were calculated using Johnson's 'D-STAT' program (version 1.11, 1995; see manual for formulae). Where the relevant means and standard deviations were not supplied in a target article, effect sizes were estimated from other information provided such as means, p, t, r or F values, or figures showing means. In the case of means and mean values estimated from figures the D-STAT enables one to work back from related test statistics to generate an estimate of the appropriate variation of the means (i.e. standard deviation of the difference scores). Unless otherwise stated

individual effect sizes generated from the studies are presented as raw-d (g) values whereas the mean effect sizes used in the meta-analysis are presented as d-values (i.e. corrected for sample size). We were also fortunate enough to be given original data from two authors (Beldarrain, 1999; and Curran, 1997) to assist in calculating effect sizes.

In Part 1 of the analysis an effect size had a positive sign when the neurological group displayed a reaction time increase after switching to a random sequence from a repeating sequence. Thus a non-significant or negative effect size in this analysis indicates a lack of an interference effect and thus an inability to demonstrate SRT-learning.

In Part 2 an effect size had a positive sign when the control group had a greater / stronger interference effect than the neurological group and a significant positive effect size indicates that the neuropathological group cannot be said to perform the SRT in a manner similar to its control group.

Where a study yielded more than one effect size from the same subjects the effect sizes were aggregated to obtain a representative effect size. If a study yielded more than one effect size obtained from separate sets of subjects these effects were include in the meta-analysis as individual effect sizes (per Hedges and Olkin, 1984).

Coding of Study Characteristics

The moderator variables coded were[5]:

A) Aetiological Meta-Category: Subjects were grouped into either Limbic System Neuropathology (LSN), i.e. early AD, Mixed Amnesia, & KS; or Basal ganglia Neuropathology (BGN), i.e. PD & HD; or Other Neuropathology (ON), i.e. frontal damage, non-specific traumatic brain injury and cerebellar lesion.

B) Individual Etiology, coded as:

Alzheimer's Disease
Non-Specific Amnesia
Korsakoff's Syndrome
Parkinson's Disease
Huntington's Disease

[5] A number of other moderator variables were coded, e.g. repeating-sequence length (8, 10 or 12-trials) but their analyses were not relevant to the main concerns of the meta-analysis and are not reported.

Cerebellar Injury
Pre-Frontal Cortex Injury
Traumatic brain injury

C) Severity of Dementia: non-dementing, very mildly dementing, mildly dementing.

D) Degree of Explicit knowledge: the degree of explicit knowledge of the repeating sequence shown by the neurological and control subjects. Subjects were classified as have 'no', 'some' or 'good' explicit sequence knowledge[6].

E) Age: subjects were classified as being 65-and-under versus over 65.

F) Raw or Log reaction times: whether or not the reaction time data was log-transformed.

G) Random Sequence Style: Per the discussion in Chapter 2 some studies employed random sequences that simply disallowed immediate stimulus repetition and / or an equal frequency of stimulus representations throughout a random block. Studies employing either (or both of) these techniques were classified as 'unconstrained' by virtue of their failure to address any variation in other statistical features between the repeating and random sequences. Whereas those studies that controlled for all statistical information except actual sequence information between repeating and random sequences were characterised as 'constrained'.

Coding and method of effect size calculation were first determined independently by the author and supervisor and any disagreements were discussed and consensus agreement reached. See Table 3A.1 in the appendix to this chapter for coding values for each study used in the meta-analysis.

Power Analyses

Statistical power for each study was calculated for small, medium and large effect sizes as defined by Cohen's (1988) conventions and mean, median, SD and minimum / maximum power calculated. In light of Pascual-Leone *et al's* (1993) cerebellar subjects and Stefanova *et al's* (2000) PD subjects who were incapable of demonstrating any interference effect it seems likely that some subjects will have very large effect sizes (i.e. be seriously, or even totally, impaired). Furthermore, preliminary data from the rat-SRT (see Chapters 6 and 7) revealed that non-lesioned rats typically produced very large effect sizes when switching from a repeating to a random

[6] Those studies which did not report this were coded with a missing value score.

sequence (i.e. d>1.0). As a result a 'very-large' effect size category was included in the power analysis (set at d=1.2) in order to examine what sort of power the human studies would have enjoyed if they too are testing for effect sizes in this range.

Statistical power was calculated using the Power & Precision (National Institute of Mental Health) software package. For Part 1 appropriate values for alpha (5%), effect size and the neurological group N were entered for a two-tailed paired t-test assuming zero difference (SD of the difference was set to 1) and power calculated. For Part 2 appropriate values for alpha (5%), effect size and neurological and control group Ns for a two-tailed t-test of means (difference scores) were entered and power calculated.

File-Drawer Analysis

It is generally acknowledged that those studies with a significant result are more likely to be published and therefore there is a bias towards such studies in the literature. This potential confound is known as the 'file-drawer problem'. Hedges and Olkin (1984) describe a method for calculating the number of unpublished studies needed to invalidate any finding of significance (i.e. reduce the combined effect size to a level of non-significance):

$$k_o=k(d-d_c)/d_c$$

Where k_o is the number of unpublished studies required to invalidate the finding, k=the number of studies included in the meta-analysis, d=the mean effect size, and d_c=an estimate of a trivial or non-significant effect size (set at 0.2 per Cohen, 1969). Note: This analysis is relevant to significant findings only and is not reported for non-significant results.

Meta-Analysis

Each meta-analysis is presented in three parts: overall analysis, moderator analyses and individual subset analyses.

The overall analysis simply analyses all effect sizes as a single group of studies, having no regard for individual study characteristics (aetiology, subject's age

etc.). Thus the mean effect size of this analysis provides a measure of performance for the entire population of effect sizes. Of particular interest in this analysis is the test of the model (QB) statistic which indicates whether or not the effect sizes differ. Obviously if the effect sizes do differ then this allows the possibility that one or more categorisations / moderators will be able to account for much of the variance and in doing so identify possible causative factors of variable SRT performance.

A moderator analysis is concerned with the 'Test of the Model' ('QB') statistic and attempts to explain the variation present between groups of effect sizes by virtue of a particular factor (e.g. age). Thus a significant moderator statistic ('QB') indicates that the various groups that go up to make the analysis (e.g. 65 years and under versus over 65 years) are significantly different from one-another. A moderator analysis is therefore the functional equivalent of a independent measures t-test or a 1-way ANOVA. The homogeneity statistic ('Qw') for a moderator analysis tests whether or not the individual effect sizes, when collapsed together, are homogenous and it is expected that effect sizes will typically be heterogeneous within a group. This is because if a group is homogenous then there is no variance between effect sizes which can be explained by a moderator variable and therefore there is nothing to analyse. The Qw test for moderator analyses is significant (i.e. the hypothesis of homogeneity is rejected) unless otherwise stated and will not be reported except in rare occasions where it is theoretically / conceptually appropriate to do so.

In contrast individual subset analyses are solely concerned with one group of a moderator analysis (e.g. subjects under 65 years) and whether or not the mean effect size for that group is significantly different from zero. The values of interest in such an analysis are: the mean effect size (ES), r & p-values. In Part 1 of the analysis significant subset effect sizes and r-values indicates that the group of neuropathological patients has an interference effect that is significantly greater than zero, whereas in Part 2 they indicate that the group has a significantly weaker interference effect than controls. The QB & p values of a subset analysis are concerned with whether or not the group is homogenous. A non-significant QB effect can be taken as confirming the group categorisation as valid and appropriate (i.e. irreducible), whereas a significant QB value indicates there is considerable variation in the effect sizes that make up the group and therefore further testing and / or subdivision of that group is warranted. However QB tests of homogeneity for subset

analyses will only be reported if appropriate.

All parts of the analysis will report file-drawer analyses (k_0) where appropriate (i.e. where a test is significant) but these will only be discussed when it is theoretically / conceptually relevant to do so.

Thus the results of the analyses for both parts will be reported in the following format:

1) Analysis of the Distribution of Effect Sizes
2) Power Analysis
3) Overall Meta-analysis result
4) Analysis of the first moderator variable
    a. Subset analysis of the first group of the first moderator variable
    b. Subset analysis of the second group of the first moderator variable…..
    N. Subset analysis of the Nth group of the first moderator variable
5) Analysis of the second moderator variable
    a. Subset analysis of the first group of the second moderator variable
    b. Subset analysis of the second group of the second moderator variable…..
    N. Subset analysis of the Nth group of the second moderator variable…

N) Analysis of the Nth moderator variable
    a. Subset analysis of the first group of the Nth moderator variable
    b. Subset analysis of the second group of the Nth moderator variable
    …..
    N. Subset analysis of the Nth group of the Nth moderator variable

Note: while it is possible to perform post-hoc comparisons between individual groups using χ^2 analyses such testing relies upon the premises of standard probability tests and thus reintroduces the very risks meta-analysis is designed to avoid. In the introduction to their 1984 book on meta-analysis Hedges and Olkin conclude “the use of conventional statistical procedures for the analysis of effect sizes….. cannot be justified on either statistical or conceptual grounds”. Therefore *post hoc* analysis of meta-analytic results will be restricted to descriptive concerns. However, confidence intervals will be reported for all effect sizes which does allow an examination of whether they (confidence intervals) overlap or not. Effect sizes with non-overlapping

confidence intervals are likely to be significantly different from each other.

Results and Discussions

Analysis of Part 1

This analysis addresses the question of whether or not the various neurological groups are capable of demonstrating a reliable (within-subject) interference effect when switching from a repeating to a random sequence.

Analysis of the Distribution of Effect Sizes

A total of 22 effect sizes were calculated from 17 studies and are presented in Table. 3.1 using Tukey's stem-and-leaf display. Across 'all aetiologies' this display reveals a moderately normal distribution with a fairly long upper tail, and outliers at both ends of the range. Analysis of the distribution of effect sizes in Statistica (StatSoft Inc. Tulsa, USA) reveals that the Kolmogorov-Smirnov test of normality is not significant ($p > 0.2$) and therefore the effect sizes are normally distributed.

Examination of the distribution of effect sizes for the three meta-aetiological categories reveals that the limbic system neuropathology group is reasonably evenly distributed throughout the small to very-large range with an extreme, upper-range, outlier. The basal ganglia neuropathology group also has an upper range outlier but otherwise have a more restricted distribution of effect sizes with peaks in both the small and medium ranges (i.e. is bimodal). In contrast the other neuropathology group includes effect sizes that spread from a small negative effect size through to a large positive effect size. The conclusions we can draw from this is firstly that while 54% of aetiologies generate effect sizes in the small to large range

36% produce larger effect sizes and 10% produce negative effect sizes. Secondly, that both the LSN and ON groups are variable within themselves, whereas the BGN group has more uniform effect sizes (bar one outlier). Both the pattern of effect sizes within the 'all aetiologies' section, and the differences between the distributions of the different meta-aetiological categories suggest there is considerable variation of effect sizes between studies and we can therefore expect this variability may be explained by one or more moderator variables.

Table 3.1, Stem-and-leaf display of 22 effect sizes for Part 1: Interference Effects for Neuropathological Subjects Only

ES Range	All aetiologies	Limbic system neuropathology	Basal Ganglia neuropathology	'Other' neuropathology
-0.2 to –0.1	-0. 12			-0. 12
-0 to 0.1	-			
0.2 to 0.3	0. 2333	0. 3	0. 233	
0.4 to 0.5	0. 54		0. 4	0. 5
0.6 to 0.7	0. 667777	0. 677	0. 677	
0.8 to 0.9	0. 99	0. 9		0. 9
1.0 to 1.1	1. 00	1. 00		
1.2 to 1.3	1. 33	1. 33		
1.4 to 1.5	1. 4		1. 4	
1.6 to 1.7	-			
1.8 to 1.9	-			
2.0 to 2.1	2. 1	2. 1		
Total # of effect sizes	22	10	8	4
Average effect size	0.56	0.78	0.51	0.17

Power Analysis for Part 1

Table 3.2 summarises the results of the statistical power analysis for Part 1 based on 22 power calculations. For a small effect size none of the studies reached the minimal power threshold (80%), and only 2 of 22, and 11 of 22, studies reached this threshold for the medium and large effect sizes, respectively. In contrast 20 of 22 studies had sufficient power when assuming a 'very large' effect size. Thus although most studies had sufficient power to detect very large effect sizes, Table 3.1 suggests that only a medium to large effect size is to be expected in most cases and therefore most studies typically have insufficient power to demonstrate any effect.

Table 3.3 breaks the power analysis down by meta-aetiological category. What is immediately clear is that limbic system neuropathology tends to have less power than the other groups at each effect size. Although both basal ganglia, and 'other', neuropathologies studies

attain (near) adequate power with a 'large' effect size the limbic system neuropathology studies do not attain this power until we assume a 'very-large' effect size. Furthermore, although the basal ganglia neuropathology has slightly weaker power than the group labelled "other neuropathology" this difference is less pronounced.

Table 3.2. Summary Statistics for the Statistical Power Analysis for Part 1 (all power values expressed as a percentage).

	← Theoretical Significance →			
	←Clinical Significance→			
Effect Size	**Small (d=0.2)**	**Medium (d=0.5)**	**Large (d=0.8)**	**Very Large (d=1.2)**
Mean Power	11.7	43.8	74.3	92.7
Median Power	10	38	75	98
Standard Deviation	4.4	20.3	21.1	12.4
Minimum Power	6	14	28	53
Maximum Power	23	86	100	100
# of studies (of 22) with greater than 80% Power	0	2	11	20
N required for 80% Power : paired t-test (2-tail)	199	34	15	8

Table 3.3. Breakdown of the Statistical Power Analysis for Part 1 by Meta-Aetiological Category (all power values expressed as a percentage)

	Average (SD) # of Neuropathological subjects in Meta-Analysis	← Theoretical Significance →			
		←Clinical Significance→			
Effect Size		**Small (d=0.2)**	**Medium (d=0.5)**	**Large (d=0.8)**	**Very Large (d=1.2)**
Mean (and SD) Power for Limbic system neuropathology	15 (10)	11 (5)	39 (24)	69 (27)	86 (16)
Mean (and SD) Power for Basal Ganglia neuropathology	17 (9)	12 (5)	46 (18)	79 (12)	98 (2)
Mean (and SD) Power for 'Other' neuropathology	18 (6)	13 (3)	51 (14)	86 (12)	99 (2)
Mean (and SD) Power for the Overall Meta-Analysis	16 (9)	11.7 (5)	43.8 (20)	74.4 (20)	92.3 (13)

The finding that most SRT studies have effect sizes in the medium to large range and yet have less than adequate power at anything but a very-large effect size (Table 3.3) is important. This finding clearly indicates that the SRT literature will benefit from the increased power of a meta-analysis. What is also interesting is that the group that contains subjects which are sometimes described as not different to controls (the LSN group) has the lowest average power at all the different effect sizes. This is probably because LSN studies have smaller sample sizes (LSN average N = 14.6 (SD=9.9)) than the other meta-aetiological groups (BGN average N = 17(9.4), and ON average N = 18.3(5.7)) and have higher within-group variability than the other meta-aetiological groups at all effect sizes (see Table 3.3).

Meta-Analysis for Part 1

A point-and-whisker plot of the overall meta-analytic result, all 22 effects sizes

that go up to make the meta-analysis, and mean effect sizes for the three meta-aetiological groupings, is presented in Fig. 3.1. A list of all effect sizes and 95%CIs for each figure in this section is provided in Table 3A.5 in the appendix to this chapter.

1) Overall Meta-Analysis Result

As can be seen from Table 3.4 the overall meta-analysis for Part 1 generates a highly significant mean effect size of d=0.56, with a 95% confidence interval that is well above zero (0.41 to 0.72). Thus, as a whole, the neurological subjects included in the analysis show a robust interference effect. However, the test of the model result (QB(21)=30.4) is marginally non-significant (p=0.084). While strictly speaking the 22 effect sizes are homogenous, the marginality of the result raises the possibility that there is some variation within the overall set of effect sizes which might be explained by moderator analyses. Some moderator analyses are appropriate in any case given the clinical and theoretical considerations under scrutiny.

Table 3.4, Overall Meta-Analytic Result

	k	d, 95% CI & r	p	QB; Test of the Model	k_0
Overall Meta-Analysis: Part 1	22	**0.56**, 0.41 to 0.72, 0.27	p<0.0001	Q(21)=30.4, p=0.084	40

2) Aetiological Meta-Category Moderator and Subset Analyses.

The moderator analysis of meta-aetiology concludes there is a significant difference between the three groups (Q(2)=8.1, p<0.025). An examination of the mean effect sizes for the each of the three groups clearly shows that the mean effect size for the ON group is substantially lower than both the LSN and BGN groups (ON d=0.17; LSN d=0.78; and BGN d=0.51). Furthermore, the confidence intervals for the LSN and ON groups do not overlap, so it seems very likely that ON subjects have a significantly weaker effect size than LSN subjects. However, as the confidence interval of the BGN group overlaps both the LSN and the ON confidence intervals the distinction between these groups is less clear. The file-drawer analysis for the LSN and BGN groups reveals that the LSN group in particular requires a large number of unpublished studies to invalidate the significant finding (k_0=29) and that a reasonably large number of unpublished studies is required to invalidate the significant finding for the BGN group (k_0=12).

Table 3.5, Aetiological Meta-Category Moderator and Subset Analyses.

Moderator Analyses	# Subjects	# effect size	d, 95% CI & r	QB; Test of the Model	k_0
Aetiological Meta-Category	345	20	**0.56**, 0.41 to 0.72, 0.27	QB(2)=8.1, p<0.025	40

Subset Analysis	# Subjects	# Studies	# effect sizes	Mean effect size, 95% CI & r	p	k_0
Limbic System Neuropathology (LSN)	148	9	10	**0.78**, 0.54 to 1.02, 0.36	p<0.001	29
Basal Ganglia Neuropathology (BGN)	136	8	8	**0.51**, 0.27 to 0.75, 0.25	p<0.001	12
Other Neuropathology (ON)	61	3	4	**0.17**, -0.19 to 0.53, 0.08	p=0.36	n/a

The subset analyses for this moderator variable tests if each independent group is different to zero. Subjects with limbic system dependent neuropathology demonstrate a large and significant interference effect (d=0.78, 95% CI = 0.54 to 1.02; p<0.001; see table 3.5). Similarly, subjects in the BGN meta-category generated a significant mean effect size (d=0.51, 95% CI = 0.27 to 0.75; p<0.001). However, in contrast, the ON group demonstrate no interference effect as their effect size, while positive, is not different to zero (d=0.17, 95% CI = -0.19 to 0.53; p=0.36) and thus display impaired SRT performance.

3) Individual Aetiological Moderator and Subset Analyses

The moderator analysis for individual aetiology (see Table 3.6) reveals there are also significant differences between specific neuropathological groups (QB(7)=17.7, p<0.025). The confidence intervals generated by pre-Frontal cortex injury subjects do not overlap with those of mixed amnesic and KS subjects and thus the pFC group is likely impaired relative to these two groups. (The effect sizes for individual aetiologies are discussed below).

Furthermore, while the MA, KS and PD file-drawer analyses reveal these results are robust (k_0=16, 12 & 10, respectively) the analyses for AD and TBI subjects demonstrated that these results need relatively few unpublished non-significant studies (k_0=7 & 5, respectively) to invalidate them.

In order to gain a better understanding of the differences between individual aetiologies moderator analyses were computed within each meta-aetiological category. The moderator analysis for the LSN meta-category (see Table 3.7) was marginally non-significant (QB(2)=1.8, p=0.09) and therefore there is no clear difference between the three constituent groups of the LSN meta-category. This is

supported by the fact that while the KS subjects have a numerically much higher effect size than AD subjects (AD d=0.57, KS d=1.36) confidence intervals for all three groups overlapped and thus there is no basis to assume they are different.

Table 3.6, Aetiology Moderator and Subset Analyses.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
Individual Aetiology	345	22	**0.56**, 0.41 to 0.72, 0.27	QB(7)=17.7, p<0.025	40

Aetiological Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	**Mean effect size, 95% CI & r**	**p**	**k_0**
Alzheimer's Disease	81	3	4	**0.57**, 0.25 to 0.88, 0.27	p<0.001	7
Mixed Amnesia	54	4	4	**1.01**, 0.61 to 1.41, 0.45	p<0.001	16
Korsakoff's Syndrome	13	2	2	**1.36**, 0.51 to 2.22, 0.56	p<0.005	12
Parkinson's Disease	111	6	6	**0.54**, 0.27 to 0.81, 0.26	p<0.001	10
Huntington's Disease	25	2	2	**0.39**, -0.19 to 0.95, 0.19	p=0.18	n/a
Cerebellar Injury	15	1	1	**-0.07**, -0.79 to 0.64, 0.08	p=0.85	n/a
Traumatic Brain Injury	24	1	2	**0.66,** 0.08 to 1.25, 0.31	p<0.05	5
Pre-Frontal Cortex Injury	22	1	1	**-0.18**, -0.77 to 0.41, -0.09	p=0.56	n/a

Table 3.7, Aetiology Moderator and Subset Analyses for LSN subjects only.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
LSN Aetiological Moderator Analysis	148	10	**0.78**, 0.25 to 0.88, 0.27	QB(2)=4.8, p=0.09	29

Aetiological Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	**Mean effect size, 95% CI & r**	**p**	**k_0**
Alzheimer's Disease	81	3	4	**0.57**, 0.25 to 0.88, 0.27	p<0.001	7
Mixed Amnesia	54	4	4	**1.01**, 0.61 to 1.41, 0.45	p<0.001	16
Korsakoff's Syndrome	13	2	2	**1.36**, 0.51 to 2.22, 0.56	p<0.005	12

Although it is not possible to discuss any differences between groups when the test of the model statistic (QB) is not significant it is possible to analyse the individual aetiological groups based on the fact that they are clinically distinct groups. The subset analysis for the three aetiologies that go to make up the LSN meta-category (Alzheimer's disease (AD), Mixed Amnesia (MA), and Korsakoff's Syndrome (KS) revealed all mean effect sizes were significantly different from zero and therefore subjects with these aetiologies demonstrate a reliable interference effect when switching from a repeating to a random sequence (AD d=0.57, 95% CI = 0.25 to 0.88, p<0.001; mixed amnesia d=1.01, 95% CI = 0.61 to 1.41, p<0.001; and KS d=1.36, 95% confidence interval0.51 to 2.22, p<0.005).

The moderator analyses for the BGN and ON meta-categories (Tables 3.8 & 3.9, respectively) were not significant (BGN QB(1)=0.22, p=0.64; ON QB(2)=5.38, p=0.1), with the confidence intervals for the constituent groups showing considerable overlap.

Table 3.8, Aetiology Moderator and Subset Analyses for BGN subjects only.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
BGN Aetiological Moderator Analysis	136	8	**0.51**, 0.27 to 0.75, 0.25	QB(1)=0.2, p=0.64	n/a

Aetiological Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	**Mean effect size, 95% CI & r**	**p**	**k_0**
Parkinson's Disease	111	6	6	**0.54**, 0.27 to 0.81, 0.26	p<0.001	10
Huntington's Disease	25	2	2	**0.39**, -0.19 to 0.95, 0.19	p=0.18	n/a

While it is not possible therefore to talk in terms of group differences it is possible to analyse the constituent aetiological groups separately from each other because they too are clinically distinct groups. The PD subjects mean effect size is significantly different to zero (d=0.54, 95% CI = 0.27 to 0.81, p<0.001) whereas the mean effect size for HD subjects is not (d=0.39, 95% CI = -0.19 to 0.64, p=0.18). Thus while PD subjects demonstrate a reliable interference effect there is an indication that HD subjects demonstrate impaired SRT behaviour.

Table 3.9, Aetiology Moderator and Subset Analyses for ON subjects only.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
ON Aetiological Moderator Analysis	8	4	**0.17**, -0.19 to 0.53, 0.09	QB(2)=5.38, p=0.1	n/a

Aetiological Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	**Mean effect size, 95% CI & r**	**p**	**k_0**
Cerebellar Injury	15	1	1	**-0.07**, -0.79 to 0.64, 0.08	p=0.85	n/a
Traumatic Brain Injury	24	1	2	**0.66,** 0.08 to 1.25, 0.31	p<0.05	5
Pre-Frontal Cortex Injury	22	1	1	**-0.18**, -0.77 to 0.41, -0.09	p=0.56	n/a

As the test of the model for the ON meta-aetiology was no significant we can only consider these results in terms of individual groups and not in terms of between-group differences. The subset analysis of the constituent ON groups (Table 3.9) reveals that both the cerebellar injury (Cb) and the pre-Frontal cortex (pFC) injury patients were not different from zero (Cb d=-0.07, 95% confidence interval = -0.79 to 0.64, p=0.85; and pFC d=-0.18, 95% confidence interval = -0.77 to 0.41, p=0.56) and

were thus impaired on the SRT. In contrast the traumatic brain injury (TBI) patients were significantly different to zero (d=0.66, 0.08 to 1.25, p<0.05) and thus demonstrated a robust interference effect.

4) Severity of Dementia Subset and Moderator Analyses

This moderator analysis compared three groups of effect sizes ('not dementing', 'very mildly dementing', and 'mildly dementing') which were based on the neuropathological subject's dementia status (see Fig. 3.2). Although the effect size for mildly dementing subjects was somewhat weaker than that for both non-dementing and very-mildly dementing subjects (d=0.45 vs. d=0.58 & d=0.6 respectively), the moderator analysis was not significant (QB(2)=0.4, p=0.82, see Table 3.10 and Fig. 3.2).

We also conducted a moderator analysis for the dementing groups only across studies (see Table 3.10) which was also not significant (QB(1)=0.3, p=0.59). Therefore severity of dementia, up to mildly dementing status, is not a reliable predictor of a subjects' ability to demonstrate a (within-subject) interference effect.

Table 3.10, Moderator and Subset Analyses for the Severity of Dementia.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
Severity of Dementia Moderator Analysis	345	22	**0.56,** 0.41 to 0.72, 0.27	QB(2)=0.4, p=0.82	35

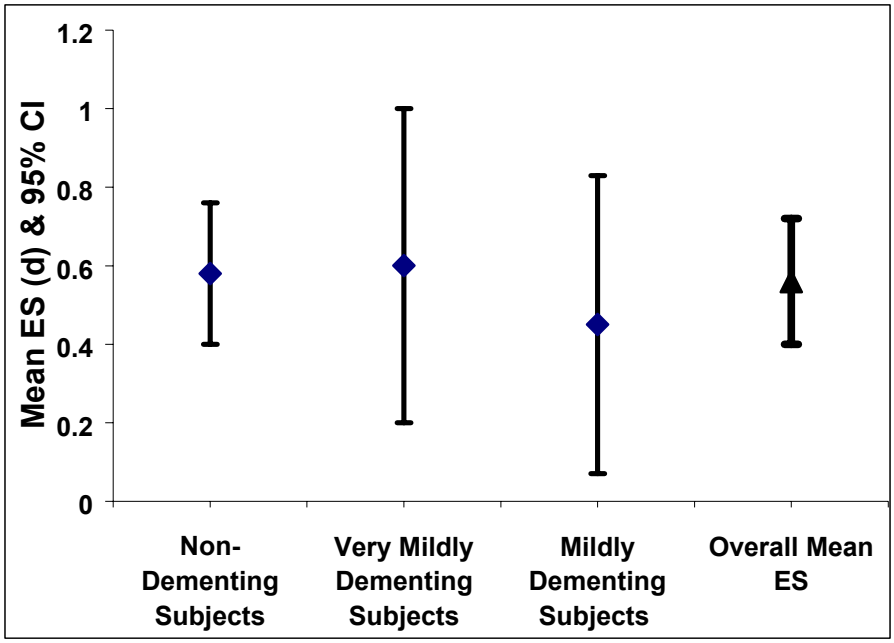
Dementia Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	Mean effect size, 95% CI & r	**p**	**k_0**
Non-Dementing Subjects	239	14	16	**0.58,** 0.39 to 0.76, 0.28	p<0.001	30
Very Mildly Dementing Subjects	51	3	3	**0.6**, 0.21 to 1.0, 0.29	p<0.005	6
Mildly Dementing Subjects	55	3	3	**0.45**, 0.07 to 0.83, 0.22	p<0.025	4

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	*QB; Test of the Model*	**k_0**
Severity of Dementia Moderator Analysis for Dementing Subjects only	106	6	**0.52,** 0.25 to 0.8, 0.25	QB(1)=0.3, p=0.59	10

Subset analysis of the individual dementing groups revealed that the effect sizes for all three groups were significantly different from zero (non-dementing d=0.58, 95% CI = 0.39 to 0.76, p<0.001; very-mildly dementing d=0.6, 95% CI = 0.21 to 1.0, p<0.005; mildly dementing d=0.45, 95% confidence interval = 0.007 to 0.83, p<0.025). However, the results for both dementing groups are somewhat weakened by the fact

that relatively few non-significant studies are required to invalidate these findings (k_0=6 & 4, respectively).

Fig. 3.2, Mean Effect Sizes And 95% Confidence Intervals For The Severity Of Dementia Subset And Moderator Analyses



5) Explicit Knowledge Subset and Moderator Analyses

Studies were categorised according to the degree of explicit sequence knowledge enjoyed by the neuropathological subjects: 'no', 'some', or 'good' (see Table 3.11 and Fig. 3.3). Those studies that did not test their neuropathological subjects for explicit sequence knowledge are excluded from this analysis. The moderator analysis was significant (QB(2)=8.5, p<0.025) and thus the effect sizes of the three groups differ.

Table 3.11, Moderator and Subset Analyses for degree of Explicit Sequence Knowledge.

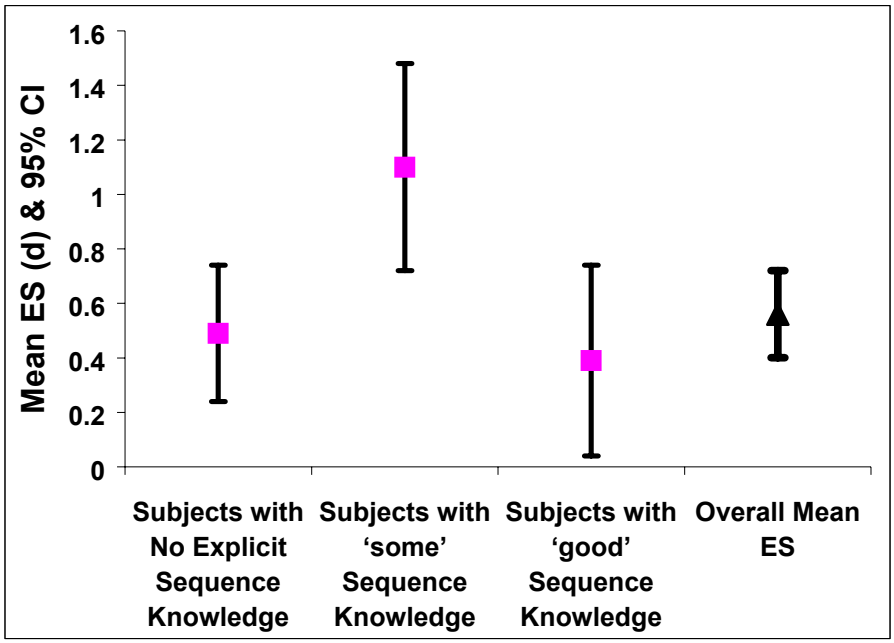
Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
Degree of Explicit Sequence Knowledge	241	17	**0.6**, 0.42 to 0.78, 0.29	QB(2)=8.5, p<0.025	32

Explicit Sequence Knowledge Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	Mean effect size, 95% CI & r	**p**	**k_0**
Subjects with 'No' explicit sequence knowledge	117	11	11	**0.49**, 0.24 to 0.74, 0.24	p<0.001	15
Subjects with 'Some' explicit sequence knowledge	61	3	3	**1.1**, 0.74 to 1.48, 0.48	p<0.001	14
Subjects with 'Good' explicit sequence knowledge	63	2	3	**0.39**, 0.04 to 0.74, 0.19	p<0.05	3

Examination of the effect sizes reveals that while the effect sizes for the 'no' explicit knowledge and 'good' explicit knowledge groups are very similar (d=0.49 & 0.39, respectively) the effect size for the 'some'' explicit knowledge group is substantially higher (d=1.1). Furthermore, both the 'no' and 'good' group's confidence intervals overlap only to a minor degree with the 'some' group. Thus we can conclude that the subjects with 'some' explicit sequence knowledge demonstrate a significantly stronger interference effect than those with 'no' or 'good' explicit sequence knowledge.

Subset analyses for degree of explicit sequence knowledge revealed that neurological subjects with 'no' explicit sequence knowledge nevertheless had a significantly greater than zero effect size and therefore a reliable interference effect (d=0.59, 95% CI = 0.24 to 0.74, p<0.001). The same was true of subjects with 'some' explicit sequence knowledge (d=1.1, 95% CI = 0.71 to 1.48, p<0.001) and those subjects with 'good' explicit sequence knowledge (d=0.39, 95% CI = 0.04 to 0.74, p<0.05). However, the file-drawer analysis revealed that only three non-significant studies would be required to invalidate the significant finding for those subjects with 'good' explicit sequence knowledge which renders the significant finding for 'good' subjects empirically weak.

Fig. 3.3, Mean Effect Sizes And 95% Confidence Intervals For The Degree Of Explicit Sequence Knowledge For Neuropathological Subjects Subset And Moderator Analyses.



Other Moderator Variables.

All other moderator analyses (on or off medication, SRT repeating sequence length, SRT style, neurological subject age, raw / log reaction times, and random sequence style) were not significant and are not reported any further.

Discussion of Part 1

The significant overall meta-analysis result provides strong empirical confirmation for the contention that neurological subjects in general are capable of performing the SRT. As a group neuropathological subjects demonstrate a significant (within-subject) interference effect when switched from a repeating to a random sequence. The fact that a large number of neurological subjects had absolutely no explicit sequence knowledge but yet had significant interference effects is strong empirical support for the multiplicity of memory and the validity of non-declarative memory in particular.

Although it was hypothesised that the BGN group would be impaired relative to

the LSN group there is no evidence to support this hypothesis. In contrast the LSN and BGN groups display relatively similar degrees of sequence learning (d=0.78 and d=0.51, respectively) as measured by the interference effect. Therefore, this analysis offers no support for the limbic system / basal ganglia model of multiple memory systems. However, the ON group has a substantially weaker interference effect than the LSN group and thus demonstrates impaired SRT performance relative to the LSN group, which does support the possibility of multiple memory systems.

The multiple-memory literature, and the SRT literature in particular, predicts subjects with LSN aetiologies will not be impaired on tests of non-declarative memory and the finding that all LSN subjects are capable of demonstrating a robust interference effect confirms this prediction. The lack of difference between the LSN aetiologies suggests that the various LSN groups included in the meta-analysis, irrespective of individual aetiology, are capable of demonstrating sequence learning when switching from a repeating to a random sequence (Part 2 will deal with their ability to perform the SRT relative to control subjects).

However, it must be noted that the AD studies included in the meta-analysis all used early-stage AD subjects and this may account for their ability to perform the SRT because entorhinal connections to the hippocampus represent the most significant neuropathology in these early stage patients (Braak and Braak, 1996; Braak *et al,*1996). AD subjects with more advanced pathology (i.e. more than 'mildly' dementing) may demonstrate a SRT impairment (see Ferraro, Balota and Connor, 1993).

The hippocampus-dependent multiple-memory theory specifically predicts that BGN based disorders will produce opposing results to LSN based disorders. As a consequence BGN subjects would be expected to display an SRT impairment in contrast to LSN subjects. However, as noted above, BGN subjects do not differ from LSN subjects in their ability to produce a robust within-subject interference effect. It is worth noting in response to the unexpected lack of any difference between BGN and LSN subjects that SRT studies with BGN subjects make it clear that BGN subjects

suffer partial rather than total impairments on the SRT task. Therefore, the ability of BGN subjects to demonstrate a within-subject interference effect is not entirely surprising. What is surprising however is that they demonstrate an interference effect that is not different to LSN subjects. However, this result is only concerned with the ability of subjects to demonstrate an interference effect relative to their own pre-interference behaviour and says nothing about the ability of subjects to demonstrate sequence learning relative to control subjects which is the more critical measure of sequence learning.

As PD and HD are both primarily disorders of the basal ganglia it is interesting therefore to find that PD subjects display interference effects that are significantly different from zero whereas HD patients do not. The obvious hypothesis is that the very restricted basal ganglia damage suffered by HD subjects produces a substantial SRT impairment, whereas the (relatively) diffuse basal ganglia dysfunction in PD subjects does not. Given that PD is a progressive disorder in which larger amounts of damage to the basal ganglia occur towards the latter stages of the disease (with increasing involvement of the dorsal caudate and frontal dopamine afferents) it would be interesting to compare early- and late-sage PD subjects. The fact that all PD subjects in this meta-analysis were in the relatively early stages of the disorder may therefore explain why they showed a relatively robust within-subject SRT behaviour. It is also worth noting that although one study within the PD group has a noticeably higher effect size than the other PD studies (Pascual-Leone, *et* al, 1993; d=1.37) that the PD group is homogenous ((Q(5)=8.42, p=0.13) and thus the unexpected differences between the PD and HD groups is not likely to be due to any inflationary effect on the PD mean effect size because of this study.

ON subjects demonstrate a SRT impairment effect when switching from a repeating to a random sequence. This suggests that the neural substrates damaged in ON subjects bears some responsibility for non-declarative sequence learning behaviour. This is not particularly surprising for the cerebellum as several authors have suggested this structure plays a role in motor-skill learning (Layforce and Doyon, 2001; Doyon *et al,* 1998; Wickelgren, 1998; Salmon and Butter, 1995) which is a substantial component of SRT behaviour. Furthermore, the finding that non-specific traumatic head injury produces an SRT impairment is not unexpected given that such

injuries often damage the frontal areas of the brain. As discussed in Chapter 2 the SRT performance requires contributions from both non-declarative memory and non-memory cognitive functions (e.g. attention). Given that frontal areas of the brain are largely responsible for attention in particular, and other more general cognitive functions, it is therefore reasonable to conclude that damage to these areas with disrupt SRT performance, without necessarily positing their involvement in non-declarative memory *'per se'.*

As increased severity of dementia is (by definition) associated with greater and more pervasive cognitive deficits, it is expected that more severely dementing subjects would suffer a greater impairment on the SRT than subjects which are less severely dementing. Contrary to this expectation severity of dementia in the mild range was not a reliable predictor of SRT behaviour. This is especially strange in light of Ferraro, Balota and Connor's (1993) finding that mildly dementing AD subjects had significantly impaired SRT performances relative to very-mildly dementing subjects. However, mildly dementing subjects do have a smaller effect size than very-mildly dementing subjects and it may be that even this meta-analysis is insufficiently sensitive to detect this small difference (i.e. has insufficient power). This seems particularly possible in light of the equally small numbers of effect sizes that go up to make both dementing groups (k=3), and the relatively small sample sizes from which those effect sizes are generated ('very-mildly dementing' N=51, 'mildly dementing' N=55). Clearly more evidence is required before one can unequivocally state that mild dementia produces a weaker SRT interference effect than very-mild dementia.

The meta-analysis found that 'some' explicit sequence knowledge produced a far stronger interference effect than either 'good' or 'no' explicit sequence knowledge. SRT studies tend to conclude that explicit sequence knowledge either benefits SRT performance (Mutter, Howard & Howard, 1994; Segar, 1997) or does not influence it at all (Stadler & Neely, 1997; Jackson & Jackson, 1995; Willingham & Korroshetz, 1993; Reber & Squire, 1884 & 1998). Therefore evidence for an 'inverted-U' relationship between SRT performance and degree of explicit sequence knowledge was unexpected. A possible explanation for this 'inverted-U' relationship is that

neuropathological subjects are unable to take advantage of greater amounts of explicit knowledge (by virtue of both their specific cognitive impairment and general cognitive impairment common to all neuropathologies examined in this analysis) and instead it becomes an interferer / distracter which degrades behaviour rather than improving it as it would in a neurologically intact subject.

In conclusion the meta-analysis for Part 1 has demonstrated that although neuropathological subjects as a whole generate a significant interference effect when switching from a repeating to a random sequence there is considerable variation between individual aetiologies. Cerebellar, pFC and HD subjects were found to suffer a SRT impairment although more studies with these patient groups are required. AD, KS, mixed amnesic, PD and TBI subjects however do demonstrate a reliable interference effect and thus show evidence of SRT learning. Furthermore, in contrast to expectations severity of dementia in neuropathological subjects is not a reliable predictor of SRT performance. While the degree of explicit sequence knowledge a neuropathological subject enjoys does reliably predict their SRT performance the relationship between explicit sequence knowledge and SRT performance is not a linear one as might be expected. Rather, a moderate degree of explicit sequence knowledge produces a stronger interference effect than either no, or good, knowledge. However, this finding only applies to neuropathological subjects and may not be true of neurologically intact subjects.

Analysis of Part 2

This analysis addressed the critical question of whether or not those subjects with a variety of neuropathological disorders perform the SRT in a manner comparable to their respective control subjects and whether the SRT performance of neuropathological patients varies as a function of neuropathology.

A total of 22 effect sizes were calculated from 17 studies and are presented in table 3.12 using Tukey's stem-and-leaf display. The overall aetiological distribution shows a moderately normal distribution of effect sizes with a long upper-range tail and several outliers. Analysis of the distribution of effect sizes in Statistica reveals that the Kolmogorov-Smirnov test of normality is not significant ($p > 0.2$) and therefore the effect sizes are normally distributed. The groups of effect sizes within the distribution suggests the possibility that these peaks may be explained by moderator variables.

Table 3.12, Stem-and-leaf display of 22 effect sizes for Part 2: The difference in Interference Effects Between Neuropathological and Control Subjects.

ES Range	All aetiologies	Limbic system neuropathology	Basal Ganglia neuropathology	'Other' neuropathology
-0.2 to –0.1	-			
-0 to 0.1	0. 1			0. 1
0.2 to 0.3	0. 222	0. 22	0. 2	
0.4 to 0.5	-			
0.6 to 0.7	0. 66777	0. 667	0. 7	0. 7
0.8 to 0.9	0. 899	0. 89	0. 9	
1.0 to 1.1	1. 0011	1. 00	1. 1	
1.2 to 1.3	1. 33	1. 3	1. 3	
1.4 to 1.5	1. 4			1. 4
1.6 to 1.7	1. 7		1. 7	
1.8 to 1.9	1. 8		1. 8	
2.0 to 2.1	2. 11		2. 1	2. 1
Total # of effect sizes	**22**	**10**	**8**	**4**
Average effect size	**0.94**	**0.68**	**1.14**	**1.43**

Even this relatively simple level of analysis suggests that control subjects as a whole display a stronger interference effect than neurological subjects. Both the relative scarcity of zero and small effect sizes (which would indicate no difference or a very weak difference respectively) support the likelihood of a difference between neuropathological and control groups. Indeed the majority of studies produce a medium, or greater, effect size.

Breaking the distribution of effect sizes down by meta-aetiology reveals that effect sizes generate by LSN subjects tend to fall within the medium to large to very-large range but also with outliers at both ends of the scale. In contrast effect sizes within the BGN and ON meta-categories are far more widely spread and show a square distribution, making it more difficult for a moderator analysis to explain the variation.

Power Analysis for Part 2

As in Part 1 none of the studies achieve adequate power assuming a small effect size (see Table 3.13). However, unlike Part 1 none of the studies achieve adequate power for a medium effect size in Part 2 either. This is most likely to be a function of between-group effects requiring larger sample sizes to achieve the same power for a given effect size than within-group effects (per the greater 'N required for 80% power' values for this table compared to the same table in Part 1; Table 3.2). It is not until we assume a 'large' effect size that any of the studies have sufficient power, and even then only 23% of effect sizes reach this goal. Even when assuming a very-large effect only slightly more than half (63%) of the studies would have adequate power. These power analyses are therefore important considerations when evaluating the many null findings reported in the literature, and especially those (LSN) studies that rely on failing to reject the null hypothesis.

Table 3.13. Summary Statistics for the Statistical Power Analysis for Part 2 (all power values expressed as a percentage).

	← Theoretical Significance →			
		←Clinical Significance→		
Effect Size	**Small (d=0.2)**	**Medium (d=0.5)**	**Large (d=0.8)**	**Very Large (d=1.2)**
Mean Power	8.5	28.1	56.1	82.8
Median Power	8	22.5	48.5	82.5
Standard Deviation	2.1	12.1	20.3	15.4
Minimum Power	6	14	27	53
Maximum Power	13	54	91	100
# of studies (of 22) with greater than 80% Power	0	0	5	14
N required for 80% Power : independent t-test (2-tail)	394	64	26	12

Breaking the power analysis down by meta-aetiological category (Table 3.14) we find a similar pattern to Part 1. LSN studies have less power than either the BGN and ON studies. However, none of the meta-aetiological categories have adequate power even at a 'large' effect size (bearing in mind most behavioural studies generate small to medium effect; Rossi, 1990; Sedlemeier & Gigerenzer, 1989). Perhaps the most interesting finding of the power analysis is that while SRT studies as a whole achieve adequate power when assuming a very-large effect size this is not true of LSN studies. Even at a very-large effect size LSN studies still have less than 80% power. Given that the question addressed by this part of the meta-analysis concerns

the sensitivity of null hypothesis testing to decide if the LSN subject's behaviour is comparable to control subjects this lack of adequate power at anything but the very-large effect sizes is of great concern, and provides a clear justification for combining these studies into a meta-analysis.

Meta-Analysis for Part 2

A point-and-whisker plot of overall meta-analysis result, all 22 effects sizes (ESs) that go up to make the meta-analysis, and mean effect sizes for the three meta-aetiological groupings, is provided in Fig 3.4. A list of all effect sizes and 95%CIs for each figure in this section is provided in Table 3A.6 in the appendix to this chapter.

Note: All sample sizes reported in tables included in this section are in the following format: numbers of neuropathological subjects / numbers of controls subjects.

Table 3.14. Breakdown of the Statistical Power Analysis for Part 2 by Meta-Aetiological Category (all power values expressed as a percentage)

Effect Size	Average (SD) # of Neuropathological / Control subjects in Meta-Analysis	← Theoretical Significance → ←Clinical Significance→			
		Small (d=0.2)	Medium (d=0.5)	Large (d=0.8)	Very Large (d=1.2)
Mean (and SD) Power for Limbic system neuropathology	15 (10) / 16 (13)	8 (2)	25 (12)	51 (23)	77 (19)
Mean (and SD) Power for Basal Ganglia neuropathology	17 (10) / 18 (14)	9 (2)	30 (13)	58 (19)	86 (11)
Mean (and SD) Power for 'Other' neuropathology	18 (6) / 26 (21)	9 (2)	33 (12)	65 (18)	91 (9)
Mean (and SD) Power for the Overall Meta-Analysis	16 (9) / 19 (11)	8.5 (2)	28.2 (12)	56 (20)	83 (15.5)

1) Overall Meta-Analysis Result

Table 3.15, Overall Meta-Analytic Result

	k	d, 95% CI & r*	p	QB; Test of the Model	k_0
Overall Meta-Analysis: Part 2	22	**0.94**, 0.781 to 1.1, 0.43	p<0.0001	Q(21)=54.2, p<0.0001	81

The overall meta-analysis (see Table 3.15) for Part 2 generated a significant large-to-very-large effect size (ES = 0.94, r=0.43, p<0.001) with a constrained 95% confidence interval (0.78 to 1.1) that is well above zero (zero being equivalent to controls in Part 2). Thus we can conclude that neurological subject as a whole do not perform the SRT in a control like manner, but are rather considerably impaired in their ability to demonstrate an interference effect when switching from a repeating to a random sequence. Furthermore, the significant test of the model (QB(21)=54.24, p<0.0001) clearly reveals that the individual effect sizes vary and therefore we can expect analysis premised on various characterisations of the studies (moderators) to reveal reliable predictors of SRT behaviour.

2) Aetiological Meta-Category Moderator and Subset Analyses

This moderator analysis (see Table 3.16; QB(2)=21, p<0.01) confirms a significant difference between the three meta-aetiological groups. Not only do they have substantially different effect sizes (LSN d=0.68, BGN d=1.14, and ON d=1.43) but the 95% confidence interval for the LSN group does not overlap with either that of the BGN or ON groups. Thus the LSN group has a significantly smaller effect size than either of the other two groups and are thus less impaired relative to these groups when compared to the appropriate controls.

Table 3.16, Aetiological Meta-Category Moderator and Subset Analyses.

Moderator Analysis	**# Subjects**	**# effect size**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
Aetiological Meta-Category	345 / 383	22	**0.92**, 0.77 to 1.08, 0.42	QB(2)=21, p<0.01	81
Subset Analysis	**# Subjects**	**# Studies**	**# effect sizes**	**Mean effect size, 95% CI & r**	**p**	**k_0**
Limbic System Neuropathology (LSN)	148 / 159	9	10	**0.68**, 0.45 to 92, 0.32	p<0.001	24
Basal Ganglia Neuropathology (BGN)	136 / 145	8	8	**1.14**, 0.93 to 1.35, 0.5	p<0.001	38
Other Neuropathology (ON)	61 / 79	3	4	**1.43**, 1.16 to 1.71, 0.58	p<0.001	25

However, the subset analysis revealed that all three meta-categories are significantly different from controls (LSN d=0.68, 95% CI = 0.45 to 0.92, p<0.001; BGN d=1.14, 95% CI = 0.93 to 1.35, p<0.001; ON d=1.43, 95% CI = 1.16 to 1.71, p<0.001). Therefore, the neuropathological subjects in all three groups have significantly weaker interference effects than controls. Thus although LSN subjects have a smaller effect size than BGN and ON subjects, and are less impaired than these groups, they nevertheless are still impaired relative to their own control group. It is especially pertinent here to note that a file-drawer analysis reveals that the results of all three meta-categories is reliable and not susceptible to invalidation by a few non-significant studies.

3) Individual Aetiological Moderator and Subset Analyses

The overall aetiological moderator analysis for all aetiologies (see Table 3.17) reveals there is a significant difference between individual aetiologies (QB(7)=20.9, p<0.005). As the 95% confidence interval for the Alzheimer's studies (0.27 to 0.93) does not overlap with that of the cerebellar study (1.4 to 2.9), these effect sizes are likely to be significantly different.

Table 3.17, Aetiology Moderator and Subset Analyses.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
Individual Aetiology	345 / 428	22	**0.92**, 0.77 to 1.08, 0.42	QB(7)=20.9, p<0.005	81

Aetiological Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	**Mean effect size, 95% CI & r**	**p**	**k_0**
Alzheimer's Disease	79 / 81	3	4	**0.60**, 0.27 to 0.93, 0.29	p<0.001	8
Mixed Amnesia	67 / 83	4	4	**0.77**, 0.39 to 1.15, 0.36	p<0.001	11
Korsakoff's Syndrome	13 / 15	2	2	**0.79**, 0.2 to 1.56, 0.37	p<0.05	6
Parkinson's Disease[1]	98 / 121	5	5	**0.85**, 0.52 to 1.18, 0.39	p<0.001	16
Huntington's Disease	25 / 24	2	2	**1.47**, 0.84 to 2.09, 0.59	p<0.001	13
Cerebellar Injury	15 / 30	1	1	**2.12**, 1.4 to 2.9, 0.73	p<0.001	10
Traumatic Brain Injury[2]	26 / 22	1	2	**0.67**, 0.07 to 1.26, 0.32	p<0.05	5
Pre-Frontal Cortex Injury	22 / 52	1	1	**0.64**, 0.1 to 1.18, 0.31	p<0.25	n/a

[1] One study (Stefanova *et al* ,2000) was removed from this analysis (only) to render the analysis homogenous.
[2] Although this analysis was heterogeneous (Q(1)=1.73, p<0.05) there were insufficient effect sizes included in it (i.e. two) to be able to remove any to achieve homogeneity.

As in Part 1 separate moderator analyses were performed for each of the meta-aetiological categories in order to identify any differences within these groups. See Fig. 3.4 for individual study effect sizes and effect sizes for each meta-aetiology.

The aetiological moderator analysis for the LSN aetiologies (see Table 3.18) was not significant (QB(2)=0.52, p=0.8), reflecting the high degree of similarity of the effect sizes between the three aetiologies. Furthermore, the three aetiologies that make up the LSN meta-category all have mean effect sizes significantly different to controls (AD d=0.60, 95% CI = 0.27 to 0.93, p<0.001; mixed amnesia d=0.77, 95% CI = 0.39 to 1.15, p<0.001; and KS d=0.79, 95% CI = 0.2 to 1.56, p<0.05) and therefore all three LSN subgroups demonstrate significantly weaker interference effects than their control groups. However, while all three aetiologies require relatively few non-significant findings to render them not different to controls, studies employing KS subjects are especially threatened by the file-drawer analysis (k_0=6). Yet this is unlikely to be a problem because it is precisely this group (KS) which would not be subject to the 'file drawer' problem because current wisdom is for there to be no difference between KS subjects and their controls in the SRT task (but see the general discussion on LSN amnesia and SRT performance).

Table 3.18, Aetiology Moderator and Subset Analyses for LSN subjects only.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
LSN Aetiological Moderator Analysis	168 / 179	10	**0.68**, 0.45 to 0.92, 0.32	QB(2)=0.52, p=0.8	24

Aetiological Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	Mean effect size, 95% CI & r	**p**	**k_0**
Alzheimer's Disease	79 / 81	3	4	**0.60**, 0.27 to 0.93, 0.29	p<0.001	8
Mixed Amnesia	67 / 83	4	4	**0.77**, 0.39 to 1.15, 0.36	p<0.001	11
Korsakoff's Syndrome	13 / 15	2	2	**0.79**, 0.2 to 1.56, 0.37	p<0.05	6

The moderator analysis of the two BGN groups (see Table 3.19) was marginally non-significant (QB(1)=3.09, p=0.08) which suggests that the mean effect size for HD subjects (d=1.47) may be higher than that for PD subjects (d=0.85). Note however, that Stefanova *et al's* (2000) PD study had to be excluded to achieve homogeneity for the PD group. Stefanova *et al* had a large N (39 PD subjects and 31 controls) and produced an effect size of 2.09, which was even higher than that shown for the HD group. If Stefanova *et al's* effect size is actually representative of the SRT performance of PD subjects relative to controls (as it may well be given the excellent sample sizes and consequent high power) this marginal PD / HD difference may be

more apparent than real. If Stefanova *et al's* effect size is actually representative of PD subjects then the exclusion of the Stefanova *et al* study to render the PD group homogenous would produce a PD effect size that is under-representative of the true effect size for PD subjects. Thus the PD effect size may actually be higher than reported for the homogenous PD group, and therefore more similar to the HD effect size.

Irrespective of any differences between PD and HD subjects both aetiologies had significantly weaker interference effects than controls (PD d=0.85, 95% CI = 0.52 to 1.18, p<0.001; and HD d=1.47, 95% CI = 0.84 to 2.09, p<0.01).

Table 3.19, Aetiology Moderator and Subset Analyses for BGN subjects only.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
BGN Aetiological Moderator Analysis[1]	123 / 145	7	**0.97**, 0.68 to 1.26, 0.44	QB(1)=3.1, p=0.08	38

Aetiological Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	**Mean effect size, 95% CI & r**	**p**	**k_0**
Parkinson's Disease[1]	98 / 121	5	5	**0.85**, 0.52 to 1.18, 0.39	p<0.001	16
Huntington's Disease	25 / 24	2	2	**1.47**, 0.84 to 2.09, 0.59	p<0.001	13

[1] One study (Stefanova *et al*, 2000) was removed from this analysis to render the analysis homogenous.

The moderator analysis for the ON aetiologies was highly significant (see Table 3.20; QB(1)=11.4, p<0.0005). Therefore, because the confidence interval for cerebellar subjects does not overlap with that of TBI and pFC subjects, cerebellar subjects are significantly more impaired relative to controls than TBI and pFC subjects. However, TBI and pFC subjects do not differ between themselves. Furthermore, all three ON aetiologies are significantly different from zero (Cerebellar d=2.16, 95% CI = 1.39 to 2.29, p<0.001; and TBI d=0.99, 95% CI = 0.26 to 1.06, p<0.001; and pFC d=0.64, 95% CI = 0.1 to 1.18, p<0.025), and thus have significantly weaker interference effects than their controls.

Table 3.20, Aetiology Moderator and Subset Analyses for ON subjects only.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
ON Aetiological Moderator Analysis	63 / 104	4	**0.97**, 0.62 to 1.32, 0.44	QB(2)=11.4, p<0.005	15

Aetiological Subset Analyses	# Subjects	# Studies	# effect sizes	Mean effect size, 95% CI & r	p	k_0
Cerebellar Injury	15 / 30	1	1	**2.12**, 1.4 to 2.9, 0.73	p<0.001	10
Traumatic Brain Injury	26 / 22	1	2	**0.67**, 0.07 to 1.26, 0.32	p<0.05	5
Pre-Frontal Cortex Injury	22 / 52	1	1	**0.64**, 0.1 to 1.18, 0.31	p<0.25	n/a

While all groups are generated from a very small number of effect sizes (i.e. 1 or 2) the cerebellar group requires a reasonably large number of non-significant studies (k_0=10) to invalidate it even though it only includes one effect size which is undoubtedly due to its extremely large effect size. In contrast the TBI group requires relatively few non-significant studies (k_0=5) to invalidate the result of its subset analyses. Thus the subset analyses for the TBI and pFC groups must be viewed with some caution. Furthermore, the two TBI effects sizes actually come from the same paper (Mutter *et al,* 1994) and subjects in the two studies vary in terms of the severity of their head injury which produced quite different patterns of SRT behaviour.

4) Severity of Dementia Moderator and Subset Analyses

In spite of the reasonably low effect size for very-mildly dementing subjects relative to non-dementing and mildly dementing subjects (d's=0.55, 1.15, & 1.1, respectively) the moderator analysis of severity of dementia (see Table 3.21 and Fig. 3.5) was not significant (QB(2)=4.6, p=0.1). Furthermore, all three dementing groups were significantly different from zero and thus demonstrate a weaker interference effect than controls (Non-dementing, d=1.15, 95% CI = 1.0 to 1.31, p<0.001; very-mildly dementing, d=0.55, 95% CI = 0.1 to 0.94, p<0.01; and mildly dementing, d=1.1, 95% CI = 0.68 to 1.53, p<0.001, see Fig.3.22).

Table 3.21, Moderator and Subset Analyses for the Severity of Dementia.

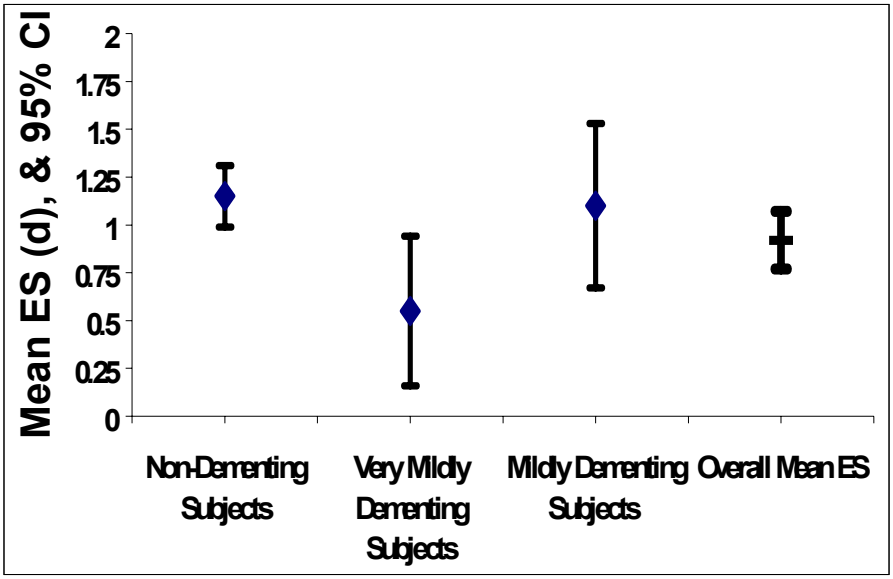
Moderator Analyses	# Subjects	# effect sizes	d, 95% CI & r	QB; Test of the Model	k_0
Severity of Dementia Moderator Analysis	345 / 383	22	**0.92,** 0.77 to 1.08, 0.42	QB(2)=4.6, p=0.1	81

Dementia Subset Analyses	# Subjects	# Studies	# effect sizes	Mean effect size, 95% CI & r	p	k_0
Non-Dementing Subjects	239 / 290	13	16	**1.15**, 1.0 to 1.31, .05	p<0.001	76
Very Mildly Dementing Subjects	51 / 55	3	3	**0.55,** 0.1 to 0.94, 0.26	p<0.01	5
Mildly Dementing Subjects	55 / 51	3	3	**1.1**, 0.68 to 1.53, 0.48	p<0.001	14

Moderator Analyses	# Subjects	# effect sizes	d, 95% CI & r	*QB; Test of the Model*	k_0
Severity of Dementia Moderator Analysis for Dementing Subjects only	106 / 106	6	**0.79**, 0.5 to 1.07, 0.37	QB(1)=3.4, p=0.07	95

A moderator analysis restricted to dementing subjects only was marginally non-significant (QB(1)=3.38, p=0.07) but does suggest that across dementing groups the more dementing subjects were more impaired relative to controls as would be expected (see the general discussion for an examination of this finding).

Fig 3.5. Mean effect sizes and 95% confidence intervals for the severity of dementia subset and moderator analyses



6) Moderator and Subset Analyses of the Degree of Declarative Sequence Knowledge Enjoyed by Neuropathological Subjects

The moderator analysis of explicit sequence knowledge enjoyed by neuropathological subjects was not significant (Table 3.22; QB(2)=4.1, p=0.13, see Fig. 3.6) and therefore the groups do not differ. This result is supported by the overlap of the 95% confidence intervals for all three groups.

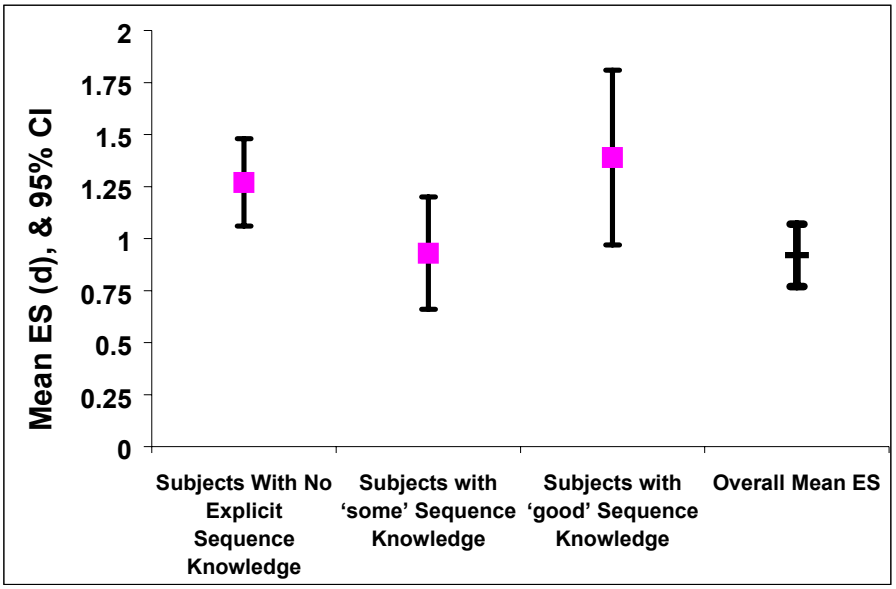
Table 3.22, Moderator and Subset Analyses for degree of Explicit Sequence Knowledge for Neuropathological Subjects.

Moderator Analyses	# Subjects	# effect sizes	d, 95% CI & r	QB; Test of the Model	k_0
Degree of Explicit Sequence Knowledge	266 / 302	17	**1.0**, 0.82 to 1.18, 0.45	QB(2)=4.1, p=0.13	68

Explicit Sequence Knowledge Subset Analyses	# Subjects	# Studies	# effect sizes	Mean effect size, 95% CI & r	p	k_0
Subjects with 'No' explicit sequence knowledge	142 / 171	11	11	**0.96**, 0.72 to 1.2, 0.43	p<0.001	42
Subjects with 'Some' explicit sequence knowledge	61 / 76	3	3	**0.82**, 0.47 to 1.18, 0.39	p<0.001	9
Subjects with 'Good' explicit sequence knowledge	63 / 55	2	3	**1.37**, 0.95 to 1.8, 0.57	p<0.001	18

All three groups (neuropathological subjects with 'no', 'some' and 'good' explicit sequence knowledge) had effect sizes that were significantly higher than zero and therefore weaker interference effects than control subjects ('no' explicit knowledge, d=1.27, 95% CI = 1.05 to 1.48, p<0.001; 'some' d=0.93, 95% CI = 0.66 to 1.2, p<0.01; and 'good' d=1.39, 95% CI = 1.97 to 1.81).

Fig 3.6. Mean effect sizes and 95% confidence intervals for the degree of explicit sequence knowledge of neuropathological subjects subset and moderator analyses



6) Moderator and Subset Analyses of the Degree of Explicit Sequence Knowledge Enjoyed by Control Subjects

The moderator analysis for the SRT performance of neuropathological subjects as a function of the explicit sequence knowledge enjoyed by the control subjects was significant (QB(2)=9.64, p<0.01, see Fig. 3.7). As can be seen in Table 3.23 the 95%

confidence intervals for subjects with 'some' and 'good' explicit sequence knowledge do not overlap and therefore these two groups are different from each other. Those studies with control subjects with 'some' explicit sequence knowledge have neuropathological subjects that are less impaired than those studies with control subjects with no or 'good' explicit sequence knowledge.

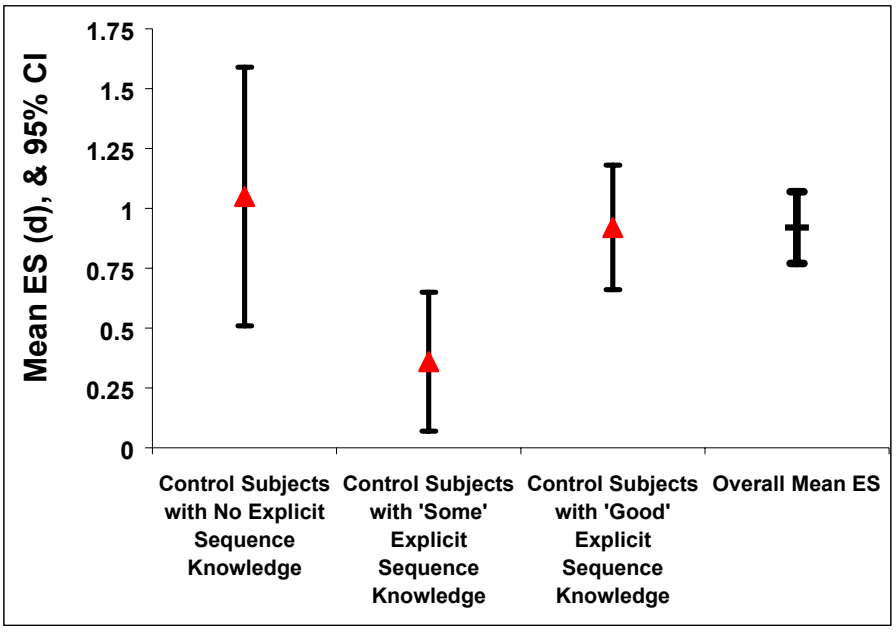
Table 3.23, Moderator and Subset Analyses for degree of Explicit Sequence Knowledge for Control Subjects

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
Degree of Explicit Sequence Knowledge	266 / 301	17	**1.0**, 0.82 to 1.18, 0.45	QB(2)=9.64, p<0.01	68

Explicit Sequence Knowledge Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	Mean effect size, 95% CI & r	**p**	**k_0**
Subjects with 'No' explicit sequence knowledge	33 / 32	11	3	**1.1**, 0.56 to 1.6, 0.48	p<0.001	14
Subjects with 'Some' explicit sequence knowledge	99 / 116	3	7	**0.66**, 0.37 to 0.95, 0.31	p<0.001	16
Subjects with 'Good' explicit sequence knowledge	134 / 153	2	7	**1.26**, 1.0 to 1.53, 0.54	p<0.001	37

As would be expected the subset analyses revealed that neuropathological subjects in each of the groups were significantly different from controls irrespective of the degree of explicit sequence knowledge enjoyed by controls (Subjects with 'No' explicit sequence knowledge d=1.1, p<0.001; Subjects with 'Some' explicit sequence knowledge d=0.66, p<0.001; Subjects with 'Good' explicit sequence knowledge, d=1.26, p<0.001).

Fig 3.7. Mean effect sizes and 95% confidence intervals for the degree of explicit sequence knowledge of control subjects subset and moderator analyses



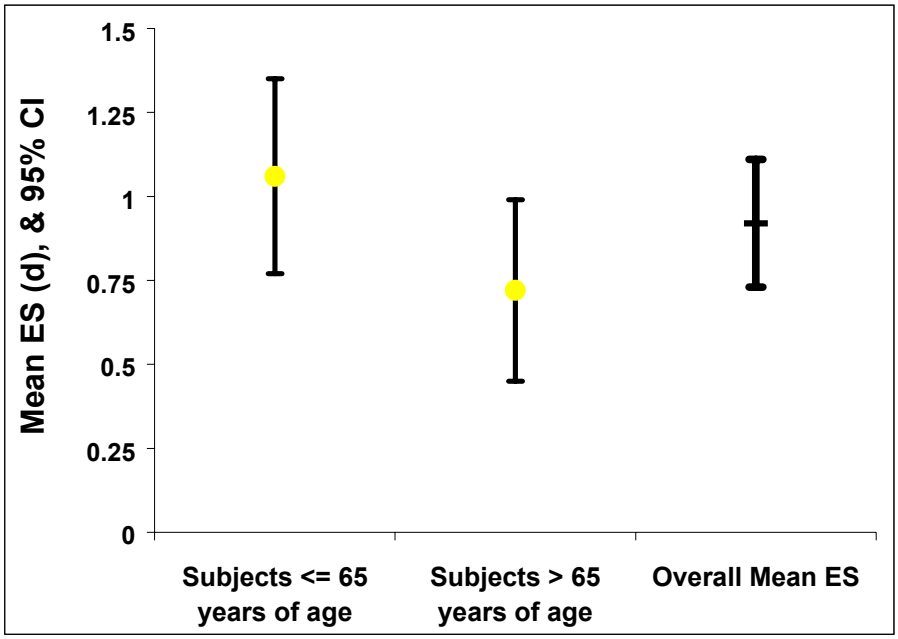
7) Age Moderator and Subset Analyses

The moderator effect for age is significant (QB(1)=3.9, p<0.05; see Table 3.24 and Fig. 3.8). The mean effect size for subjects over 65 was significantly lower than the effect size for subjects ≤65 (d=1.06 & 0.72, respectively), as reflected by the minimal overlap in 95% confidence intervals. Thus subjects *over* 65 are relatively *less* impaired relative to controls compared to subjects ≤65. This is unexpected as age is generally positively related to cognitive impairment and thus we would expect the older cohort to be more impaired than the younger cohort. However, the younger cohort includes the effect sizes for the HD, cerebellar and pFC subjects which inflate the mean effect size for this group. This argument is supported by a clear lack of homogeneity in the younger group (Qw=39.76, p<0.001). A moderator analysis with a homogenous under-65 group (the cerebellar study [Pascual-Leone *et al,* 1993] and the outlier PD study [Stefanova *et al,* 2000], were removed) is not significant (QB(1)=0.41, p=0.52). Thus the homogenous 'under-65 group' is not impaired relative to the 'over 65 group' which is what would typically be expected of a younger cohort (the younger cohort are usually expected to be equal to, or *less* impaired, than the older cohort). Therefore it is the inclusion of those studies in the younger cohort that have large effect sizes (Pascual-Leone *et al,* cerebellar d=2.16; Stefanova *et al,* PD d=2.09) which are due to the neurologic condition of the patients that renders the non-homogenous '65-and under' group impaired relative to the 'over-65' group, rather than the age of the subjects *'per se'*.

Table 3.24, Moderator and Subset Analyses for Age of Subjects.

Moderator Analyses	# Subjects	# effect sizes	d, 95% CI & r		QB; Test of the Model	k_0
Age Moderator Analysis	345 / 395	22	**0.92,** 0.77 to 1.08, 0.42		QB(1)=3.9, p<0.05	81
Dementia Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	Mean effect size, 95% CI & r	**p**	**k_0**
Subjects ≤ 65 years	231 / 268	12	15	**1.06**, 0.86 to 1.25, 0.47	p<0.001	65
Subjects > 65 years	114 / 127	5	7	**0.72,** 0.45 to 0.99, 0.34	p<0.001	18

Fig 3.8. Mean effect sizes and 95% confidence intervals for the age subset and moderator analyses



The (homogonous) 'over 65' age group has an effect size that is significantly different from zero and these subjects therefore have weaker interference effects than their control subjects ('> 65' d=0.72, p<0.001). Both the heterogeneous '65 and under 'age group and the homogenous '65 and under' group do have effect sizes significantly different to controls (heterogeneous '≤ 65' d=1.06, p<0.001; homogeneous '≤ 65' d=0.83, <0.0001) and are impaired relative to controls.

8) Moderator and Subset Analyses for Presence or Absence of Medication in Neuropathological Subjects during SRT performance

This moderator analysis revealed that studies in which neuropathological subjects were on medication during SRT performance produced larger effect sizes than studies in which neuropathological subjects were not on medication during SRT performance (QB(1)=6.4, p<0.025).

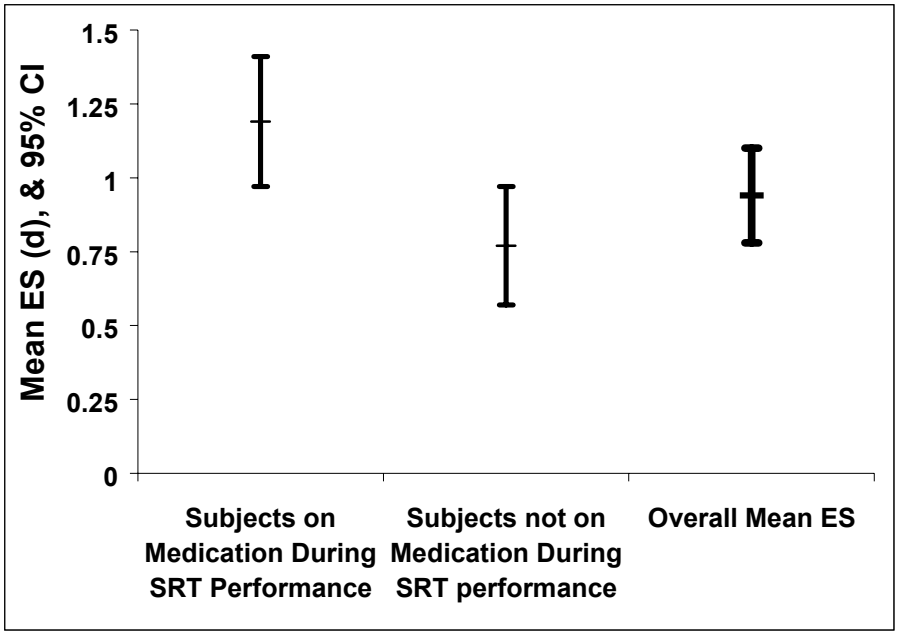
Table 3.25, Moderator and Subset Analyses for Subjects On / Not On Medication

During SRT Performance.

Moderator Analyses	**# Subjects**	**# effect sizes**	**d, 95% CI & r**	**QB; Test of the Model**	**k_0**
Presence/Absence of Medication Moderator Analysis	335 / 396	22	**0.92,** 0.77 to 1.08, 0.42	QB(1)=6.4, p<0.025	81

Dementia Subset Analyses	**# Subjects**	**# Studies**	**# effect sizes**	Mean effect size, 95% CI & r	**p**	**k_0**
Subjects on Medication	136 / 146	12	8	**1.19**, 0.93 to 1.45, .051	p<0.001	40
Subjects not on medication	199 / 250	5	14	**0.77,** 0.57 to 0.97, 0.36	p<0.001	40

Fig 3.9. Mean effect sizes and 95% confidence intervals for the Subjects On / Not On Medication During SRT Performance subset and moderator analyses



Subjects on medication were significantly more impaired relative to controls than those subjects not on medication. Thus it would appear that medication worsens SRT performance. However, the difference between the medicated and unmedicated groups is confounded by the fact that all medicated subjects suffered BGN dysfunction (all medicated subjects were either PD or HD sufferers, and all PD and HD subjects were medicated during SRT performance). Thus, given that this test is essentially between BGN studies and all other studies (i.e. the 'non-medicated' group is simply the LSN and ON groups collapsed together) the possibility exists that the difference between the two medication groups is due more to meta-aetiological status rather than

purely as a function of medication, and consequentially this result must be interpreted with some caution.

Other Moderator Analyses

All other moderator analyses (different repeating sequence lengths, raw or log-transformed reaction times, and 'constrained' or 'unconstrained' random sequences) were not significant (all p's>0.1) and are not reported further.

Discussion of Part 2

The primary conclusion that can be drawn from the analysis of Part 2 is that neurological subjects as a whole differ from control subjects in that they have a significantly weaker interference effect. In contrast to the SRT literature this also holds true for LSN subjects which comprise the group typically described as *not* different to controls in the literature. The most parsimonious explanation for this discrepancy is that those studies which conclude LSN subjects perform the SRT in a control-like manner did so because they lacked the statistical power to find even the medium-to-large differences between LSN and control subjects, as a consequence of using small sample sizes.

Subjects in all three aetiological meta-categories are impaired relative to controls and they also differ between themselves. While LSN subjects *are* impaired relative to controls, in contrast to what is usually reported in the SRT literature, LSN subjects are also less impaired relative to controls than both BGN and ON subjects. Hence there is clear support for the hypothesis that BGN subjects are impaired in the SRT relative to LSN subjects and for the hippocampal / basal ganglia memory systems dissociation. However, the dissociation is only partial because the LSN deficit is not consistent with the more specific hypothesis of a complete dissociation of multiple-memory systems.

While the individual LSN aetiologies (AD, MA, & KS) were not different from one-another there was some indication that there were differences between the individual BGN aetiologies. However, the complete PD group in this analysis was markedly heterogeneous ($Q(5)=19.33$, $p<0.0025$) and only attained homogeneity once the extremely large effect size from the Stefanova *et al* (2000; d=2.09) study was

removed. Including the Stefanova *et al* study in the analysis (ignoring issues of heterogeneity) would substantially weaken any differences between the PD and HD groups and this which must be taken into account when considering any possible differences between PD and HD subjects.

All constituent ON aetiologies (cerebellar lesions, non-specific TBI and pFC injury) are impaired relative to controls which is not surprising in light of their inability to demonstrate a robust interference effect in Part 1. However, while the individual ON aetiologies did not differ between themselves in Part 1 they did so in Part 2. Specifically, cerebellar subjects demonstrated a profound impairment relative to controls (d=2.16) whereas TBI and pFC subjects demonstrated an impairment more in line with LSN subjects (TBI d=0.66, pFC d=0.64, & LSN d=0.68). Furthermore, although the effect size for cerebellar subjects is only generated from a single study it is sufficiently large that it requires a reasonable number of non-significant results to invalidate it (k_0=10).

It was expected that severity of dementia would be a reliable predictor of SRT performance, the analysis for severity of dementia repeats the findings for the same analysis in Part 1. While all 'non-dementing', 'very-mildly dementing' and 'mildly dementing' groups were significantly impaired relative to controls they did not differ between themselves and thus once again severity of dementia, at least in this range, is not a useful predictor of SRT performance. However, the analysis restricted to dementing subjects only was marginally non-significant which suggest the test may have been insufficiently sensitive to the relatively large numerical differences in effect sizes between these two groups ('very-mildly dementing' d=0.55, 'mildly dementing; d=1.1). More evidence is needed, particularly from more severe dementia, before one can conclude that dementia status *'per se'* is not an important variable in SRT effects.

Studies differ as to whether there is any advantage of explicit sequence knowledge for SRT performance (Segar, 1997; Stadler & Neely, 1997; Jackson & Jackson, 1995; Mutter, Howard & Howard, 1994; Willingham & Korroshetz, 1993; Reber & Squire, 1884 & 1998). A high degree of explicit sequence knowledge undoubtedly aids SRT performance by allowing subjects to confidently and deliberately predict the locus of the next stimulus. Although the moderator effect for

explicit sequence knowledge enjoyed by *neuropathological* subjects was not reliable, the moderator of neuropathological subjects performance as predicted by *control* subject's explicit sequence knowledge was. Thus the degree of explicit sequence knowledge enjoyed by a control subject accurately predicts the SRT performance of a neuropathological subject. Specifically, when control subjects have 'some' explicit sequence knowledge neuropathological subjects are less impaired relative to control subjects than when control subjects have 'no' or 'good' sequence knowledge. This may be because control subjects perform the SRT more poorly when they have 'some' explicit sequence knowledge (because this limited amount of explicit sequence knowledge acts as a relative interferer) than when they have 'no' or 'good' explicit sequence knowledge. However, this explanation would require additional evidence and replication before it could be considered as anything other than a theoretical possibility.

In summary Part 2 has reported four important findings. First, although most SRT studies have medium-to-strong effect sizes they have low power and are thus insensitive to group differences, which is especially problematic for those studies that rely on failing to reject the null hypothesis in order to be able to conclude their groups do not differ (which is typical of LSN SRT studies in particular). Second, LSN subjects generally *are* impaired relative to controls, irrespective of which aetiology causes the LSN damage, in contrast to what is usually reported in the SRT literature. Third, BGN subjects are impaired relative to LSN subjects which is evidence for both the hippocampus / basal ganglia memory-systems dissociation and multiple-memory systems in general, although the strength of the evidence is weakened because of the incorrect assumption that LSN subjects are completely unimpaired in the SRT. And finally, contrary to expectations severity of dementia is not a reliable predictor of SRT performance although this finding may be due to the meta-analysis being insensitive to differences between the (non-)dementing groups.

General Discussion

Overview of the Goals and Results of the Power and Meta Analyses.

The purpose of the analysis was firstly to test the hypothesis that those SRT studies which reported no difference between neuropathological group and controls did so because such studies lacked adequate statistical power. Even though most studies produced medium to large effect sizes the results of the power analysis conclusively demonstrated that the majority of studies lacked adequate power even at these effect sizes (which were unusually high for behavioural studies) and as a result were insensitive to group differences. Thereafter the meta-analysis compared between aetiologies and meta-aetiological categories to ascertain if individual neuropathologies and / or groups of neuropathologies had particular consequences for SRT performance.

One goal of these analyses was to test the widespread assumption in the SRT literature that subjects with limbic system dependent amnesia were not impaired on the SRT. In contrast to the literature the meta-analysis found that subjects with limbic system amnesias *do* have impaired SRT performance, but not as impaired as the other neuropathologies (BGN and ON) tested. Another goal of the SRT was to determine the degree of the deficit produced by basal ganglia disorders and whether different basal ganglia disorders resulted in different degrees of SRT impairment. While both PD and HD subjects suffer markedly impaired SRT performance relative to controls and LSN subjects there is little clear evidence as yet of any difference between the abilities of PD and HD patients to perform the SRT. A surprise finding was that subjects grouped into the 'other' meta-aetiological category were substantially impaired in the SRT, although perhaps this should not be so surprising given that cerebellar subjects in particular demonstrated absolutely no sequence learning of any type while performing the SRT task. Finally, a number of moderator analyses were performed but only the degree of explicit sequence knowledge enjoyed by both neuropathological and control subjects was found to be a reliable predictor of SRT performance. All other moderator variables were not reliable predictors of SRT performance.

Limbic System Amnesia and the SRT.

One of the primary reasons for performing this meta-analysis was to discover if subjects with disorders of the limbic system are unimpaired in the SRT relative to control subjects, as is often claimed in the SRT literature. The meta-analysis clearly demonstrated that LSN subjects are reported as being unimpaired relative to controls because such studies have low power and are thus insensitive to even the medium-to-strong differences between LSN and control subjects. The meta-analysis shows that the reason LSN studies have low power is because they typically have sample sizes that are too small. Once LSN subjects were aggregated into a single group by the meta-analysis LSN patients were shown to actually have significantly *weaker* interference effect than controls and therefore can not be said to be unimpaired in the SRT relative to controls.

The finding that LSN subjects are impaired relative to controls is at odds with virtually all of the studies that contributed effect sizes to the LSN group. While Knopman and Nissen (1987) found no difference between the interference effects for the AD and control groups in the first SRT study, but examination of the respective means indicated that there might be one. While the average difference score for the AD subjects was 110msec, it was 181msec for the control subjects. Similarly the standard deviations of each group are substantially different, the control subjects SD was 84msec whereas the AD subjects SD was twice as large at 162msec. As a result the effect size when using a pooled SD (i.e. not just the SD of the controls) for the group differences is reasonably large (d=0.62) and is indicative of a possible real difference between the two groups. Thus the reason for Knopman and Nissen and the meta-analysis reaching opposite conclusions is that Knopman and Nissen's test of the null hypothesis was insensitive to any actual group differences because of low power (due in particular to the small sample size of the control group: N=13).

In fact of the nine studies included in the meta-analysis that used LSN subjects only two (Ferraro, Balota and Connor, 1993; and Curran, 1997) concluded that their LSN subjects were different to control subjects. However, careful examination of all LSN studies suggests that it is actually Ferraro, Balota and Connor who reached the correct conclusion and not the other studies (including Curran who appears to have

reached the correct conclusion by error).

Curran (1997) concluded his mixed-amnesic patients were impaired relative to controls but there is actually evidence that there was no difference between groups in his study. His conclusion that the mixed-amnesics were impaired in the SRT relative to controls rests on the finding that amnesic patients demonstrated less 'higher-order' learning than controls. This conclusion was based on a marginally non-significant test result ($p=0.07$) of the difference in reaction times for random and repeating-sequence sets performed by amnesic subjects. Furthermore, this marginal test result directly contradicts the more reliable, and non-significant, test of any difference between the strength of interference effects of the amnesic and controls groups. We were fortunate enough to obtain the actual data used in Curran's study and used these data to directly test any difference in interference effects between mixed-amnesics and controls. The t-test of difference scores between groups was not significant ($t(9)=0.35$) and produced a small effect size of $d=0.16$. Therefore there is currently no firm evidence that Curran's groups differed, and thus at this stage amnesics do not seem to be are impaired on his version of the SRT task.

In contrast to the majority of LSN studies Ferraro, Balota and Connor (1993) report that their very-mildly dementing AD subjects were not different to controls whereas a deficit was present in their mildly dementing AD subjects. They concluded the reason for the different SRT performance between their AD groups was simply because the mildly dementing AD subjects had a SRT impairment by virtue of their more severe dementia. Their finding that very-mildly dementing subjects are not impaired relative to controls contrasts with most other SRT studies using LSN subjects. Their assumption that SRT differences vary with severity of dementia also contrasts with the meta-analysis which concluded that severity of dementia was not a reliable predictor of SRT behaviour. While the issue of dementing severity and SRT performance will be discussed below, their finding that very-mildly dementing subjects were not impaired in the SRT task seems valid for the following reasons. Not only did the very-mildly dementing AD subjects have a very similar interference effect to control subjects (including a small effect size for the difference between the two groups, $d=0.22$) but the very-mildly dementing AD subjects did not have slower overall reaction times than control subjects. This contrasts with *all* other LSN subjects (including Ferraro, Balota and Connor's mildly dementing AD subjects) who did have

slower overall reaction times. Irrespective of the meta-analyses finding that severity of dementia does not predict SRT performance it appears obvious that the very-mildly dementing AD subjects demonstrated no SRT impairment because they had such a mild cognitive disorder.

Overall LSN subjects are reliably impaired in the SRT relative to controls, but they are also less impaired relative to other forms of neuropathology. Subjects with LSN-dependent amnesia produce a far stronger interference effect than subjects with generalised traumatic brain injury or cerebellar lesion (Part 1). Furthermore LSN subjects are less impaired relative to controls than subjects with any other form of major neural disorder (Part 2), and BGN and ON subjects are more impaired relative to controls than LSN subjects (in Part 2). By demonstrating that LSN subjects are substantially less impaired in the SRT than BGN subjects (irrespective of the LSN subject's impairment relative to controls) the meta-analysis provides some support of a (partial) dissociation between non-declarative memory and limbic system neuropathology.

An obvious criticism of aggregating AD, KS and mixed-amnesia studies into a single group is that these are clinically and functionally different aetiologies, and in particular have reasonably different neuropathological profiles. By definition of being a dementia AD produces other neuropathology outside the limbic system and KS often produces relatively diffuse neural damage in addition to its primary neuropathology. It is possible therefore that the individual aetiologies produce quite different patterns of impairment / sparing in the SRT and hence the relatively mild deficit in the LSN group may be due to the averaging effect on effect sizes produced when combining the groups. However, it is readily apparent from Fig. 3.4 that this is not the case, the individual aetiologies, and even individual studies, that go to make up the LSN group are remarkably consistent between themselves. Even thought here are only two KS studies they are particularly consistent with each other. Similarly the mixed amnesics study are consistent within themselves as are the AD studies (the exception in each of the AD and amnesic groups, Curran, 1997, and Ferraro, Balota and Connor, 1993, are discussed above). Not only are the effect sizes of individual studies consistent within aetiologies but the mean effect sizes for each aetiology are very similar to each other. Finally, the test of homogeneity for each LSN aetiology and for the LSN group as a

whole are all non-significant, demonstrating that both the individual groups and the LSN meta-aetiology are valid characterisations of their constituent effect sizes. Hence the three LSN aetiologies all produce a very similar degree of impairment in the SRT and therefore can be treated as a single group where useful to do so.

Another possible confound within the LSN meta-aetiology is that 'mixed amnesic' groups include subjects with clinically dissociated aetiologies, in particular Korsakoff's Syndrome. A closer inspection of the three studies that go to make up the 'mixed amnesia' aetiological grouping in the meta-analysis reveals that the 'amnesiac' groups in each study included a number of different (albeit related) aetiologies. Table 3.26 shows the amnesic group breakdown for each study and as can be seen 'mixed amnesia' groups are made up of a variety of neuropsychological disorders which seriously questions the feasibility of treating them as a homogenous group. As KS subjects (on average) make up a large minority sub-group (about a 3rd) of the mixed amnesic designs overall, the distinction between the mixed amnesic and Korsakoff's aetiologies may not be valid. A greater problem is that KS subjects may not always be representative of pure ('organic') amnesia, even if they do not show clear signs of other (i.e. frontal) pathology.

Table 3.26. Subject Numbers in Aetiological Sub-Groups Within the 'Mixed Amnesic' Classification by Individual Study.

Study	KS	Encephalitis	Diencephalic Lesion	Hippocampal Lesion	Anoxia	Infarct	Unknown	Total
Curran, 1997	4	2	-	-	3	1	-	10
Reber & Squire, 1994	1	-	6	2	-	-	-	9
Reber & Squire, 1998	2	1	-	-	-	1	1	5
Total	**7**	**3**	**6**	**2**	**3**	**2**	**1**	**24**

Finally, Aggleton and Brown (1999) comment on a study by Hanley *et al* (1994) who reported that one subjects with an anterior communicating artery aneurysm evidenced damage to the left hemisphere, including damage to the anterior thalamus *and* the caudate. This subject showed a verbal recall deficit but not a recognition memory deficit. Although this pattern of injury is presumed uncommon in ACoA patients it provides additional evidence that human amnesics often fail to have restricted neural damage which serves to reinforce the case for experimental lesion work in animals.

Basal Ganglia Neuropathology and the SRT

An examination of the studies with subjects suffering basal ganglia dependent neuropathology reveals that the literature and the meta-analysis agree that BGN patients are impaired in the SRT. In particular the meta-analysis extended the literature by specifying the size of the effect and providing quantitative evidence that subjects with BGN disorders are impaired on the SRT relative to both controls and LSN-dependent amnesics.

No SRT study to date has directly compared the performance of PD and HD subjects and in doing so in the meta-analysis provides a unique and important comparison of these disorders. There is an initial suggestion that PD and HD may produce differing amounts of impairment in the SRT task. However, this suggestion is dependent on the removal of an outlier effect size generated by one PD study (Stefanova *et al,* 2000). While the inclusion of this study in the PD group renders the PD group heterogeneous Stefanova *et al* is more empirically reliable than most PD-SRT studies by virtue of having much larger-than-normal sample sizes and thus possibly more indicative of the real ability of PD subjects in the SRT (Note, there was no obvious reason why the PD subjects in Stefanova *et al* produced such a larger effect size than other PD subjects). Thus the effect size of the homogenous PD group is likely to underestimate the true effect size for PD subjects and therefore likely to overestimate the difference between the effects of PD and HD on SRT performance. Hence, while there is a clear and obvious SRT deficit for BGN subjects as a whole there is little evidence to suggest the constituent aetiologies within the BGN meta-category vary between themselves.

However, the finding that LSN subjects are less impaired relative to BGN subjects rather than LSN subjects simply being unimpaired relative to BGN subjects conflicts with the multiple memory literature which claims that a clean dissociation exists between LSN and BGN subjects in non-declarative memory tasks. This lack of a clear dissociation between the SRT performance of LSN and BGN subjects, and the mild LSN-SRT impairment it stems from, is a serious challenge for the multiple memory systems theory.

Other Neuropathology and the SRT

In contrast to both LSN and BGN groups the 'other' neuropathologies are relatively underrepresented in the SRT literature. Of the three studies included in the meta-analysis one used subjects with cerebellar lesions, one used subjects with pFC lesions and one used subjects with (closed head) traumatic brain injury (from which two effect sizes were generated). While the test of homogeneity was not significant when combining the two TBI studies together the extent and severity of brain injury differed markedly between subjects in the two TBI conditions which made combining them into a single group conceptually problematic. Because there are so few studies in each of the individual aetiologies they (the separate aetiological groups) suffer the greatest problems with test sensitivity and interpretation and their results are also especially vulnerable to invalidation via unpublished studies. For these reasons it was especially useful to combine these effect sizes into a meta-aetiological group even if the neuropathological and clinical profile of the individual aetiologies isn't particularly consistent within the meta-category.

As there are so few studies in the SRT literature employing subjects with these aetiologies there is no general consensus about the effects of these aetiologies on SRT performance beyond that non-specific brain injury (i.e. anything that does not include LSN or BGN dysfunction) will probably result in some degree of impairment of SRT performance, but a weaker impairment than basal ganglia dysfunction. Consequently the results of the meta-analysis provides good empirical evidence that non-specific brain injury does indeed impair SRT performance. Two findings in particular are of interest as regards ON-SRT studies. Firstly, that as a group the ON subjects are impaired relative to all other subjects (ON subjects are impaired relative to control and LSN subjects in Part 1, and relative to control, LSN and BGN subjects in Part 2). Surprisingly, this indicates that there are other aetiologies that produce a more severe SRT impairment than even basal ganglia dysfunction. Secondly, that there are considerable differences between the individual aetiologies that make up the ON meta-category. Not only are the individual aetiologies different in terms of the strength of their effect sizes but also in terms of the pattern of effect sizes.

The least impaired of the three individual ON aetiologies are the non-specific TBI subjects (Mutter *et al,* 1994) who produce evidence of a moderately-strong within-subject interference effect (Part 1, d=0.66; and thus moderately-strong evidence of non-declarative sequence learning) and a moderately-strong impairment relative to

controls (Part 2, d=0.67). The finding that subjects with mild head injury are not particularly impaired is not surprising as such injury is likely to produce little brain damage and therefore little consequence for cognitive function. In contrast subjects with explicit, confirmed brain insult (such as the moderately head injured subjects) can be expected to suffer a marked degree of brain damage and thus a more serious cognitive impairment. Most interestingly the effect size for mildly head injured subjects in Mutter *et al,* (1994) has a numerical similarity with control subjects in the meta-analysis (d=0.07). This strong similarity between mild head injured and control subject's SRT performance is the perhaps the best evidence that the mild head injured subjects demonstrate a substantial difference to more severely injured subjects in terms of their SRT performance and thus, irrespective of the TBI group's homogeneity, should perhaps not be grouped together with the more severely head injured subjects.

Subjects with pFC lesions demonstrate a negative within-subject interference effect that was not different to zero (Part 1; d=-0.18, i.e. their reaction times get *faster* after being switched to a random sequence) and thus they demonstrate absolutely no evidence of a within-subject interference effect. However pFC subjects are only moderately impaired relative to controls in Part 2 (d=0.64). It is difficult to understand how a group that does not display any within-subject interference effect can only be 'moderately' impaired relative to a control group. It would be expected that control subjects would generate a strong interference effect and therefore be substantially different to pFC subjects who fail to demonstrate any form of interference effect. However, the calculations for the effect sizes for the control and pFC groups are not in question as we were able to obtain some of the original data from the study using pFC patients (Beldarrain *et al,* 1999) and to calculate effect sizes directly from those data.

In contrast to both the TBI and pFC subjects cerebellar (Cb) subjects are completely impaired in the SRT. Like pFC subjects Cb subjects display no within-subject interference effect (Part 1; d=-0.07) and thus no evidence of non-declarative sequence learning. Unlike pFC subjects however Cb subjects also display a more profound impairment relative to controls (Part 2; d=2.12). Not only is the within-subject interference effect for Cb subjects substantially weaker than those BGN patients who are traditionally regarded as impaired on the SRT but Cb subjects display a greater impairment relative to controls than even that of the severely impaired HD subjects (HD d=1.47).

Power and Effect Size

No discussion of the meta-analytic results would be complete without some mention of statistical power. As noted in the discussions for both Parts 1 and 2 SRT studies suffer a serious lack of power, largely as a result of small sample sizes. This is a concern in its own right but even more so because many SRT studies rely on a failure to reject the null hypothesis to conclude there is no difference between groups, which is especially vulnerable to type-II errors in conditions of low power. One of the critical findings of this meta-analysis has been that many SRT studies, and especially those employing subjects with LSN, simply lack sufficient power to be able to draw the conclusions that they report. This is the main reason for the discrepancy in results between individual studies and the meta-analysis as regards the ability of LSN patients to perform the SRT. Because these studies had low power they were insensitive to even the medium-to-large differences between LSN and control groups and thus incorrectly concluded that LSN patients were not impaired in the SRT. This fundamental error has distorted the results of SRT studies, since the very first study. It is only with the findings of the meta-analysis that we finally begin to get a clear and accurate (and potentially more interesting) understanding of spared / impaired SRT performance in a range of different neurological disorders.

As previously discussed the main cause of low power in SRT studies is low sample size. Another important benefit of the meta-analysis is providing valid estimates of effect sizes for different neuropathological populations which enables future researchers to tailor their research to fit the relevant effect size, and in particular ascertain an adequate sample size (see below) prior to beginning any research.

Moderator Variables

One of the surprises of the meta-analysis was the failure of severity of dementia to demonstrate a meaningful consequence for SRT performance. (See discussion of Parts 1 and 2). While it was expected that severity of dementia would be directly and inversely related to SRT performance the meta-analysis concluded there is no effect of dementia on SRT performance. There is currently no good explanation for this unexpected pattern of results except to repeat the point made in the discussion of Parts 1 and 2. Irrespective of the fact that these tests are part of a meta-analysis they still have relatively few subjects (the two dementing groups have ~50 subjects

each) and thus may still be somewhat insensitive to relatively small group differences.

While some studies conclude that explicit sequence knowledge is advantageous for SRT performance other studies conclude it has little, if any, effect (Segar, 1997; Stadler & Neely, 1997; Jackson & Jackson, 1995; Mutter, Howard & Howard, 1994; Willingham & Korroshetz, 1993; Reber & Squire, 1884 & 1998). The consistent finding across both parts of the meta-analysis was that those patients with 'some' explicit knowledge performed better and / or were less impaired relative to controls than those subjects with 'no' or 'good' explicit sequence knowledge.

Although the moderator analyses of 'Age' and 'On / Off Medication' were statistically good predictors of SRT performance both suffered conceptual difficulties as detailed in the discussion of Part 2. Briefly, the 'Age' analysis is confounded by the presence of very high effect sizes due to aetiological condition rather than age in the younger cohort. Similarly the on / off medication analysis is confounded by systematic differences in group composition other than medication status.

Recommendations for Future Research.

In light of the power and meta-analytic results and the discussion presented above a number of recommendations can be made as to the design and direction of future SRT research.

Future SRT Study Design Recommendations

1) It is critical that a study have sufficient power to detect any differences between groups. Minimum sample sizes, to obtain 80% power with a two-tailed test and alpha set at 0.05, for the five primary aetiologies used in this meta-analysis are presented in Table 3.25 based on the mean effect size for each aetiology as calculated in the meta-analysis.

Table 3.25. Subject numbers required for 80% power according to effect sizes calculated during the Meta-Analysis

Aetiology	Part 1[2]		Part 2[3]	
	Mean effect size[1]	N Required for 80% Power (Dependent-means t-test)	Mean effect size[1]	n Required for 80% Power (Independent means t-test)[4]
Alzheimer's Disease	0.57	27	0.6	45
Mixed amnesics	1.01	10	0.77	28
Korsakoff's Syndrome	1.36	7	0.79	27
Parkinson's Disease	0.54	29	1.1	15
Huntington's Disease	0.39	54	1.47	9

[1] As calculated in the meta-analysis
[2] For studies examining the ability of neurological subjects to demonstrate an interference effect different from zero
[3] For studies comparing the interference effects of neurological and control subjects
[4] t-test of difference scores, n given is the number of neuropathological subjects required for 80% power and assumes equal n for the control group.

2) Subject's should ideally have no explicit sequence knowledge at all, or at least it should be keep to a bare minimum. Furthermore, it is preferable that if one group achieves a degree of explicit sequence knowledge that the other group do so to the same degree in order to better equate the influence of explicit sequence knowledge between groups. However, the mechanics of doing this raise a number of serious methodological issues (e.g. do subjects employ non-declaratively 'acquired' explicit sequence knowledge in the same way as knowledge deliberately provided by the experimenter?). Furthermore, some subjects (e.g. amnesics) may be incapable of retaining and using deliberately provided explicit sequence knowledge. Thus overall it is methodologically and theoretically preferable to ensure all subjects have no explicit sequence knowledge. However, just how this somewhat idealised goal might be accomplished, especially in neurologically intact control subjects, without introducing other methodological confounds (e.g. dual-task conditions for controls subjects only), is not clear.

Suggested Directions For Future SRT Studies

1) A study that would be of particular value at this time is a comparison between dementing and non-dementing PD subjects. The utility of such a study is two-fold. It may help to explain the variation in the SRT performance of PD subjects (effect sizes for PD studies in the Part 1 of meta-analysis ranged from 0.23 to 1.42 and were widely distributed in that range, and in Part 2 ranged from 0.16 to 1.8, not counting the outlier, Stefanova *et al,* d=2.09).

2) The study that found a greater SRT impairment in more dementing AD subjects (Ferraro, Balota and Connor, 1993) suggests dementia may be a critical predictor of SRT performance at least in this disorder. Coupled with the possibility that the meta-analysis failed to find a difference between severity of dementia because it also lacked the power to detect any difference argues strongly for a study designed specifically to address the issue of severity of dementia irrespective of aetiology. In particular a study that deliberately examines differing severities of dementia within and across aetiological conditions. Obvious aetiological candidates for this include: AD, and dementing PD and HD patients.

General Conclusions

Overall the meta- and power-analyses have reinforced the notion that the SRT is a sensitive test of non-declarative memory. In particular the meta-analysis has shown that the SRT is sensitive to neuropathological condition, and in particular is capable of dissociating limbic system and basal ganglia dysfunction. Furthermore, the meta-analysis has substantially increased our level of understanding of the relationship between neuropathological condition and SRT performance, and in particular shown that the relationship is more subtle and complex than originally thought. Most importantly the meta-analysis has produced conclusive evidence in opposition to the commonly held assumption that subjects with LSN-dependent amnesia are not impaired on the SRT; in fact they *are* impaired, just not to the same degree as other neuropathological populations. Not only does this contradict the majority of the SRT literature but the lack of a clean dissociation between LSN and BGN SRT performance also challenges the commonly held view that these two substrates are responsible for completely different memory systems. A central tenant of the multiple memory systems theory is the dissociation between neural substrates responsible for different memory systems. Taken together the LSN SRT impairment and the lack of a clear distinction between LSN and BGN performance are therefore a serious theoretical and empirical challenge to the theory.

The other major finding of this study is that the vast majority of SRT studies have inadequate power, rendering them insensitive to group differences. This is a

serious problem when applied to typical research practices but is even worse in conditions of atypical experimental design (relying on a failure to reject the null hypothesis) which are so common in SRT studies with LSN-dependent amnesics. Thus extreme care must be taken when interpreting the results of most SRT studies, especially when they conclude there is no difference between groups, and future studies must be designed with due concern for statistical power.

Both the lack of power in SRT studies and, in particular, the serious challenge to the multiple memory system that a LSN SRT impairment produces strengthens the case for animal lesion work. The primary advantage of animal studies over human is the certainty associated with both the site and extent of neural injury. It is readily apparent from the results and discussion above that the two main issues within the human literature are a result of the (understandable) difficulty with accurately determining the neurological condition of a subjects and / or the site and extent of the neural damage a subject suffers (especially in neurodegenerative disorders, see Reber, 1992), and the often serious difficulty in sourcing sufficient neurological pure subjects to ensure adequate power. The use of animal subjects in research can overcome these problems by allowing the use of accurate, specific and restricted brain lesions and by ensuring the availability of large numbers of subjects.

These advantages of animal research were part of the reason we developed an animal analogue of the SRT, in order to better test the various neural substrates implicated by the SRT literature and this meta-analysis. The other primary reason was the lack of an analogical valid animal model of a human non-declarative memory task. The following chapter (Chapter 4) introduces the literature concerned with animal memory work and multiple memory systems in particular to illustrate the successes and limitations of prior attempts to demonstrate multiple memory systems in animals. Chapter 4 will focus on the poor fit between animal and human ‘non-declarative’ memory tasks and will demonstrate that an animal-SRT task is a practical and useful addition to the multiple memory systems literature. Modelling human non-declarative sequence learning in animals offers the opportunity to demonstrate a double-dissociation of neural substrate (basal ganglia lesions and limbic system lesions) and memory task (allocentric spatial learning and the animal-SRT task) using animal memory tasks that are both valid and reliable models of their analogous human

memory tasks, which hitherto has not been possible.

Appendix to Chapter 3

Table 3A.1. Coding of Moderator Variables by Study

	Meta-Aetiology	Individual Aetiology	Severity of Dementia	On Meds (Y/N)	SRT Length	SRT Style	Expl Knowl Patient
Beldarrain et al, 1999	3	7 (pFC)	1	No	4 & 10[1]	1	1
Curran, 1997	1	2 (Mixed Amnesiacs)	1	No	12	1	1
Ferraro Balota & Connor, 1993	2	4 (PD)	1	Yes	10	1	Not Rep
Ferraro Balota & Connor, 1993	1	1 (AD)	2	No	10	1	Not Re
Ferraro Balota & Connor, 1993	1	1 (AD)	3	No	10	1	Not Re
Jackson et al 1995	2	4 (PD)	1	Yes	11	2	1
Knopman & Nissen,1987	1	1 (AD)	3	No	10	1	1
Knopman & Nissen, 1991	2	5 (HD)	2	Yes	10	1	1
Knopman, 1991	1	1 (AD)	2	No	10	1	1
Mutter et al, 1994	3	7 (Head Injury)	1	No	10	1	3
Mutter et al, 1994	3	7 (Head Injury)	1	No	10	1	3
Nissen & Bullemer, 1987	1	3 (KS)	1	No	10	1	1
Nissen, Willingham & Hartman, 1989	1	3 (KS)	1	No	10	1	Not Re
Pascal-Leone et al, 1993	2	4 (PD)	1	Yes	8, 10 & 12[1]	1	2
Pascal-Leone et al, 1993	3	6 (Cerebellar Injury)	1	No	8, 10 & 12[1]	1	1
Reber & Squire, 1994	1	2 (Mixed Amnesiacs)	1	No	10	1	1
Reber & Squire, 1998	1	2 (Mixed Amnesiacs)	1	No	12	1	1
Sommer et al, 1999	1	4 (PD)	1	Yes	10	1	2
Stefanova et al, 2000	2	4 (PD)	1	Yes	10	1	4
Stefanova et al, 2000	2	2 (ACoA amnesia)	1	No	10	1	2
Westwater et al, 1998	2	4 (PD)	1	Yes	10	1	Not Re
Willingham & Korroshetz, 1993	2	5 (HD)	3	Yes	12	1	1

[1] Coded as a 'missing' value in the meta-analysis
[2] All unreported variables were coded as 'missing'.
Note: A 'missing' value means the mean ES associated with that study is not included in the moderator analysis for that variable (only).

Legend for Coding Variables

Aetiological meta-category: LS=1, BG=2, Other=3
Individual Etiology: AD=1, Mixed Amnesiacs=2, KS=3, PD=4, HD=5, Cerebellar Lesion=6, pFC & Non-specific (frontal) head injury =7
Dementing (the neurological group): Yes/No
Severity of Dementia (in the neurological group): 1=Non-dementing, 2=Very mildly dementing, 3= Mildly dementing or worse
On Medication (the neurological group): Yes/No
SRT Length: Actual length used as coding variable
SRT style: 1= Knopman & Nissen style 4-button; 2= 4-choice box (Jackson et al, 1995)
Amount of explicit knowledge: 1=none, 2= 'some', 3= 'good'.
Age: Under, or equal to, 65 and over 65
Logged RT: Yes/No
Random Sequence Style: 'Unconstrained' (random sequences were only constrained by disallowing immediate repetition and / or ensuring equal frequency of stimulus presentation of a training block) & 'Constrained' (random sequences that only varied from repeating sequences in terms of the actual sequence information presented).

Table 3A.2. Detail of Methods Used For Generating Effect Sizes by Study

Author and Neurologic Aetiology	Part 1 Analysis (Within subject)			Part 2
	Data Source	(Values given are Mean & (SD) of difference scores)	**Mean ES (g)**	**Data Source**
Beldarrain et al, 1999[1] pFC Injury	Original data provided by author	Long SRT; Ipsilateral hand = -54(96), N=22, ES = -0.56 Contralateral hand = -45(130), N=22, ES = -0.34 Short SRT= -135(392), N=18, ES = 0.34	**-0.19**	p-values in text & original data provided by author
Curran, 1997 Mixed Amnesics	F-value in text	F=4.21, Amnesics N=10	**0.65**	Original data provided by author
Ferraro et al 1993 PD	Assume significance (Difference = 51ms)	p=0.05, PD N=17	**0.71**	Interaction F-value in text
Ferraro et al 1993 Very mild AD	Assume significance (Difference = 62ms)	p=0.05, Very Mild AD N=27	**0.55**	Interaction p-value in text
Ferraro et al 1993 Mild or worse AD	Assume no effect (Difference = 15ms)	p=0.5, Mild or worse AD N=15	**0.25**	Interaction F-value in text
Jackson et al 1995, Experiment 2 : PD	Mean difference & SD_{diff} in text	PD = 9.3(33.9), N=11	**0.27**	Difference scores t-value in text
Knopman & Nissen,1987 AD	Mean difference & SD_{diff} in text	AD = 110(162), N=28	**0.68**	Difference scores t-value in text
Knopman & Nissen, 1991 HD	RTs in Table 2, SD_{diff} calculated	HD = 62(112.19), N=13	**0.55**	Interaction F-value in text
Knopman, 1991, Session 1, AD	Unlogged mean difference score in text. SD_{diff} calculated.	AD 80(83.63), N=11	**0.96**	Interaction F-value for Log RTs in text
Mutter et al, 1994, Mild HI	t-values in text	Expt. 1, Mild HI, t=3.765, N=12, ES = 1.08 Expt. 2, GCS>13, t=3.075, N=12, ES = 0.89	**0.91**	Table 3; Mean Differences and SD_{diff} scores
Mutter et al, 1994, Moderate HI	t-value in text	Experiment 2, GCS≤13, t=1.819, N=12	**0.53**	Table 3; Mean Differences and SD_{diff} scores
Nissen & Bullemer, 1987, KS	$Mean_{diff}$ scores estimated from Fig. 8, SD_{diff} calculated from F-value for Block	KS = 95(46.3), N=6	**2.05**	$Mean_{diff}$ scores estimated from Fig 8, SD_{diff} calculated from interaction F-vaule
Nissen, Willingham & Hartman, 1989, Session 1, KS	$Mean_{diff}$ scores estimated from Fig. 1, SD_{diff} calculated from F-value for Block	KS 85(67.03), N=7	**1.27**	$Mean_{diff}$ scores estimated from Fig 1, SD_{diff} calculated from Block F-values for individual groups
Pascal-Leone et al, 1993 PD	$Mean_{diff}$ scores estimated from Fig. 1 & 3, SD_{diff} calculated from p-value for Block in experiment 1	All ESs; SD_{diff} 70.87, N=20 Expt. 1: PD = 110, ES = 1.56 Expt. 2: SRT 8, PD = 167.5, ES = 2.36; SRT 10 PD = 110, ES = 1.54; SRT12 PD = 17.5, ES = 0.24	**1.42**	$Mean_{diff}$ scores estimated from Fig. 1 & 3, Pooled-SD_{diff} calculated from p-values for Block in experiment 1
Pascal-Leone et al, 1993 Cerebellar	$Mean_{diff}$ scores estimated from Fig. 1 & 3, SD_{diff} calculated from p-value for Block in experiment 1	All SD_{diff} = 100.33, N=15 Expt 1: Cerebellar = -15, ES = -0.15. Expt 2: SRT8, Cerebellar = 10, ES = 0.1; SRT10 Cerebellar = -15, ES = -0.15; SRT12 Cerebellar = -10, Es = -0.1	**-0.08**	$Mean_{diff}$ scores estimated from Fig. 1 & 3, Pooled-SD_{diff} calculated from p-values for Block in experiment 1
Reber & Squire, 1994 Mixed Amnesics	Assume significance	Experiment 1: p=0.05, N=9	**1.04**	$Mean_{diff}$ scores estimated from Fig. 1. SD_{diff} calculated from interaction F-value
Reber & Squire, 1998 Mixed Amnesics	$Mean_{diff}$ score from Table. 1, SD_{diff} calculated.	Session 1 Amnesics = 24.8(28.1), N=5	**0.88**	$Mean_{diff}$ scores from Table. 1, SD_{diffs} calculated.
Sommer et al, 1999 PD	$Mean_{diff}$ & SD_{diff} values in text	PD = 78.3(218.1), N=11	**0.36**	$Mean_{diff}$ & SD_{diff} values in text

Stefanova et al, 2000 PD	$Mean_{diff}$ scores estimated from Fig. 1, SD_{diff} calculated from F-value for Block	PD = 20(85.78), N=39	**0.23**	Mean scores estimated from Fig. 1, SD_{diff} calculated from Interaction F-value
Stefanova et al, 2000 ACoA	$Mean_{diff}$ scores estimated from Fig. 1, SD_{diff} calculated from F-value for Block	ACoA = 110(85.78), N=30	**1.28**	Mean scores estimated from Fig. 1, SD_{diff} calculated from Interaction F-value
Westwater et al, 1998 PD	t-value in text	t=2.6, PD N=13	**0.72**	Interaction F-value in text
Willingham & Korroshetz, 1993. HD	Assume no difference	p=0.5, HD N=12	**0.28**	Difference scores t-value in text

[1] The harmonic mean (37) was entered for the N of controls subjects in the meta-analysis

[2] The interaction F of the raw-RT data for session one were used as it is consistent with what is shown in the graph (the log-RT interaction F is not).

Table 3A.3. Power of Studies Included in the Meta-Analysis Given Idealised ESs : Within Neurological Group Effect (Two-Tailed)

			← Theoretical Significance →			
					← Clinical Significance →	
Study:	**# Subjects**	**Actual ES (d)**	**Small (d=0.2)**	**Medium (d=0.5)**	**Large (d=0.8)**	**Very Large (d=1.2)**
Beldarrain et al, 1999 (pFC)	22	-0.19	15	61	95	100
Curran, 1997 (Mixed amnesics)	10	0.65	9	29	62	92
Ferraro Balota & Connor, 1993 (PD)	17	0.71	12	49	87	100
Ferraro Balota & Connor, 1993 (Very mild AD)	27	0.55	17	71	98	100
Ferraro Balota & Connor, 1993 (Mild AD)	15	0.25	11	44	82	99
Jackson et al 1995 (PD)	11	0.27	9	32	67	95
Knopman & Nissen,1987 (AD)	28	0.68	18	72	98	100
Knopman & Nissen, 1991 (HD)	13	0.55	10	38	75	98
Knopman, 1991 (AD)	11	0.96	8	26	56	88
Mutter et al, 1994 (Mild traumatic head injury)	24	0.92	16	65	96	100
Mutter et al, 1994 (Moderate traumatic head injury)	12	0.53	10	35	71	97
Nissen & Bullemer, 1987 (KS)	6	2.05	7	17	36	66
Nissen, Willingham & Hartman, 1989 (KS)	7	1.27	7	20	43	75
Pascal-Leone et al, 1993 (PD)	20	1.42	14	56	92	100
Pascal-Leone et al, 1993 (Cerebellar)	15	-0.08	11	44	82	99
Reber & Squire, 1994 (Mixed amnesics)	9	1.04	8	26	56	88
Reber & Squire, 1998 (Mixed amnesics)	5	0.88	6	14	28	53
Sommer et al, 1999 (PD)	11	0.36	9	32	67	95
Stefanova et al, 2000 (PD)	39	0.23	23	86	100	100
Stefanova et al, 2000 (ACoA)	30	1.28	19	75	99	100
Westwater et al, 1998 (PD)	13	0.72	10	38	75	98
Willingham & Korroshetz, 1993 (HD)	12	0.28	10	35	71	97

Table 3A.4. Power of The Studies Included in the Meta-Analysis Given Idealised ESs : Between Neurological & Control Group Effect (Two-Tailed)

# of Subjects				← Theoretical Significance →			
				← Clinical Significance →			
Study:	**Neuro.**	**Controls**	**Actual ES (d)**	**Small (d=0.2)**	**Medium (d=0.5)**	**Large (d=0.8)**	**Very Large (d=1.2)**
Beldarrain et al, 1999 (pFC)	22	52	0.65	12	49	87	99
Curran, 1997 (Mixed amnesics)	10	10	0.16	7	19	40	72
Ferraro Balota & Connor, 1993 (PD)	17	26	0.73	10	35	71	96
Ferraro Balota & Connor, 1993 (Very mild AD)	27	26	0.22	11	43	82	99
Ferraro Balota & Connor, 1993 (Mild AD)	15	26	1.22	9	32	67	95
Jackson et al 1995 (PD)	11	10	1.7	7	19	41	74
Knopman & Nissen,1987 (AD)	28	13	0.62	9	30	64	94
Knopman & Nissen, 1991 (HD)	13	12	1.32	8	22	48	82
Knopman, 1991 (AD)	11	16	0.58	7	21	45	79
Mutter et al, 1994 (Mild traumatic head injury)	24	11	0.07	8	27	57	89
Mutter et al, 1994 (Moderate traumatic head injury)	12	11	1.4	7	21	45	78
Nissen & Bullemer, 1987 (KS)	6	8	0.9	6	14	28	53
Nissen, Willingham & Hartman, 1989 (KS)	7	7	0.8	6	14	28	54
Pascal-Leone et al, 1993 (PD)	20	30	1.05	10	40	78	98
Pascal-Leone et al, 1993 (Cerebellar)	15	30	2.16	9	34	70	96
Reber & Squire, 1994 (Mixed amnesics)	9	12	0.97	7	19	41	73
Reber & Squire, 1998 (Mixed amnesics)	5	10	0.68	6	14	27	53
Sommer et al, 1999 (PD)	11	15	0.16	8	23	49	83
Stefanova et al, 2000 (PD)	39	31	2.09	13	54	91	100
Stefanova et al, 2000 (ACoA)	30	31	0.98	12	48	87	100
Westwater et al, 1998 (PD)	13	9	0.89	7	20	42	75
Willingham & Korroshetz, 1993 (HD)	12	12	1.77	8	22	47	80

Table 3A.5. Effect Sizes and 95% Confidence Intervals for All Results Figures in Part 1.

Figure:	Mean ES	95% CI
3.1, All Effects Sizes Included in Part 1		
Ferraro Balota & Connor, 1993 (Very mild AD)	0.55	0.546
Ferraro Balota & Connor, 1993 (Mild AD)	0.25	0.716
Knopman & Nissen,1987 (AD)	0.68	0.537
Knopman, 1991 (AD)	0.96	0.878
Alzheimer's Disease Mean ES	0.57	0.31
Curran, 1997 (Mixed amnesics)	0.65	0.898
Reber & Squire, 1994 (Mixed amnesics)	1.04	0.986
Reber & Squire, 1998 (Mixed amnesics)	0.88	1.248
Stefanova et al, 2000 (ACoA)	1.28	0.558
Mixed Amnesia Mean ES	1.01	0.4
Nissen & Bullemer, 1987 (KS)	2.05	1.398
Nissen, Willingham & Hartman, 1989 (KS)	1.27	1.151
Korsakof's Syndrome Mean ES	1.36	0.86
Limbic System Neuropathology Mean ES	0.78	0.24
Ferraro Balota & Connor, 1993 (PD)	0.71	0.697
Jackson et al 1995 (PD)	0.27	0.843
Pascal-Leone et al, 1993 (PD)	1.42	0.702
Sommer et al, 1999 (PD)	0.36	0.841
Stefanova et al, 2000 (PD)	0.23	0.448
Westwater et al, 1998 (PD)	0.72	0.793
Parkinson's Disease Mean ES	0.54	0.27
Knopman & Nissen, 1991 (HD)	0.55	0.785
Willingham & Korroshetz, 1993 (HD)	0.28	0.8
Huntington's Disease Mean ES	0.39	0.56
Basal Ganglia Neuropathology Mean ES	0.51	0.24
Mutter et al, 1994 (Mild traumatic brain injury)	0.92	0.83
Mutter et al, 1994 (Moderate traumatic brain injury)	0.53	0.81
Traumatic Brain Injury Mean ES	0.66	0.59
Beldarrain et al, 1999 (pFC)	-0.19	0.595
Pascal-Leone et al, 1993 (Cerebellar)	-0.08	0.719
Other Neuropathology Mean ES	0.17	0.36
Overall Mean ES	0.56	0.16
Figure 3.2, Severity of Dementia		
Non-Dementing Subjects	0.58	0.18
Very Mildly Dementing Subjects	0.6	0.4
Mildly Dementing Subjects	0.45	0.38
Overall Mean ES	0.56	0.16
Figure 3.3, Degree of Explicit Sequence Knowledge		
Subjects with No Explicit Sequence Knowledge	0.49	0.25
Subjects with ‘some’ Sequence Knowledge	1.1	0.38
Subjects with ‘good’ Sequence Knowledge	0.39	0.35
Overall Mean ES	0.56	0.16

Table 3A.6. Effect Sizes and 95% Confidence Intervals for All Results Figures in Part 2.

Figure:	Mean ES	95% CI
3.4, All Effects Sizes Included in Part 2		
Ferraro Balota & Connor, 1993 (Very mild AD)	0.22	0.543
Ferraro Balota & Connor, 1993 (Mild AD)	1.26	0.693
Knopman & Nissen,1987 (AD)	0.62	0.672
Knopman, 1991 (AD)	0.58	0.784
Alzheimer's Disease Mean ES	0.6	0.33
Curran, 1997 (Mixed amnesics)	0.16	0.878
Reber & Squire, 1994 (Mixed amnesics)	0.97	0.917
Reber & Squire, 1998 (Mixed amnesics)	0.68	1.102
Stefanova et al, 2000 (ACoA)	0.98	0.533
Mixed Amnesia Mean ES	0.77	0.38
Nissen & Bullemer, 1987 (KS)	0.9	1.101
Nissen, Willingham & Hartman, 1989 (KS)	0.8	1.089
Korsakof's Syndrome Mean ES	0.79	0.77
Limbic System Neuropathology Mean ES	0.68	0.24
Ferraro Balota & Connor, 1993 (PD)	0.73	0.631
Jackson et al 1995 (PD)	1.7	1.003
Pascal-Leone et al, 1993 (PD)	1.05	0.601
Sommer et al, 1999 (PD)	0.16	0.782
Stefanova et al, 2000 (PD)	2.09	0.581
Westwater et al, 1998 (PD)	0.89	0.889
Parkinson's Disease Mean ES	1.1	0.22
Knopman & Nissen, 1991 (HD)	1.32	0.841
Willingham & Korroshetz, 1993 (HD)	1.77	0.941
Huntington's Disease Mean ES	1.47	0.62
Basal Ganglia Neuropathology Mean ES	1.14	0.21
Mutter et al, 1994 (Mild traumatic brain injury)	0.07	0.804
Mutter et al, 1994 (Moderate traumatic brain injury)	1.4	0.898
Traumatic Brain Injury Mean ES	0.66	0.4
Beldarrain et al, 1999 (pFC)	0.65	0.444
Pascal-Leone et al, 1993 (Cerebellar)	2.16	0.767
Other Neuropathology Mean ES	1.43	0.28
Overall Mean ES	1.06	0.14
Figure 3.5, Severity of Dementia		
Non-Dementing Subjects	1.15	0.16
Very Mildly Dementing Subjects	0.55	0.39
Mildly Dementing Subjects	1.1	0.43
Overall Mean ES	0.94	0.16
Figure 3.6, Degree of Explicit Sequence Knowledge : Neuropathological Subjects		
Neuropathological Subjects with No Explicit Sequence Knowledge	1.27	0.21
Neuropathological Subjects with ‘some’ Sequence Knowledge	0.93	0.27
Neuropathological Subjects with ‘good’ Sequence Knowledge	1.39	0.42
Overall Mean ES	0.94	0.16
Figure 3.7, Degree of Explicit Sequence Knowledge : Control Subjects		
Control Subjects with No Explicit Sequence Knowledge	1.05	0.54
Control Subjects with 'Some' Explicit Sequence Knowledge	0.36	0.29
Control Subjects with 'Good' Explicit Sequence Knowledge	0.92	0.26
Overall Mean ES	0.94	0.16
Figure 3.8, Age		
Subjects ≤ 65 years of age	1.2	0.15
Subjects > 65 years of age	0.72	0.27
Overall Mean ES	0.94	0.16

Figure 3.8, Medication Status		
Subjects on Medication During SRT Performance	1.19	0.22
Subjects not on Medication During SRT performance	0.77	0.2
Overall Mean ES	0.94	0.16

Chapter 4

An Introduction to Multiple Memory Systems in Animals

General Introduction

The previous chapter presented a quantitative review of the human SRT literature and concluded that patients with limbic system dysfunction *are* impaired in the SRT. Not only does this directly contradict most SRT studies that use such patients but also the multiple memory systems theory itself. As a consequence there is a need for restricted lesion work in animal subjects to support and expand on the human SRT literature, especially as regards determining the neural substrates for non-declarative sequence learning. This chapter will show that while there are several valid animal analogues of human declarative memory, there is as yet no good animal analogue of human implicit memory and implicit sequence learning in particular. While the performance of animals across memory tasks is dissociable, which implies multiple memory systems, the tasks used to demonstrate non-declarative memory are not comparable / analogous to human non-declarative tasks and therefore the memory system(s) they rely on may not be the same as those used in human non-declarative memory.

This chapter begins by showing that there is good evidence for multiple-memory systems in animals and that these systems appear analogous to the appropriate human memory systems. Next this chapter will present a discussion of memory tasks in animals that are presumed to be analogous to declarative and non-declarative memory tasks in humans and establish that the animal non-declarative tasks in particular are poorly connected to the appropriate human tasks. Finally, it will be concluded that rats are capable of appreciating and responding to serially ordered

information. The ability of rats to respond to serially ordered information suggests that animal subjects could perform a SRT-type task.

4.1 Evidence of Multiple-Memory in Animals

In order to ascertain the likelihood of rats being able to perform a SRT task it is first necessary to demonstrate that animals can demonstrate multiple and dissociable forms of memory. If they can not, then rats are unlikely to be able to show a dissociation between SRT behaviour and allocentric spatial learning (the primary rat-analogue of human declarative memory). It is not necessary at this point, however, to demonstrate that animals have memory systems analogous with human declarative and non-declarative memory, merely that they have independent and dissociable forms of memory.

Multiple Memory in Rats

A large number of studies have demonstrated that rats posses multiple memory systems and there is wide agreement that what memory systems rats do enjoy are broadly similar to human memory systems (Packard and Teather, 1998; Cohen, Poldrak and Eichenbaum, 1997; Gluck, Ermita, Oliver and Myers, 1997; Squire, 1992; Horn, 1991; Kesner, 1990).

The case for multiple memory systems in rats gains strong support from several studies which successfully demonstrate double-dissociations between memory type and lesion site. In an example of this kind of work Packard, Hirsh and White (1989) tested rats with either caudate nucleus or fimbria / fornix lesions on win-shift and win-stay tasks. The win-shift task requires a subject to shift their response strategy to alternative locations based on the presence of the rewarding stimulus in the previous choice and is considered a test of working memory analogous to human declarative memory. In contrast the win-stay task simply requires a subject to learn an approach response to a particular stimulus and to repeat the response within a session, which is comparable to human habit / skill learning, and is considered a test of procedural memory. A clear double-dissociation was found as rats with hippocampal systems damage (fimbria / fornix lesions) were impaired on the win-shift task but not in the win-stay task (they even out performed control rats in this task) whereas caudate lesioned

animals were not impaired in the win-shift task but were impaired in the win-stay task.

Similarly Kesner, Farnsworth and DiMattia (1989) demonstrated a double-dissociation between spatial localisation (egocentric and allocentric) and lesion site (medial prefrontal cortex and parietal cortex). Rats performed both an 'adjacent arm task' in a standard 8-arm radial maze as a test of egocentric spatial memory, and a 117-hole cheese board task that tested allocentric spatial memory. They found that medial pre-frontal cortex lesions impaired the adjacent arm task but not the cheese board task, whereas parietal cortex lesions impaired cheese-board performance but not adjacent arm performance.

A single-dissociation was demonstrated by Packard and White (1990) who found that caudate nucleus lesions impaired rats' performance on a baited / unbaited 8-arm radial maze. Although caudate lesioned rats learned to approach baited arms (the pattern of baited / unbaited arms was held constant across sessions) they were severely impaired in their ability to learn to avoid entering the unbaited set. Furthermore, examination of response patterns ruled out the possibility that rats (both lesioned and controls) were employing an egocentric response pattern. Thus caudate lesioned rats can be characterised as demonstrating a reference memory impairment but not a working memory impairment (Olton and Papas, 1979). However Packard and White go on to note that the working / reference memory distinction is 'incomplete' and instead suggest that the caudate lesion impairment is a consequence of a deficit in stimulus-response (S-R) associations. Specifically, they suggest that while rats with caudate lesions are capable of learning a general S-R association (i.e. radial-maze arms have food at the end of them) they are unable to discriminate between baited and unbaited arms because they can neither strengthen a S-R association (learned pre-lesion) between baited arms and food, nor weaken the association between unbaited arms and a lack of food. However, because the caudate lesioned animals had intact hippocampi they were still able to learn the relationship among stimuli, and in particular the stimulus properties of reinforcers learned before animals received a lesion remained intact. Furthermore, caudate lesioned rats do not repeat within trial errors because they do not have a general working memory impairment. This, therefore, is clear evidence that one form of memory relies of the caudate nucleus whereas another form does not.

In a demonstration of a double-dissociation between memory system and neural substrate in rats, Packard and White (1991) demonstrated a dissociation of task (win-shift and win-stay; per Packard and White, 1989) and dopamine (DA) agonist infusion site (hippocampus and caudate). Rats with DA agonists injected into the hippocampus showed improved retention of a win-shift task, but not a win-stay task, whereas rats with DA agonists injected into the caudate nucleus showed improved acquisition of a win-stay, but not a win-shift, task. As the win-shift task is considered a working (declarative) memory task, and the win-stay a reference (procedural) memory task, this good evidence of dissociable memory systems that rely on independent neural substrates in rats.

In a compelling attempt to dissociate memory and neural lesion, McDonald and White (1993) tested three different lesion sites (fimbria/fornix, dorsal striatum, and lateral amygdala) on three different tasks (win-shift, win-stay, and conditioned cue preference (CCP, a simple stimulus (light) –reward (food) association); being tests of declarative, procedural and reward associative -memory respectively). They found a triple-dissociation between memory task and lesion site. Damage to the hippocampal system (fimbria / fornix lesions) impaired acquisition of the win-shift task but not of either the win-stay or CCP tasks; damage to the basal ganglia (dorsal striatum lesions) impaired acquisition of the win-stay task but not of either the win-shift or CCP tasks; and damage to the lateral amygdala impaired acquisition of the CCP alone. The win-stay deficit produced by the striatal lesions supports Packard and White (1990) above in that McDonald and White conclude that “the dorsal striatum is not necessary for acquisition of a [simple] stimulus-reward association”, but is required for acquisition of a relatively more complex stimulus-response association. These results were also replicated with neurotoxic lesions (cochicine and kanic acid for hippocampal lesions, and *N*-methyl-D-asparate lesions for both amygdala and caudate lesions) instead of the radio-frequency lesions used in the primary study. Replicating the study with neurotoxic lesions serves two functions: it provides further evidence of the validity and reliability of the primary finding, and it increases the specificity of the neural substrate under examination (from gross neuroanatomical region to more specific neural structures). These studies provide clear evidence of dissociable and independent memory systems in rats and substantially strengthens the case for multiple memory systems in animals.

In a series of more recent studies Packard and Teather (1997) provided new pharmacological evidence of dissociation of function between the hippocampus and dorsal striatum by using intra-cerebral injections of 2-Amino-5-Phosphonopentanoic acid (AP5; a glutamatergic NMDA antagonist). Rats performed a both a spatial and a cued task in the Morris water maze. The standard spatial water-maze task, in which rats learn to approach from different directions an escape platform that is always hidden (submerged) in the same quadrant on all trials, is known to be a hippocampal dependent (Morris, Garrud, Rawlins and O'Keefe, 1982) spatial working memory task. By contrast, the cued water-maze task, in which rats learn to approach a visible platform that is moved between quadrants after each trial, is known to be sensitive to caudate lesions (McDonald and White, 1994; Packard and McGaugh, 1992). As predicted rats with intrahippocampal injections of AP5 were impaired on the spatial, but not the cued task. Rats with intracaudate AP5 injections were impaired on the cued, but not the spatial, task. Thus these results are consistent with findings from the radial arm-maze (Packard *et al,* 1989; McDonald and White, 1993), and therefore provide support from another set of behavioural tasks that the hippocampus and dorsal-striatum are responsible for independent memory systems (see also McDonald and White, 1994).

A further specification of the neural architecture responsible for the different types of memory is found in Floresco, Seamans and Phillips (1997) who demonstrated an ability to briefly impair task performance via temporary inactivation of the relevant neural structure. Rats were injected with lidocaine in either the hippocampus, medial prefrontal cortex or nucleus accumbens in order to produce a transient inactivation of the structure. Subjects performed a delayed spatial win-shift radial-maze task and a non-delayed random foraging radial-maze task. Transient inactivation of the hippocampus disrupted performance on both tasks, whereas inactivation of the hippocampus on one side of the brain and the prefrontal cortex on the other side of the brain disrupted the delayed win-shift, but not the undelayed foraging task. In contrast inactivation of the hippocampus on one side of the brain and the nucleus accumbens on the other disrupted foraging behaviour but not delayed win-shift behaviour. Similar results have been found with the Morris water-maze task (Seamans and Phillips, 1994).

In conclusion, there is good evidence that rats demonstrate multiple, dissociable and independent memory systems. In particular that the neural substrates of animal memory systems are similar to human memory substrates in that the extended-hippocampal system is strongly implicated in 'declarative' memory and the basal ganglia is implicated in 'non-declarative' memory, for both animals and humans. This would suggest that modelling human memory behaviour in animals is possible, valid and a useful method for testing various hypotheses that can not easily be examined in humans.

Multiple Memory in Non-Human Primates

The demonstration of multiple memory systems in animals would, however, be especially compelling if a dissociation could also be demonstrated in animals that are more closely related to humans. The following section will provide an account of the demonstration of multiple-memory systems in non-human primates which, much like the memory systems found in rodents, are analogous to human memory systems and thus further strengthen the case that animal memory work can provide valuable insights into human memory. Non-human primates demonstrate evidence of multiple memory systems in much the same way found in rats. For example, Bueger, Gross and Rocha-Miranda (1974) report that caudate putamen lesions impair retention of visual, but not auditory, discriminations or delayed alteration in monkeys. See also: Baxter and Murray, (2001); Murray, Gaffan, and Flint, (1996); Gaffan, (1994); Cohen and Eichenbaum, (1993); Squire, (1992); Gaffan and Harrison (1989); and Mishkin (1978) for similar findings. It is sufficient for the purposes of this section to discuss the detail of a single comprehensive demonstration that non-human primates have multiple memory systems reasonably analogous to those seen in humans.

As part of an attempt to present clear evidence of multiple memory systems in macaque monkeys Gaffan (1994) describes object recognition as the "paradigmatic example of declarative memory in monkeys" and proposes that it depends on the medial temporal lobe in monkeys in much the same way declarative memory in humans depends on analogous structures (but see Aggleton and Brown, 1999). Gaffan also notes that it should be possible to double-dissociate between fornix and perirhinal damage by virtue of the fact that fornix damage produces the least

behavioural impairment of any temporal lobe damage in object recognition tasks and perirhinal cortex damage in particular produces substantially greater object recognition impairments than fornix dysfunction. Gaffan suggests this is because axons of the fornix originate and / or terminate within the medial temporal memory systems whereas “nonfornical neocortical inputs and outputs of the medial temporal memory system are more functionally important than the fornix”. The experiments reported in the study unequivocally support his claims. Lesions to both the perirhinal cortex and the fornix substantially impair post-operative performance of a pre-operatively learnt delayed-matching-to-sample task (i.e. within-session recognition memory), but the perirhinal lesions produced a substantially greater impairment than fornix lesions. In contrast fornix lesions, but not perirhinal cortex lesions, impaired a spatial discrimination task which is considered analogous to human declarative memory tasks. Gaffan then went on to dissociate these structures from the amygdala by demonstrating that neither fornix or perirhinal lesions resulted in an impairment during the acquisition of systematic preferences (for novel foods) whereas amygdala dysfunction severely impaired acquisition. He also noted that difference in effect for the lesions is not simply a matter of differences in quantitative severity as the effects has been doubly-dissociated from each other. The clear conclusion therefore is that the fornix and perirhinal cortex belong to functionally distinct (albeit not strictly independent) memory systems. Furthermore, lesions to the human perirhinal and adjacent cortex produce ‘semantic dementia’ (Hodges, 1993) analogous to the behavioural deficits in monkeys with perirhinal lesions. Thus monkeys not only have multiple memory systems, but these systems are functionally similar to that found in humans.

Not only does the evidence of multiple memory systems in non-human primates enjoy considerable conceptual and functional similarities with human multiple memory systems but there have also been two very recent studies examining the ability of monkeys to perform the SRT task. Procyk, Dominey, Amiez and Joseph (2000) demonstrated that monkeys can learn a SRT task. Two monkeys were trained to perform a SRT task that only differed from the human task by the use of a 9-choice response panel (a 3 x 3 square array) and a short, 4-trial, repeating sequence. One of the monkeys demonstrated both a learning effect during repeating sequence blocks and, more importantly, an interference effect when switched from a repeating to a

random sequence. The second monkey's performance was more problematic. While its reaction times actually slowed over repeating-sequence sessions it nevertheless displayed some evidence of an interference effect as reactions slowed even more markedly when the it was switched to a random sequence. Interestingly the author substituted new repeating-sequences at regular intervals and both monkeys were able to learn each new sequence and respond accordingly. Humans fail to demonstrate any ability to transfer identical abstract structure between different sequences (which requires learning the underlying abstract structure of the sequence) in the SRT and the authors noted that monkeys were also incapable of abstract sequence learning and thus concluded that monkeys show the same kind of SRT learning as humans in an 'implicit' [learning] condition.

Another SRT study with monkeys (Nixon and Passingham, 2000) reports similar results as well as supporting the finding in the meta-analysis that cerebellar subjects are substantially impaired on the SRT. Monkeys with cerebellar lesions were substantially impaired on a (4-trial) SRT task. Although they demonstrated post-surgical retention of a repeating sequence (learned pre-surgically) their reaction times were consistently slower post surgically in comparison to both control monkeys and their own pre-surgical reaction times. Furthermore, they displayed little ability to learn a new repeating sequence post-surgically whereas control monkeys quickly learned the new sequence. Note: because monkeys were not switched to a random sequence after a repeating sequence there was no opportunity for them to demonstrate an interference effect. However, monkey's preoperative response times improved noticeably more when they were switched from random to a repeating sequence (characterised as an 'assistance' effect). Not only did cerebellar monkeys never learn the new sequence to the same degree they had learned the first sequence but they were perpetually slower while performing the new sequence in comparison to their (pre-operative) old sequence behaviour. Although the utility of this study is limited by the fact that the study uses a single dissociation approach and does not employ any interference effect (the primary measure of non-declarative sequence learning in humans), it is still evidence that monkeys can demonstrate a SRT-like learning-effect phenomena and that such performance is dependent on at least one brain structure (the cerebellum) that is also involved in human SRT performance.

While both monkey-SRT studies provide some support that monkeys can perform a SRT-like task in a human-like manner, the Procyk *et al*. (2000) study in particular provides the best evidence of the ability of non-human primates to perform a SRT task (albeit evidence from only one animal). Ultimately however both studies offer evidence that the an animal SRT task would be a valid and useful device for examining the neural substrates of non-declarative sequence learning in humans.

Interim Conclusions on Multiple-Memory Systems in Animals

Although far from a complete discussion of the animal multiple-memory literature the studies cited above provide clear evidence that animals can demonstrate dissociations of memory task and neural substrate. The purpose of this chapter is not to engage in a protracted discussion of the mechanisms and characterisations of the different memory types, and the relevant neural structures in animal subjects, nor is it to attempt to decide which of the competing theories of multiple-memory systems (both animal and human) is the more valid based on the evidence provided. However, it does show that it would be feasible and useful to demonstrate that rats show some type of sequence learning ability. Part of the impetus for this thesis is that there is currently no memory task for animal subjects with good analogical validity with human non-declarative memory tasks, beyond the recent pilot work with monkeys. Therefore, we are forced to rely on indirect evidence, for example that rats are capable of demonstrating learning and memory of a type that is dissociable from another form of learning and memory which is analogous with human declarative memory. All the studies discussed in this chapter so far demonstrate than rats display evidence of a memory system, or systems, that is independent from another memory system which is analogous with human declarative memory. Furthermore, this first memory system is generally reliant on basal ganglia structures as opposed to the extended hippocampus or temporal memory system and thus mimics the basal ganglia / hippocampus dissociation found in humans (Chapters 1, 2 & 3). Therefore, there is good evidence not only that animals are capable of demonstrating multiple-memory systems but that they do so in a manner which is analogous to that of humans. Thus there is considerable justification for exploring the possibility that rats can demonstrate some form of 'non-declarative' memory, and sequence learning in particular. Moreover, the demonstration in the meta-analysis in Chapter 3 that amnesic subjects

show a degree of impairment in the SRT task is a serious theoretical and empirical challenge for the multiple-memory systems theory which needs addressing, and an animal-SRT would be an ideal way to explore the causes of this theoretically unexpected impairment.

4.2 Animal Analogues of Human Memory Tasks

At this stage in the discussion little attempt has been made to marry animal systems directly to human systems, which is required if results of experimental work in animals is to be generalised to human subjects. As noted in Chapter 1 even though there is good conceptual grounds for positing strong similarities between animal and human memory systems (i.e. Reber's principle of commonality, 1992) comparison between different species can be problematic (Sherry and Schacter, 1987). In order to make the extrapolation from animal to human behaviour as valid as possible it is necessary to demonstrate that the structures and processes upon which the comparison relies are as similar as possible in both species. While there are a number of widely accepted animal analogues of human declarative memory there are few valid animal analogues of human non-declarative memory. The following section will discuss a number of both commonly used animal memory tasks in order to demonstrate that while there are good analogues of human declarative memory tasks there are none of human non-declarative memory tasks.

Allocentric Spatial Memory

Allocentric spatial memory is regarded as the primary rat analogue of human declarative memory (Aggleton, Vann and Good, 2000; Aggleton and Brown, 1999; and Gaffan, 1998). Commonly used tasks of this type in animal research are designed to test an animal's spatial memory and include such tasks as radial-maze performance (e.g. Cohen and Eichenbaum, 1993; Colombo, Davis and Volpe, 1989; Dunnett, 1983; Floresco, Seamans and Phillips, 1997; Kesner, Bolland and Dakis, 1993; Squire, 1992; Squire and Zola-Morgan, 1988; Zola-Morgan et al, 1992), water-maze (spatial) learning (e.g. Gallagher and Holland, 1992; McDonald and White, 1994; Packard, Hirsh and White, 1989; and Packard and Teather, 1998), various discrimination tasks (e.g. Eichenbaum *et al*, 1986, 1988; Zola-Morgan and Squire, 1985), and delayed non-matching to sample (e.g. Aggleton, 1985; Gaffan, 1974; Otto and Eichenbaum, 1992; Rafaelle and Olton, 1988; Rothblatt and Kromer, 1991)

For example, the radial arm maze has a long history of use within memory research and studies of this type typically report that rats with hippocampal systems damage show impairments on this task, in much the same way humans with limbic system damage are impaired on analogous spatial tests (Hopkins, Kesner and Goldstein, 1995). However, animal subjects are by no means completely impaired as they learn to run down maze arms to collect rewards and to search among maze arms for reward and therefore are capable of learning the constituent procedures necessary for the task performance. Furthermore, rather than a global behavioural impairment, or even a global memory impairment, rats with hippocampal system damage display a specific behavioural deficit that can be characterised as an inability to learn and / or express the variant / flexible requirements of the task, particularly in terms of spatial working memory (Cohen and Eichenbaum, 1993). Unlike intact rats, hippocampally lesioned rats will continue to make spatial working memory errors within a session by entering previously baited arms that they have already visited within the session. Hence the operational requirement for working memory usually exacerbates their spatial memory deficit (Jarrard, 1995).

Similar conclusions can be drawn from studies employing Morris' (1981) spatial water-maze task. Rats with hippocampal lesions are impaired in contrast to sham-lesion rats as they take longer to escape from the water on to a submerged, and thus unseen, platform at a fixed location. Once again, however, the impairment is not total as hippocampal rats reduce their escape latencies and also demonstrate learning when a single start location is employed (Cohen and Eichenbaum, 1993).

Delayed Non-Match to Sample

Studies employing a delayed non-match-to-sample (DNMS) procedure require subjects to discriminate between two stimulus, one of which they experienced in an earlier sample phase, and are rewarded for attending to the novel stimuli during the test phase. A number of variations on this procedure are commonly used (e.g. 'Y'-mazes) but while some variations of the DMTS task are sensitive to hippocampal damage (i.e. those employing positional cues, Aggleton et. al. 1989; Gaffan, 1974, 1977; Gaffan and Saunders, 1985; Otto and Eichenbaum, 1992; Zola-Morgan and Squire, 1985) others are not (i.e. those using object cues). What is of particular

interest is that subjects with compromised hippocampal systems show no impairment at short delays but as the delay (between sample and test phase) increases these rats are progressively more impaired

This ability to learn and remember task procedures and the presence of a delay-dependent impairment is highly analogous with the performance of human amnesic subjects in recognition memory tests (Cohen and Eichenbaum, 1993) and provides support for both generalising from animals to human, and for multiple memory systems in general. (But see Aggleton and Brown, 1999, who argue that recognition memory depends on familiarity judgements which are not dependent on episodic memory).

Basal Ganglia Dependent Animal Memory Systems.

Although not as widely accepted or as well detailed as the hippocampal studies, there is general agreement that basal-ganglia, and especially the caudate nucleus, are primarily responsible for procedural / reference forms of memory in animals (Packard and Teather, 1998; White, 1997; Kesner, Bolland and Dakis, 1993; Packard and White, 1991; Packard, Hirsh and White, 1989). In consequence, a number of studies have examined the performance of rats with caudate lesions in the radial-maze in an attempt to contrast these findings with those of hippocampal lesion subjects (see above). However, while although these forms of memory are dissociable in rats and although the dissociation mimics the hippocampal / basal ganglia distinction found in humans there is no ready analogue of reference memory tasks in humans.

Furthermore, a strict interpretation of the working / reference memory dissociation predicts that while rats with hippocampal lesions will suffer (spatial) working memory but not reference memory impairments, and rats with basal ganglia dysfunction will show the opposite pattern of impairment / sparing. However, as discussed above rats do sometimes show severe reference memory deficits (McDonald and White, 1990; Packard and White, 1990). But in other tasks (e.g. the water maze) caudate lesions do not impair reference memory (McDonald and White, 1994; Packard and Teather, 1998). Thus reference memory does not clearly dissociate caudate and hippocampal damage. Therefore, there is not only a need for a

valid animal-analogue of a human non-declarative memory task but a need for one that can reliably dissociate caudate and hippocampal injury.

The Analogical Validity of Animal Models of Human Memory tasks.

There is good agreement as to the validity of animal allocentric spatial memory tasks as a model of human declarative memory but there is no similarly well accepted model of human non-declarative memory tasks in animals. It is not that animal subjects are incapable of displaying dissociable memory systems. The studies presented earlier show very clearly that animal memory systems are functionally and neuroanatomically very similar to human memory systems. However, what is lacking is a functional and conceptual analogue of a human non-declarative memory tasks in animals. Although several animal memory tasks have proven to be dissociable from animal analogues of human declarative memory tasks (i.e. allocentric spatial memory tasks), and thus serve to demonstrate dissociable and independent memory systems in animals, these animal memory tasks have no clear connection to human non-declarative memory tasks other than being dissociable from tasks that are conceptually related to human declarative memory tasks. The SRT task appears to be a good candidate as an animal model of one human non-declarative memory task. Also, the evidence suggests that animals are likely to employ the same neuroanatomical structures to perform the SRT as those responsible for human SRT performance. However, the ability of rats to demonstrate sequence learning is not directly supported by the evidence discussed above. Rather it is supported by the general evidence in the literature which demonstrates rats are capable of responding appropriately to serially ordered information even if the serial order of the stimuli contains no sequence information.

Serial Pattern learning in Rats

In order to be able to perform any SRT task animal (rat) subjects would need to be able to respond to serially ordered information. Fortunately there is considerable evidence that they can do so.

The 5-Choice serial reaction time task has been used extensively with rats throughout the last decade as an animal analogue of the continuous attention

performance test in humans (Muir, Fischer and Björklund, 1999; Muir, Bussey, Everitt and Robbins, 1996; Sirviö *et al*, 1993; Carli and Samanin, 1992; Jäkälä *et al*, 1992A&B; Muir, Robbins and Everitt, 1992; Robbins *et al*, 1989; Cole & Robbins, 1987 & 1989; Carli, Robbins, Evenden and Everitt, 1983; and Eysenck, 1982). Primarily used to dissociate neural structure and cognitive task (and often contrasted with spatial memory) it appears at first glance to be all but identical to the human-SRT. However, no 5-choice SRT task that is reported in the literature has ever used a repeating sequence of stimuli. Because it is employed solely as a test of visual attention the stimuli are always presented in a random fashion. Nevertheless, the fact that it requires rats to respond to serially ordered stimuli (albeit randomly ordered) indicates the possibility that they may be capable of learning a repeating sequence.

Other studies (Burns and Dunkman, 2000; Compton, 2001 and 1991; Compton, Bishop and Dietrich, 1996; Fountain, 1990) have also shown that rats can learn serial ordered information including demonstrating anticipation of forthcoming elements of a 3-element sequence. Compton, (2001) trained rats to acquire a three element series of 21, 7, or 0 sucrose pellets, and to associate a particular response cue (texture and brightness of the runway floor) with a particular element size. Rats ran faster (i.e. demonstrated anticipation) in response to cues associated with larger element sizes relative to cues associated with smaller element sizes. Compton compared the ability of neurologically intact control rats, hippocampal or caudate lesioned rats to learn the element size series (stimulus-stimulus associations) and response cue (stimulus-response) associations. While anticipation developed more slowly in both groups of lesioned rats than in intact rats all three groups eventually demonstrated an ability to track the elements of the series. Reversal of the response ('explicit') cues disrupted hippocampal lesioned rats and, to a lesser degree, caudate lesioned rats, but did not disrupt control rat behaviour. Reversing the order in which the element sizes (number of pellets) were presented markedly disrupted anticipatory behaviour in caudate rats, but did not effect hippocampal or control rats.

Thus rats appear to have the ability to perceive and retain serially ordered information and to act on it meaningfully. Therefore, it is likely rats would be capable of learning a repeating sequence in a SRT-like task and thus be capable of showing the desired interference effect when switched to a random sequence.

4.3 Conclusions

Animal subjects at least appear to have comparable memory systems with humans and therefore provide valid targets for memory research that can not be undertaken in humans, or can only be undertaken in humans with considerable difficulty and / or with frequent experimental confounds. The fact that animal subjects demonstrate dissociable memory systems, a memory system analogous to human declarative memory, and an 'other' memory system somewhat analogous to non-declarative human memory behaviour strongly suggests that, should a valid animal-analogue of the human SRT be developed, animal subjects are very likely to exhibit similar phenomena to humans in such a task. Not that animals can be expected to behave precisely the same as humans, but rather that within the constraints of the task and their abilities animal subjects should demonstrate behaviour that is the animal equivalent of human behaviour in such a task.

There is good evidence to suggest that rats can attend and respond to serial ordered information which is a necessary prerequisite for any attempt to model SRT behaviour in animals. Furthermore, based on the evidence that basal ganglia lesions typically impair procedural / reference memory tasks it seems likely that this brain area would also be involved in sequence learning / SRT behaviour in rats in much the same way this area is clearly involved in human sequence learning / SRT behaviour (Chapters 2 and 3). If a valid animal-SRT task could be developed it could be used to examine the neural and neurochemical substrates responsible for human non-declarative sequence learning. It would also provide an opportunity to test the apparent SRT impairment demonstrated in limbic system amnesics in the meta-analysis presented in Chapter 3 and furthermore to contrast limbic system and basal ganglia involvement in the SRT under rigorous experimental conditions, i.e. with better experimental controls, focused and restricted brain injury, and large sample sizes (to ensure adequate power).

As a consequence of the arguments presented in this chapter there is good reason to suspect that rats could perform an animal-analogue of the human SRT task. Therefore, the following chapter (Chapter 5) will present an initial attempt to adapt the

SRT task for use with rodent subjects in order to provide an opportunity to examine this form of memory in animals.

Chapter 5

The Fan-Maze Serial Reaction Time Task

General Introduction

As discussed in Chapter 4, there is currently no valid animal analogue of a human non-declarative memory task. Given the uncertain nature of the neural locus of non-declarative memory in humans and the considerable variation in how different neurological disorders effect non-declarative memory performance (see Chapter 3) there is a clear need for an animal analogue of a human non-declarative memory task. Previous studies have suggested that radial maze tasks employing unbaited arms (reference memory: Packard and White, 1990) or a win-stay procedure (Packard, Hirsh and White, 1989) are representative of non-declarative memory in animals. Unfortunately, it is not clear how those two tasks relate specifically to non-declarative memory tasks used with humans. Another example of a 'non-declarative' animal task that is assumed to be similar to human tasks is Wang *et al's*, 1990. 24-hr concurrent discrimination task. In the context of Wang's task however Squire (1992) noted that this, and perhaps other, examples of 'non-declarative' memory in animals are problematic because while animals appear to be using processes akin to non-declarative memory humans appear to rely more on episodic memory during such tasks.

Chapter 2 explained that the SRT is a widely accepted behavioural task that, in its modified form (i.e. accounting for Shanks and St. John's concerns), is a valid and sensitive test of human non-declarative memory. Chapter 4 provided supporting evidence that rats demonstrate the necessary pre-requisites for SRT performance. Specifically, they display dissociable multiple-memory systems which have considerable points of similarity with human memory systems, and are capable of

recognising and responding to serially ordered information. Therefore, it seems likely that rats will be able to perform a SRT-like task and that such a task will rely on brain structures in the rat that are functionally comparable to those structures that are responsible for SRT performance in humans.

Deficits on the SRT task may be a reflection of an impairment in expressing serially ordered information, rather than a general impairment in learning new associations (Jackson *et al*, 1995). Two recent animal studies that examined the neural substrates of serially-ordered sequences add strong support for the suggestion that the basal ganglia are important in the execution of sequential behaviour. Kermadi, Jurguet, Arzi and Joseph (1993) found neuronal activity in the caudate that appeared to be consistent with the neural coding of serial order sequencing in primates. Aldridge, Berridge, Herman and Zimmer (1993) studied the role of the neostriatum in controlling serially-ordered sequences in rodent grooming behaviour and reported lesions of the neostriatum disrupted grooming sequences but not the individual behavioural elements.

The literature presented in Chapter 4 suggests that animals with limbic system neuropathology would be unimpaired in a SRT task. The results of the meta-analysis in Chapter 3, however, contradicts that assertion. Contrary to expectations the meta-analysis found that human subjects with limbic system neuropathology (LSN) were in fact impaired in the SRT. In particular, once collapsed into a group in order to assure adequate power LSN subjects demonstrate a significantly *weaker* interference effect than control subjects, which is evidence of an SRT impairment. This contradicts the conclusions of the individual LSN SRT studies and threatens the multiple-memory systems theory which relies on the tenant of independent and dissociable memory systems. LSN should not impair SRT behaviour as the limbic system is responsible for declarative and not non-declarative memory. The fact that LSN subjects *are* impaired in a non-declarative memory task, albeit less so than subjects with damage to other neural systems, is thus a serious theoretical and empirical challenge to the multiple memory systems theory. Furthermore, this human LSN SRT impairment reinforces the need for an animal-SRT task to address this important issue as well as then more general one of which specific neural systems are involved in SRT learning.

As demonstrated in Chapters 1 & 2 it is possible to dissociate between different forms of memory in humans and thus the unexpected results of the meta-analysis are more likely due to the complex relationship between declarative and non-declarative memory as expressed in SRT performance (albeit dominated by non-declarative memory), rather than simply due to the inability of the SRT to dissociate the different types of memory. An animal-SRT could well provide an opportunity to ascertain what role limbic system structures play in a non-declarative task that should be independent of limbic system structures. Furthermore, it could well be possible to also to discover the role that brain structures responsible for cognitive functions secondary / auxiliary to memory function (e.g. attention) play in SRT task performance.

Clearly a major problem with the human SRT literature is the uncertainty surrounding the precise location and extent of neural injury in subjects. Even with modern neuroimaging techniques there is still relative uncertainty as to the precise nature of a subject's neural pathology and how it relates to SRT behaviour (see Chapter 2 'Neuroimaging and the SRT'). Therefore, the development of a rat SRT will be particularly useful for evaluating the consequences of restricted and precisely targeted brain injury and in doing so shed light on the neural correlates of human non-declarative memory. It would expected that dorsal caudate lesions in rats would result in a SRT impairment (Jackson *et al*, 1995; Knopman & Nissen, 1991; Willingham, Nissen & Bullemer, 1989; Willingham & Koroshetz, 1990; and Ferraro, Balota & Connor, 1993) whereas hippocampal lesions would not (Nissen & Bullemer, 1989; and Nissen Willingham & Hartman, 1989). Conversely, hippocampal system lesions should impair performance of a spatial working memory test in animals but not performance on the SRT.

After consideration of many standard animal memory tasks (i.e. the radial-maze, the water-maze, and various operant chamber designs) it was initially decided to adapt the radial-maze design to something that could be used in an SRT type procedure. Our original intent was to demonstrate a double dissociation between animal-analogues of human declarative and non-declarative memory tasks *within the same apparatus* so that non-specific (e.g. motivational and locomotor) factors would largely be equal. Thus, we required an apparatus and procedure that were flexible enough to allow for some form of SRT task *and* enable us to generate a radial maze-

like working memory task, which is one of the most accepted animal-analogues of human declarative memory. We designed a novel apparatus called a 'fan-maze' which was essentially a 16-arm sunburst-maze collapsed horizontally such that all the arms were serially adjacent rather than spatially separated. It is interesting to note that the radial maze also had its origins in the sunburst maze (Harley, 1979). The reasoning was that a limited number of arms could be employed for the SRT-type task and the full set of arms employed in some form of spatial memory task.

This chapter describes the development of the 'Fan-maze SRT and will report the initial study designed solely to demonstrate that rats are capable of SRT-type sequence learning from the perspective of demonstrating an interference effect when switched from a repeating to a random sequence.

These initial findings in particular are valuable as they provide a clear indication that rats can learn a basic SRT-like task and demonstrate a robust interference effect. Thus rats' behaviour is conceptually and behaviourally similar to that shown by humans in the human SRT task.

Methodology

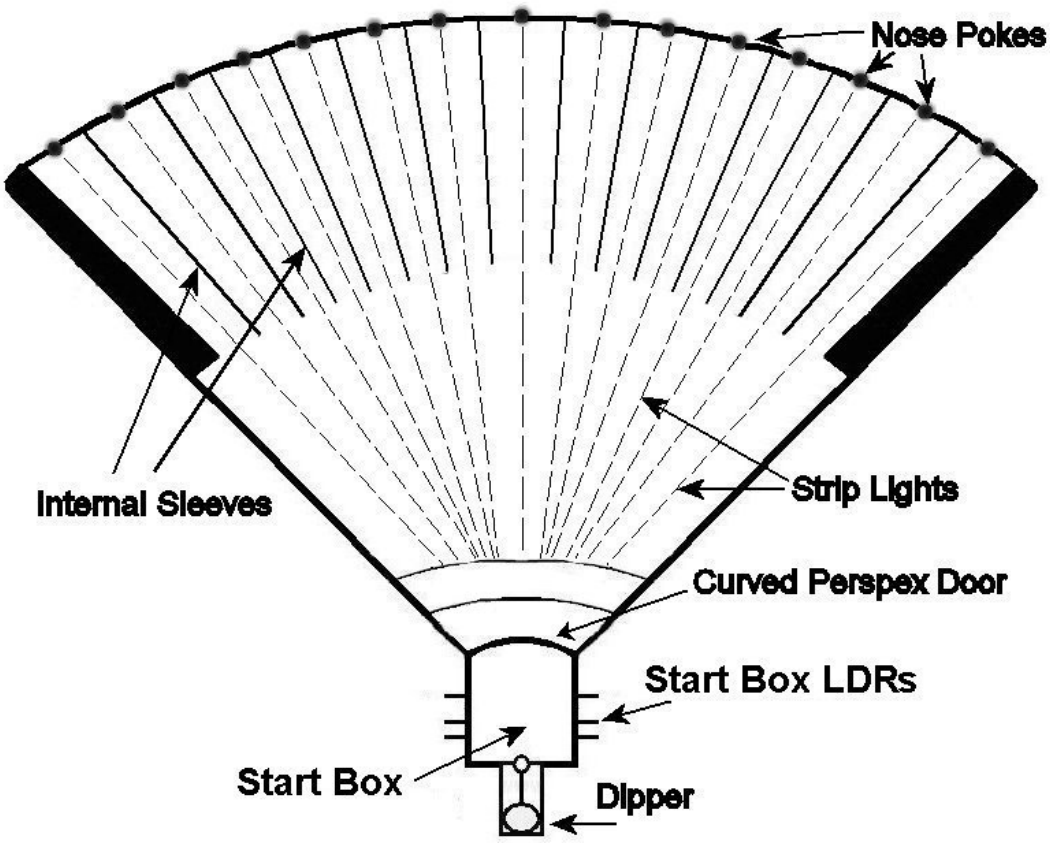
Subjects.

Eleven female 13 month old Wistar rats were individually housed in standard laboratory cages in a constant temperature controlled holding room (21˚C) with an reversed 12 hour light/dark cycle (lights off from 6am). All animals had water available *ad libitum* and were maintained at 80% of free feeding body weight during the course of the study. All studies were reviewed and approved by the Canterbury University Animal Ethics Committee.

Apparatus

The 'Fan-maze' (see Fig. 5.1) was a novel device comprising an aluminium body (85cm from start-box to peripheral wall, 132cm wide at the periphery; 15cm wide at the leading edge) with 15 equispaced nose-pokes around the peripheral wall, each of which could be separately illuminated from behind. The fan-maze was located in a dimly lit room.

Fig 5.1. A Schematic of the 15-hole Fan-Maze



Each nose-poke was a 5cm diameter circle cut into the distal wall of the maze and illuminated by a bright LED mounted behind an opaque Perspex shield / screen. Two infra-red light-detecting-relays (LDRs) were mounted immediately outside the nose-poke housing (but prior to the Perspex shield) to register head entry into the nose-poke. Each nose-poke was centrally located at the end of short arms which were separated by 25cm long, 15cm high, internal aluminium sleeves and covered by a clear Perspex lid (25cm long). Attached to the leading edge of the apparatus was a 'start-box' (15cm high, 15cm wide, 20cm long) with a clear Perspex lid. The start-box was separated from the body of the maze by a curved Perspex door that was computer operated and motor-driven. A hole in the start-box wall distal to the maze allowed access to a remote controlled dipper for reinforcement delivery (0.05ml of condensed milk diluted 1:1 with water). Opaque Perspex was mounted under fifteen 1cm wide by 55cm long strips cut in the aluminium floor of the maze and illuminated

from beneath by 5 serial, equispaced, 28v/40ma bulbs. Each strip light led directly to one of the nose-pokes and started 10cm from the start-box door and terminated 5cm before the appropriate nose-poke. Three infra-red light-detecting-relays (LDRs) were mounted at different heights in the walls of the start-box such that movement away from all three LDRs, after the dipper had returned to its resting (down) position, started the timer. The three LDRs were positioned in such a way as to make it all but impossible for a rat to not break at least one of them while attending to the dipper port. Distal nose-pokes were illuminated individually after a inter-trial interval of 10 seconds during which the Perspex door prevented the rat re-entering the maze but allowed it to view the maze. The onset of the nose-poke light, strip-lights and lowering of the Perspex door were simultaneous such that the rat could observe the lit nose-poke slightly before it could gain access to the maze. A response at the correct (illuminated) nose-poke at the perimeter of the maze turned the timer off and operated the dipper (which remained in position for 5 seconds after the rat broke the start-box LDRs when it re-entered the start-box after making a nose-poke).

Behavioural Testing Procedure

Preliminary training consisted of having the central striplight and nose-poke (#8) lit and allowing the rat to freely explore the maze but only making reinforcement available after a response at this central nose-poke. After all rats had been shaped to shuttle between the dipper and the central nose-poke they were switched to a random sequence of nose-pokes that only ever included 4 positions (left-to-right, #'s 3, 6, 10 & 13; these positions were used for all sequences, repeating and random, in all fan-maze studies thereafter). Rats were run once per day for a single session of 96 massed trials. For each trial a record was kept of the running time (the time taken between leaving the dipper area and making a correct nose-poke; latency data from the occasional error response was not included in the analysis).

For the rat SRT we modified the 4-choice random and repeating sequences used by Knopman & Nissen (1987). The repeating 10-trial sequence employed by Knopman and Nissen in the their SRT task was D-B-C-A-C-B-D-C-B-A, such that the four stimuli were presented unequally. So that the density of reinforcement associated with the different nose-poke locations would be equal we employed a 12-trial sequence: A-D-A-B-C-D-C-A-D-B-C-B (where A=nose-poke #3, B=#6, C=#10, &

D=#13). In our random sequence condition the order in which the nose-pokes were presented had to satisfy several criteria: a nose-poke position never immediately repeated, and during the course of each 12 trials all four nose-poke positions were presented with equal frequency. All sessions, repeating and random, consisted of 96 individual trails. Individual 12-trial random-sequences were combined per Fellows, 1967, at the start of each session to make up a unique set of 96 random trials which was used for all rats during that particular session. Care was taken to ensure that neither the repeating sequence nor any significant (4-trial) portion of it was included in the random sequences. Due to the novel apparatus and procedure we thought it sufficient at this stage only to replicate the procedure used by most of the human literature and control for frequency of stimulus presentation within the random sequences, not for any other simple frequency information between repeating and random sequences (see Chapter 2). The intent was to demonstrate that rats could learn *something* while performing the SRT task in order to show that the task was a valid analogue of the human SRT. It was intended that the presence / absence of different simple frequency information in the repeating and random sequences would be manipulated later to demonstrate that the rats were capable of learning actual sequence information. For example, humans will learn actual sequence information when all other statistical frequency information is held constant between repeating and random sequences (see Reed and Johnson, 1994).

Random sequences (R) and repeating sequences (S) were imposed using three sets of random sequence sessions interspersed with two sets of repeating sequence sessions (RSRSR). The rats were first trained on random sequences (R1) and once all subjects had developed stable running times (nine sessions) they were then switched on the next day to repeating sequence sessions (six daily sessions; S1) before returning to the random sessions (two daily sessions; R2). Four days after the end of the R2 set of sessions subjects were returned to the maze for another six repeating sequences sessions (S2) before finally being switched immediately to a final set of five random sequence sessions (R3)

Results

The running time latency for each trial was log-transformed per Knopman and

Nissen (1987) to achieve homogeneity of variance in these scores and was used in all analyses. The other measures recorded during performance was an error measure of the percentage number of correct trials throughout a session. The primary measure of sequence-learning is the deterioration in behaviour (interference effect) when switching from a repeating-sequence to a random-sequence and difference scores were computed for each subject for each measure and averaged across the group. However, as the experimental design also included switches from a random-sequence to a repeating-sequence it is possible that subjects might demonstrate a behavioural advantage as a result of the switch. This advantage might be evident even when first switching to a novel repeating-sequence but is more likely when switching back to a previously encountered repeating-sequence. Thus we also analysed the data to test for any improvement in behaviour (labelled as an 'assistance' effect) when switching from a random-sequence to a repeating-sequence.

General analysis of behaviour across a single set of sessions was undertaken via repeated-measures one-way MANOVAs (using the ANOVA/MANOVA module in Statistica, StatSoft Inc. Tulsa, USA) for each measure (Log-RT, percent correct, and light / orientation difference). Interference effect analyses compared the last two sessions (192 trials) of the repeating sequence to the first two sessions (192 trials) of the random sequence. Assistance effect analyses compared the last two sessions (192 trials) of the random sequence to the first two sessions (192 trials) of the repeating sequence Thus both interference and assistance effect analyses were a: two (sequence condition) by two (session) MANOVA, with repeated measures on both factors.

General Analysis of log reaction time

Each block of sessions of any given type was analysed by separate repeated-measures one-way MANOVAs to test for any effects (e.g. learning) within any of the five blocks of session types. As shown in Fig. 5.2 both the first random and the first repeating sequence sessions (R1 & S1) had significant reductions in reaction times over sessions (R1, $F(8,80)=27.3$, $p<0.0001$; and S1, $F(5,50)=3.55$, $p<0.01$). However, neither the second block of random sequences (R2) nor the second block of repeating sequences (S2) produced significant effects ($F's<1$). The last block of sessions (R3) also produced evidence of a significant session effect ($F(6,54)=3.88$, $p<0.0025$) but

this was due to a significant *increase* in reaction time across sessions.

Fig 5.2, Log-Reaction Time Scores Across Sessions (error bars ± 1 SEM).

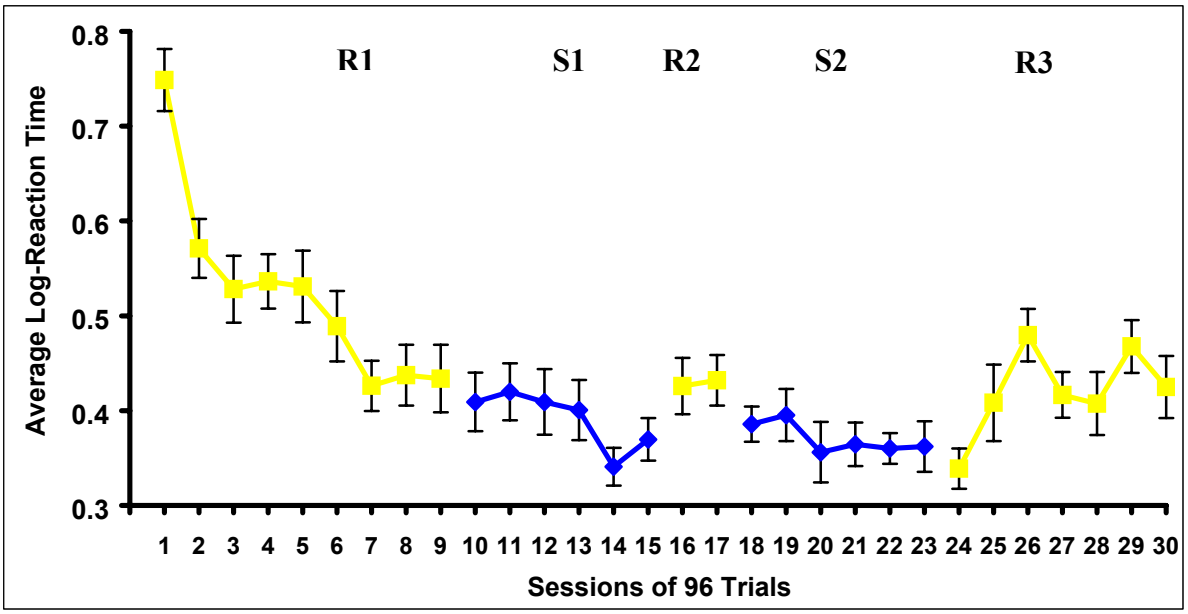


Fig. 5.2 also shows that there is an overall effect of sequence type. Although reaction times were generally stable at the end of the first block of sessions ('R1', random sequences only) there is an indication of a decrease in reaction times once subjects are switched to a block of repeating sequence sessions ('S1'). Once switched back to random sequence sessions (R2) reaction times deteriorated back to the same level as at the end of the first random-sequence block, strongly suggesting that subjects had used some form of sequence knowledge to improve performance during the repeating-sequence sessions. Furthermore, when switched back to the repeating-sequence sessions (S2) reaction times immediately improved to the same point as during the first repeating-sequence block. It is also clear that the last four sessions of the second repeating-sequence block (sessions 20-23) are both stable and substantially faster than any random-sequence blocks to that point. Although the mean reaction time then unexpectedly improved again immediately after switching back to the random-sequence sessions (session #24) the general trend for this third block of random-sequence sessions (R3) was for reaction times to be more consistent with those of the previous two random-sequence blocks (R1 & R2), i.e. slower than during repeating-sequence sessions.

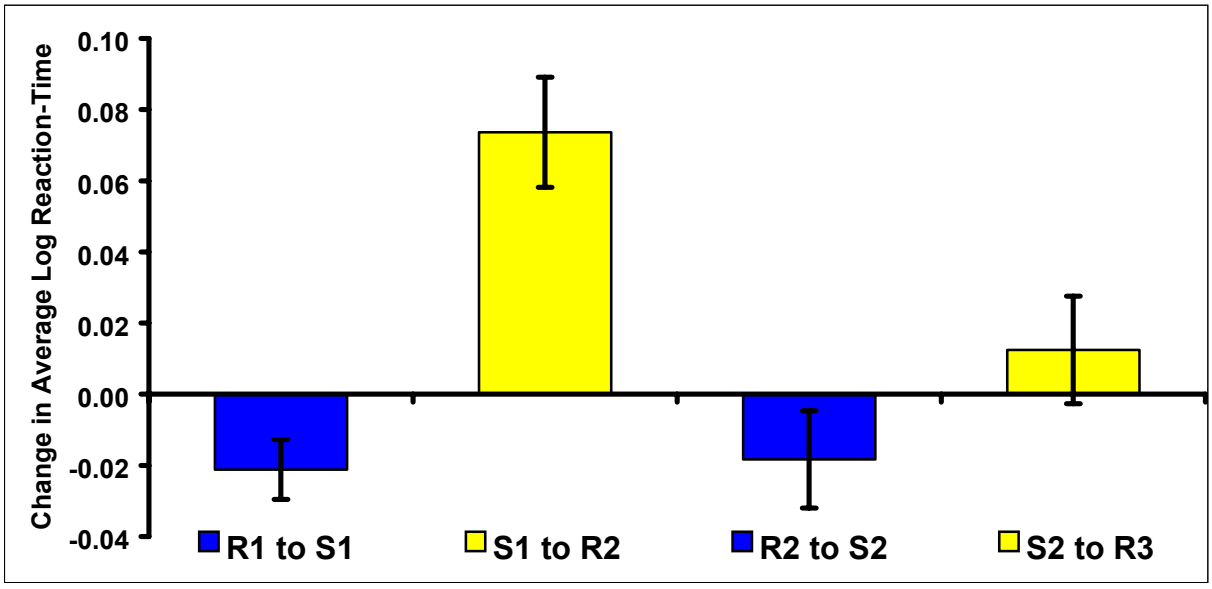
Interference and Assistance Analysis of log reaction time

Individual switches from / to a repeating / random sequence were analysed via 2 x 2 MANOVAs (sequence condition by session number) with repeating measures on

both factors. The interest here is the degree to which behaviour changes as a function of sequence condition and thus interference / assistance effects are displayed in terms of the difference in behaviour between one sequence condition and the other.

The first sequence-switch analysis was for the switch from the initial set of random sessions (R1) to the first set of repeating sessions (S1; an 'assistance' effect). As shown by the difference score shown by the first bar in Fig. 5.3 ('R1 to S1') the switch to a repeating sequence provided an immediate advantage as reaction times were significantly faster during the two repeating sessions compared to the preceding two random sessions ($F(1,10)=6.29$, $p<0.05$, see Figs. 5.2 & 5.3).

Fig 5.3, Assistance and interference effects for the log reaction time measure when switching to / from a block of random sequence sessions (error bars ± 1 SEM).



The next switch (S1 to R2) was a conventional interference effect. While there was no main effect or session or an interaction effect (F's<1), the effect of this critical manipulation (S1 vs. R2) was clearly confirmed ($F(1,10)=22.67$, $p<0.0001$; Fig. 5.3). Reaction times slowed significantly when subjects were switched from a repeating to a random sequence, which in the human SRT is considered clear evidence of sequence learning.

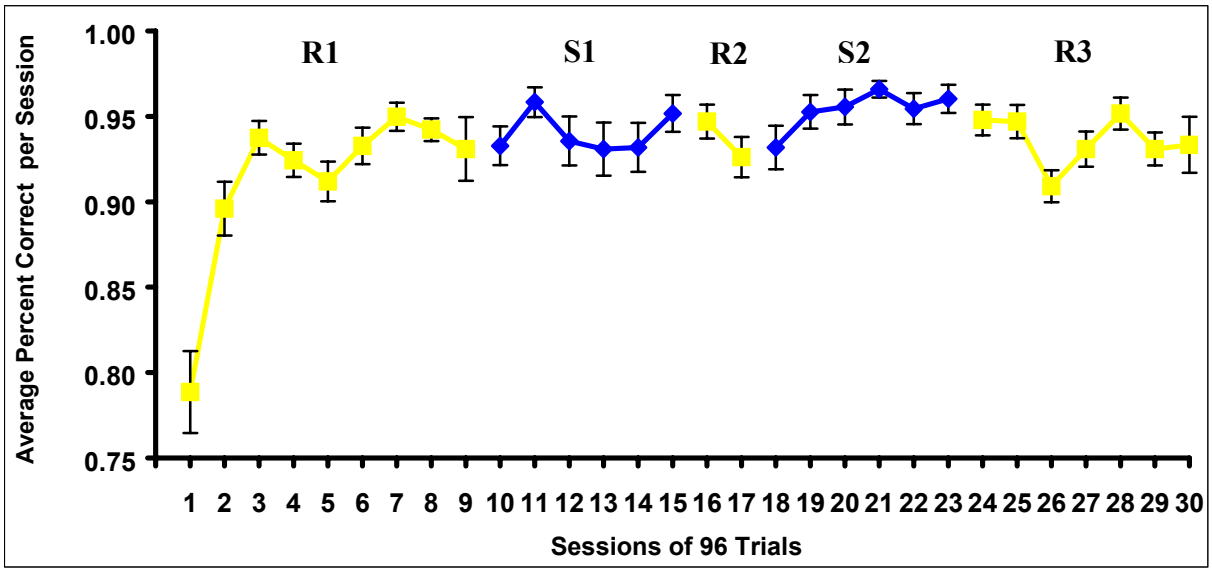
The next switch (R2 to S2) was another 'assistance' effect. The effect of switching from two blocks of random sequence sessions (R2) to two blocks of repeating sequence sessions (S2; same sequence as S1) was also significant ($F(1,10)=5.92$, $p<0.05$) while session and interaction effects were again absent (F's <1.0). Thus, as in the first random to repeating switch rats demonstrated an immediate

advantage when changing to a repeating sequence.

The final switch (S2 to R3) was a conventional interference effect but this switch did not produce any effects (main effect of sequence condition, F<1; main effect of session, F(1,10)=1.54, NS; interaction effect, F(1,10)=1.14, NS). Thus subjects did not repeat the interference effect that they had demonstrated when first switched from repeating to a random sequence. However, examination of Fig. 5.2 clearly shows that, other than the first session, times for the third block of random sequence sessions (R3) were generally slower than that of the (immediately preceding) second block of repeating sequence sessions (S2). Examination of individual subject performance during the first random sequence session of R3 (session #24) reveals no anomalies or atypical behaviour patterns and while the unusually fast reaction times (for a random sequence block) in this session explains the lack of an interference effect we cannot explain why subjects reacted so quickly to random stimuli during this session.

General Analysis of the Percentage of Correct Trials per Session

Fig 5.4, Percentage number of correct trials per session across all sessions (error bars ± 1 SEM).

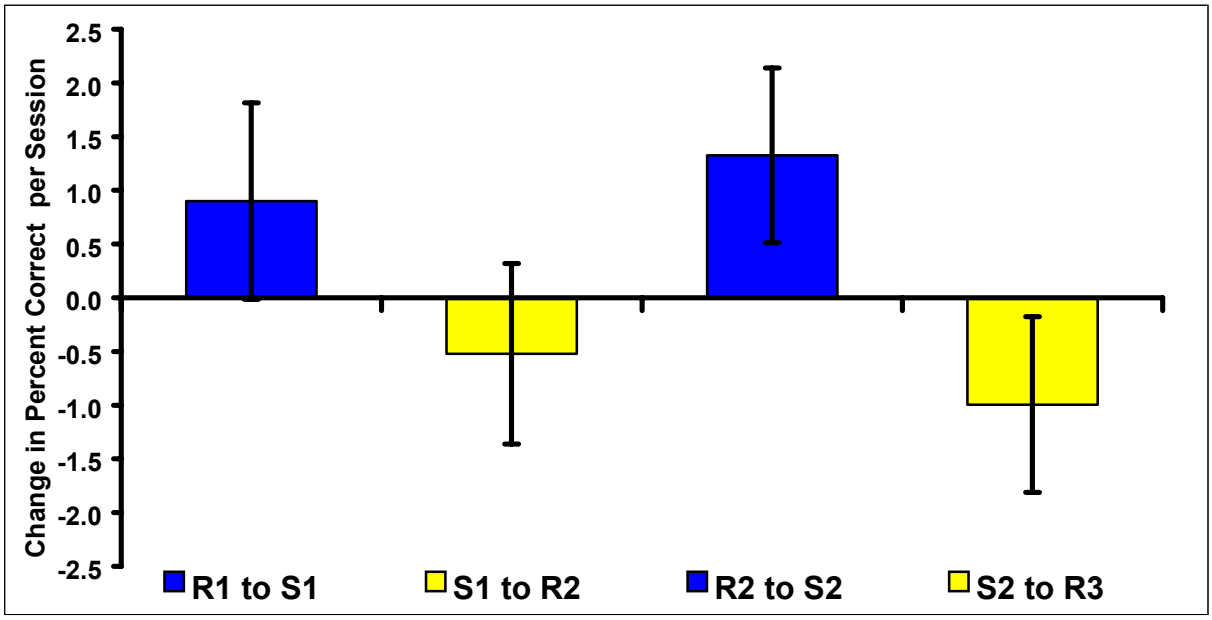


With the exception of the first block of (random) sessions (R1) there is little evidence of any systematic variation between sequence type for this measure (see Fig. 5.4). A one-way repeated-measures MANOVA of the first block of random sequences (R1) was significant (F(8,80)=4.2, p<0.0005) indicating that subjects made fewer errors across sessions. However, separate one-way repeated-measures

MANOVAs for the first block of repeating sequence sessions (S1), the second block of random sequence sessions (R2), and the second block of repeating sequence sessions (S2) were not significant although the latter block of sessions approached significance (S1, $F(5,50)=1.7$, NS; R2, $F(1,10)=1.13$, NS; and S2, $F(5,50)=2.13$, $p=0.078$). Thus there was no systematic change in error rate across these three middle blocks of sessions. However, a one-way repeated-measures MANOVA of the last block of random sequence sessions (R3) was significant ($F(6,54)=2.44$, $p<0.05$) but in this case it is due to subjects making *more* errors during some sessions in this block.

Interference and Assistance Analysis of the Percentage of Correct Trials per Session

Fig 5.5, Assistance and interference effects for the percentage number of correct trials per session when switching to / from a block of random sequence sessions (error bars ± 1 SEM).



As in the previous assistance / interference effect analysis this analysis consisted of 2 x 2 (sequence condition by session) repeated-measures MANOVAs for each switch. Throughout all switches for this (error) measure all main effects of sequence condition and session were not significant (all F's<1, see Fig. 5.4). Thus at no point did the subjects demonstrate either assistance or interference effects when switching from a random to a repeating or a repeating to a random block of sequences (respectively) for this measure. Although the interaction effect for the first (assistance effect) switch (R1 to S1, Fig. 5.5) was not significant ($F(1,10)=2.13$, NS) the interaction for the next (interference effect; S1 to R2) switch was significant ($F(1,10)=9.09$,

p<0.025). This effect is likely due to error rates improving during the last two repeating sequence sessions of S1 but worsening during the two random sequence sessions of R2 (see blocks 14 & 15 and 16 17 in Fig. 5.4). The interaction effect for the second assistance effect switch (R2 to S2) just failed to reach significance ($F(1,10)=4.27$, $p=0.06$) and the interaction for the final (interference effect) switch (S2 to R3) was not significant ($F<1$).

General Discussion

Although not an unqualified success the study produced favourable evidence that rats can learn a 12-trial repeating sequence in a SRT-like task. The log reaction time data in particular strongly suggests that rats were capable of demonstrating SRT sequence learning similar to human SRT behaviour. Rats demonstrated a log reaction time interference effect when first switched from a repeating to a random sequence in exactly the same manner that humans do. Although the second repeating-to-random switch was not significant due to unusually fast reaction times in the first random sequence session (session #24; Fig. 5.2) it is clear that for the remainder of this block of random sequence sessions reaction times were substantially higher than during the preceding block of repeating sequence sessions. In general rats reacted faster during repeating sequence sessions than during random sequence sessions. Thus rats also demonstrated learning effects consistent with those found in humans.

The evidence from the other behavioural measure is less clear. Although the pattern of behaviour as measured by the percent-correct measure was consistent with the sequence switches (i.e. behaviour deteriorated when switched from a random sequence to a repeating sequence session, and improved when switched from a random to a repeating sequence session) none of the interference / assistance effects were significant. Nevertheless, some initial percent-correct learning effects were consistent with human SRT behaviour, but as indicated elsewhere (Chapter 2) such changes are far less convincing than interference effects.

The evidence of sequence learning from the reaction time measure must be tempered by the acknowledgement that statistical structure was not held constant between repeating and random sequences per the discussion on sequence structure

in Chapter 2. Specifically that frequency information (e.g. transition frequency), other than simple stimulus frequency, was not constrained across sequence types. Thus any evidence of sequence learning (i.e. interference, assistance or learning effects) may be due to the rats learning the simple frequency differences between the repeating and random sequences and not due to learning the repeating sequence (Jackson and Jackson, 1995; Reed and Johnson, 1994; Curran, 1997; and Stadler and Neely, 1997). Nonetheless, the fact that the rats demonstrated some form of sequence learning is sufficient at this point to conclude they learned something in a manner analogous to that of humans.

Although the primary aim of this study was to develop an animal-analogue of the human SRT task it will be apparent that the fan-maze SRT task has some conceptual and behavioural differences from the human SRT task.

The sensorimotor demands of the animal and human SRT tasks are very different. Firstly, while humans makes simple key presses rats have to travel almost a full meter before making a response, and then travel back again to collect their reward. Secondly, because the strip-lights extended so close to the entrance point into the maze from the start-box, and these strip-lights were a perfect cue for nose-poke identification, rats might be simply adopting a 'follow the light' strategy. Thus rats may not have suffered as strong a behavioural disadvantage when responding to random sequences than they might otherwise have done had they not been able to employ this simply strategy. In fact under these conditions it is the sequential nature (or otherwise) of *strip light* presentation rather than nose poke presentation that provides the operant cues necessary for faster responding. The fact that the nose poke associated with the strip light was also lit might be irrelevant from the rat's point of view, as it can instead rely on a far more immediate cue. Thus, the point at which the rat must decide how to respond may occur sooner after entering the maze than desired, which substantially minimises the behavioural differences associated with the different sequence types as measured within the maze. It may even be that the point at which a rat 'responds' is actually prior to entering the maze (i.e. having sighted the lit strip light while still inside the start-box, or very shortly after entering the maze) and therefore any real difference in behaviour occurs prior to any behavioural measurement taking place. In light of these concerns and the consequent decision to

completely redesign the animal-SRT task the development of a spatial memory task in the fan-maze was abandoned.

Furthermore, the open nature of the fan-maze and its spatial environment permit the use of spatial cue / strategies to aid in accurately predicting the next stimulus location. Given that human SRT is a non-declarative memory task, and that spatial learning is the primary animal-analogue of human *declarative* memory tasks, this confound substantially reduces the analogical validity of the fan-maze SRT task.

However, these issues notwithstanding there was a strong suggestion that rats can learn repeating sequences and respond to changes in sequence information in a similar manner to that of humans in the SRT. In light of this conclusion, and the methodological and procedural issues discussed above, the following points and recommendations are made in respect of future attempts to develop an animal analogue of the human SRT.

- ❖ As discussed in the introduction to this chapter the primary concern with current examples of procedural memory tasks in animals is the lack of contact these tasks make with human non-declarative memory tasks. Therefore, rat-SRT procedures should conceptually and behaviourally resemble human SRT procedures as closely as possible. Firstly, animal subjects should perform the task within as few sessions as possible. Secondly, any switch from one sequence condition to another should take place *within* a session, and preferably towards the end of the session, as is done in human studies. Thirdly, all random sequences should be more closely constrained such that the only statistical and / or frequency information that is allowed to vary between the random and repeating sequences is the actual repeating sequence. Fourthly, the apparatus and procedure should encourage the rat to perform the task as quickly as possible with the minimal amount of gross motor behaviour.

- ❖ The relatively large surface area of the fan-maze and the obvious environmental cues introduce an unavoidable spatial component into the task. Due to the fact that we were attempting to model a non-spatial memory task it is preferable therefore that rat-SRT performance is not dependent on /

confounded by spatial strategies, in the same way human SRT is a not dependent on spatial factors. Thus a more compact apparatus minimising extra-maze spatial cues during performance (which would also limit locomotion) is preferable.

In light of the general finding in this chapter that rats display SRT-behaviour in a similar fashion to human SRT behaviour (irrespective of how they do so) and the points concerning the procedural and conceptual limitations of the rat-SRT task the next chapter (Chapter 6) will present the initial study of a second new animal SRT task. It is hoped that the nascent ability of rats to perform an animal-SRT analogue demonstrated in the studies above will be more readily observed in a study which substantially increases the points of similarity between the animal and human SRT. Demonstrating that rats can perform the SRT under more rigorous conditions would provide far stronger evidence that the rat-SRT is a valid animal-analogue of the human SRT.

Chapter 6

The Intracranial Self-Stimulation Serial Reaction Time Task: A Control Group Study

Introduction

A Second Rat SRT TASK

The fan-maze design / protocol was a reasonably successful initial design (see Chapter 5). However, the points of similarity between the rat SRT task and the human task were relatively limited and the fan-maze task was prone to procedural problems and technical difficulties. The primary measure of SRT performance, the interference effect, is a systematic measure of behavioural variation. Thus anything that acts to produce unwanted behavioural variation increases the risk of losing the effect of the sequence manipulation in behavioural 'noise', and the fan-maze appeared particularly prone to behavioural issues that increased such variation. The possibility that rats could employ spatially based strategies to perform the task also weakens the task's validity as an animal-analogue of a human *non-declarative* memory task. Mechanical failures and the shuttling requirement also suggested the need for a batter task.

In light of these concerns it was decided to abandon the fan-maze in favour of a more restricted environment which would allow us to employ a procedure that has several advantages. The new procedure is methodologically more similar to the human-SRT and is expected to highlight differences between repeating and random sequence behaviour. This chapter will begin with a discussion of the arguments for

these changes before reporting the results of a control study that was undertaken to test the new equipment, the new procedures, and discern the optimal parameters for generating an interference effect in rats. It will be clear from the results that the changes were successful.

The purpose of developing the rat-SRT was to model human SRT behaviour in rats in order to test assumptions and theories about human non-declarative sequence learning. Although there was some evidence that rats performed the fan-maze SRT in a similar fashion to how humans perform the SRT there were obvious differences between the task requirements for the human SRT and the fan-maze SRT. Five main points of dissimilarity were identified between the human and fan-maze SRT. These were: the delivery of a food reward for every trial, a much lower number of trials per session, a far greater number of sessions overall, switching between sequence types occurred between sessions not within sessions, and there were a number of behavioural differences in terms of how the SRT task was performed in the fan-maze (i.e. locomotor responding in a maze as opposed to the brief motor requirements of simply pressing a button). Each of these points is discussed briefly below.

While unavoidable the fact that animal subjects need to be rewarded reliably and regularly in order to continue behaving introduces a behavioural artefact into SRT performance that is not present in the human task. Of particular concern is the fact that while the human SRT is performed in a continuous fashion during which separate trials follow on quickly and smoothly, trials in the fan-maze SRT are performed in a more discrete fashion by virtue of the rat deliberately stopping to gain the reward and in doing so interrupting SRT performance. A number of alternatives were mooted such as increasing the number of trials per reward (into ‘chunks’) but while this might well improve within chunk behaviour it would still require the division of a session into relatively many chunks. We even thought it possible to increase the number of trials per reward to the total number of repeating-sequence trials (e.g. 12 trials) and in this manner ensure the subject quickly and accurately completed a full sequence before being rewarded. Unfortunately, this doesn’t defeat the chunking problem, it only reduces the number of chunks experienced by the animal. More problematically reward at the end of a sequence encourages recognition of the presence of a repeating sequence and even of the sequence itself, in direct contrast to the human

SRT and the desired aim of the protocol. We also considered employing a variable reinforcement schedule to encourage subjects to run quickly, but again this just redistributes the chunking and also does not eliminate the need to halt behaviour to gain the reward and may even interfere with sequence learning by forcing the animal to experience / organise stimuli in a variable fashion (see Frensch, Buchner and Lin 1994; Jacoby et al, 1989; Stadler, 1995; and Stadler, 1993, in Chapter 2). It was apparent that as long as a subject was rewarded in a discrete and regular fashion it would be impossible to avoid reward delivery interfering with SRT behaviour.

As a function of the food-reward system, and especially satiation effects, it was not possible for rats to perform anywhere near as many trials per session as human subjects do. Consequently rats performed both far fewer trials per sessions and far more sessions overall than human subjects. Obviously the main concern with this procedure is that the rats did not have the same opportunity to continuously learn the repeating sequence as humans do. The rats had experienced fewer trials within a session and their sessions were far further apart than in human studies (days as opposed to minutes). This problem is further compounded by the problem that rats continually experienced interrupted performance during exposure to the repeating-sequence due to constantly stopping to obtain food reward.

Another difference between the human and fan-maze SRT tasks is that switching between sequence types in the fan-maze was done between sessions in contrast to switching within a session in the human work. Given that the sequence switch is critical to demonstrating non-declarative sequence learning it is clearly desirable to maximise such an effect. Hence it is more desirable to switch sequence types during continuous responding rather than at the beginning of a new session. Switching between sessions denies an important point of similarity between the rat and human versions and switching from a repeating to a random sequence within a session could be expected to increase the ability of animal subjects in the SRT to demonstrate a strong interference effect.

Rats in the fan-maze are required to run almost a full meter to register a response before having to run back again to receive their food reward whereas humans respond in the SRT task by simply pressing a button. This also decreases

behavioural similarities between the two tasks. The relatively long response time in the case of rats also substantially increases behavioural variability within the fan-maze task. Given that a stated aim of the fan-maze SRT was to model *as closely as possible* the human-SRT task the profound difference in the mechanics of the response behaviour is a concern.

When considering the source of these problems in the fan-maze SRT it quickly became apparent there were three main causes: reward delivery characteristics, response mechanics and the opportunity to employ spatial strategies. The latter two of these causes were not only relatively easy to address but could be solved in concert. To minimise the requirement for locomotion and increase the procedural points of similarity between the animal and human tasks we developed a far smaller apparatus which, by virtue of its small size, could be placed in an environment with minimal spatial cues. Allowing subjects to respond simply and quickly with a minimum of motor behaviour reduced the influence of behavioural artefacts produced by the response mechanics in the fan-maze (e.g. fatigue from repetitive, medium-distance, locomotion). Eliminating any spatial cues greatly reduced the possibility of animals using (allocentric) spatial memory to perform the task and thus encouraged the use of behavioural strategies based on non-declarative memory.

The new technique we used employed a task in which rats nose-poking one-of-four holes directly in front of the rat (see Fig. 6.1 below). This new task reduced behavioural artefacts by minimising the amount of motor response required to make a response. A head turn and a relatively shallow nose-poke was all that was required. It thus more closely approximated the button-pressing response of the human-SRT in terms of the nature of the response, especially the ability to quickly move from one stimulus response position to another with minimal motor behaviour.

The Intracranial Self Stimulation

The other primary cause of problems in the fan-maze, the reward delivery system, was less easily overcome. While the food restricting procedure has a long and useful history in animal behaviour experiments any experimenter who has used this procedure will readily acknowledge it is not without difficulties and drawbacks. No matter how carefully their weight is monitored (subjects in the fan-maze SRT studies

were weighed daily) a degree of variability in body weight may occur, which is likely to affect behavioural motivation. When combined with the necessity of interrupting behaviour in order to deliver a reward (be it one per trial or whatever) the problem of varying motivation, both between and within sessions, introduces substantial opportunity for behavioural variation.

It was this concern led us to consider employing intracranial self-stimulation (ICSS) as a reward delivery system because (among other reasons discussed below) it has an extremely quick onset and very short duration (e.g. 2msec and 500msec, respectively) and thus results in minimal behavioural interruption during task performance. On examination it seemed possible that ICSS might be ideally suited for addressing the concerns identified with the fan-maze as it not only is it minimally disruptive but it is also extremely salient and can be designed to ensure a constant level of reinforcement within a subject. Furthermore, by virtue of setting the degree of simulation a rat receives to a fixed proportion of the current that produces maximal response rates it is also possible to ensure a constant level of reinforcement *between* subjects (see “Rate-Response Function” in the methods section below). Furthermore, ICSS is not subject to satiation (Gallistel, 1964; Gallistel *et al*, 1974; Waraczynski *et* al, 1987)

Olds and Milner (1954) first discovered that very mild electrical stimulation of certain brain areas was rewarding, so much so that if stimulation was made contingent on behaviour, e.g. bar pressing, a rat would respond rapidly and repeatedly for long periods of time (i.e. hours). Since then a considerable body of work has examined the neural and procedural characteristics of ICSS (see the “Parametric Analysis of Brain Stimulation Reward in the Rat” series of articles, Gallistel et. al. 1974, for a detailed review) such that it is now possible to detail a number of the characteristics of the protocol. 1) It is largely a function of electrical stimulation of one of the two main dopamine (DA) reward pathways, either the mesolimbic or nigrostriatal pathways (Fiorino, et. al 1993; Gallistel et. al, 1981; McCown et. al. 1986; Nicolaysen et. al 1988; Phillips and Fibiger, 1978; Ranaldi and Beninger, 1994; Stephens and Herberg, 1977). 2) The relationships between a variety of stimulation parameters (pulse-frequency, current strength, train-duration, pulse style, etc.) can be empirically quantified. For example, Gallistel et. al (1981) showed that the ‘required’ strength (current value) of a stimulation train (a series of pulses) is a hyperbolic function of train

duration (see also Edmonds et. al. 1974; Edmonds and Gallistel, 1974; Gallistel, 1964, 1974 and 1978; Leon and Gallistel, 1992; Stellar and Gallistel, 1975; Mazur *et al,* 1987; and Waraczynski et. al. 1987). And 3) It has a number of idiosyncratic features. It does not suffer from satiation, and (given appropriate stimulation parameters) is extremely reinforcing and any behavioural task learnt via ICSS reward is however prone to rapid extinction. Any simple parametric manipulation can render a previously rewarding stimulation aversive and vice-versa.

A pilot study was first performed with a few animals to examine the feasibility of ICSS in the modified rat-SRT (the data not presented). Rats demonstrated reliable interference effects when switching from repeating to random sequences while receiving ICSS protocol in the new SRT apparatus. For these reasons it was decided to undertake a formal control study designed to ascertain the optimal parameters for reliable SRT behaviour in rats.

Experimental Design

The purpose of the study presented in this chapter is to manipulate experimental conditions in order to determine the optimal parameters for generating an interference effect in rats. Given that the interference effect is the primary measure of SRT performance establishing the optimal parameters for generating interference effects in rats will therefore provide the optimal parameters for studying rat-SRT behaviour in general.

It was hypothesised that spaced learning (i.e. experiencing the repeating sequence over separate sessions) might accentuate any interference effect. It was decided to compare the effect of performing the SRT over three sessions with that of performing it within a single session. Given that humans perform 500 trials in a typical SRT study it was decided to roughly double this figure for use with rats. The figure of 940 trials per session was settled for ease of use with different sequence lengths (see Chapter 7). Consequentially individual triple condition sessions were 940 trials long. Hence the single session was 2820 trials long, and in this way all rats experienced the same number of total trials irrespective of number of sessions (i.e. three sessions x 940 trials per session = 2820 trials in total).

In order to demonstrate that any interference effect produced by switching from a repeating to a random sequence was due to the random sequence information interfering with prior representations of sequence information a strong experimental test would be to show that either random sequences alone and / or repeating sequences alone (i.e. without any switch) do not produce a similar effect. Thus three sequence conditions were used within each of the two number of sessions conditions: random-only, repeating-only, and repeating sequences with a switch to random sequences. Rats that only ever experienced random sequences should display a modest improvement in behaviour over sessions, due simply to a practice effect with the motor requirements of the task, but no interference effect. However, repeating-only rats should also fail to show any interference effect as they never actually switch to random sequences.

Thus the experimental design consisted of three factors: sequence type (random or repeating), number of sessions to complete the total of 2820 trials (one or three), and presence or absence of a switch from a repeating to random sequences. However, the design was incomplete in that not all possible combinations of these factors were used. In particular the triple-session condition used a modified ‘repeating only‘ condition. Rather than just receive repeating information across all three sessions, as a strict application of experimental conditions would demand, it was decided to switch this group to a random sequence at the end of their third, and last, session. Doing this provided a direct comparison with the single-session repeating plus switch-to-random condition, i.e. an examination of the effect of switching to random sequences once only at the end of the overall exposure to repeating sequences, irrespective of how many sessions the rat had experienced the repeating sequences over.

However, unlike the repeating-to-random group in the single session condition the repeating-to-random group in the triple session condition was switched from repeating to random sequences at the end of all sessions. Thus it differs from all other groups in that it experienced multiple repeating to random sequence switches.

Due to the use of a novel apparatus and untested reward delivery system in this experiment it was decided to employ a shorter, 4-trial, repeating sequence in

order to ensure sequence learning. A pilot study (not reported) using the SRT chamber, the ICSS reward system, and a 4-trial repeating sequence demonstrated that rats could learn that repeating sequence under these conditions. Hence it was decided to retain this sequence length in the current study in order to ensure rats would be capable of demonstrating the optimal parameters for sequence learning. A parametric analysis of rats' abilities to learn longer sequence lengths is presented in the next chapter.

The purpose of this study was to maximise rat SRT performance in order to establish that the rat-SRT is a robust and sensitive test capable of demonstrating any impairment or sparing of SRT performance as a consequence of damage to different neural structures. For this reason the rats in the condition that generated the strongest interference effect, and the respective random-only control group, went on to constitute the control groups for animals with various lesions (see Chapter 7). Although the control and lesion studies are presented separately for purposes of clarity (this chapter and Chapter 7, respectively), they were actually undertaken in a partially overlapping fashion and conducted in exactly the same fashion.

Method

Rats.

57 Norwegian hooded rats were used aged between 120 & 150 days at the time of electrode implantation for intracranial self-stimulation. Rats were housed individually with food & water available *ad libitum* and trained / tested during the dark portion of a reversed 12hr light / dark cycle. The change in rat strain from Chapter 5 was brought about through colony changes imposed on the facility.

Surgery: ICSS Electrode Implantation:

Subjects were treated 20 minutes pre-operatively with 0.18mg/kg of atropine sulphate (concentration=0.13mg/ml, dose=0.18mg/kg; Phoenix Pharmaceutical Distributors, Auckland, New Zealand) before being anaesthetised with 100mg/kg of sodium pentobarbital (concentration=50mg/ml, dose=100mg/kg). Twisted wire ICSS bipolar electrodes (Plastics One Inc. model MS303/1) were cut to length (1.3cm) and had a small amount (~1mm) of insulation removed from the tip. The electrode tips

were separated by ~1mm before being implanted into the medial forebrain bundle at the level of the lateral hypothalamus. Flat skull stereotaxic coordinates adapted from the Paxinos and Watson (1986) atlas were: from Bregma, A/P –2.6mm, M/L ±1.8mm, and from dura, -8.6mm ventral. Electrodes were held in place by a dental acrylic head-cap that was fastened to the skull with 4 small screws. All subjects received one electrode implanted into either the left or right hemisphere (balanced across rats). Subjects were left for seven days after surgery before familiarisation training began.

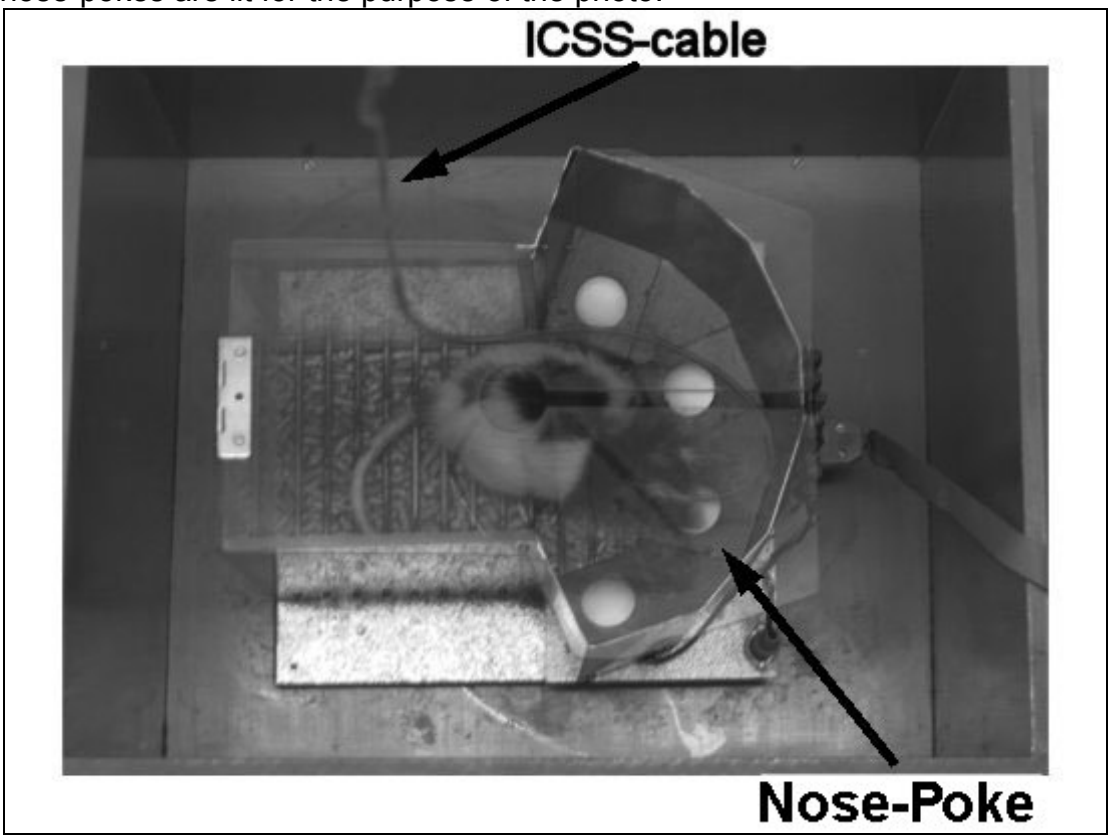
Apparatus

The novel ICSS-SRT chamber (Fig. 6.1) had an open-ended rectangular aluminium body, 20cm long by 15cm wide by 15cm high, with a Perspex lid and a brass-rod floor, underneath which was a shallow tray containing bedding material. The open end of the rectangle was fastened to a slightly wider (27cm) semi-circular aluminium wall which has a small ledge (5cm wide) mounted at an angle of 15º between the floor and the wall. The ledge holds four equispaced nose-pokes (3cm diameter, 7cm apart from centre to centre) and designed to be easily reached from a central position in the chamber. The aluminium wall was painted with a non-conducting paint (Dulux Paint, Auckland, New Zealand; 'HammerFinish Grey') to prevent interference from the electronics associated with the nose-pokes. The Perspex lid included a channel and hole to allow the ICSS cable easy access to the chamber. The ICSS cable ran from a 2-channel commutator (Plastics One, model SL2C) fastened to an aluminium arm, that projected to a point 40cm directly above the centre of the chamber down to the subject. The ICSS-SRT chamber was placed within the SRT-training box (see below) to minimise spatial cues. The chamber was controlled by an IBM-PC running Med Associates (St. Albans, Vermont) WinMPC operant software connected to a MED-PC computer interface, which also operated the stimulator.

The Stimulator: A Lafayette Instruments Co. (Lafayette, Indiana) model 82408 sine wave stimulator was used. The stimulator was capable of delivering from 0.01µa to 10µa with a train duration between 0.1 and 11 seconds in either a bipolar or monopolar fashion at a fixed pulse frequency of 50Hz. Current strength variation can be achieved in three ranges (0.01µa to 0.1µa, 0.1µa to 1.0µa, 1.0µa to 10µa) by a variable-resistance relay that was calibrated to an accuracy of 1% of the range

maximum. For this reason any current variation was performed in steps of at least 5%. While actual stimulation strength varied according to individual subjects (see below) stimulation was always bipolar, and the train duration was always 0.5 seconds. Note: no subject used in the experiments reported in this thesis required stimulation above 0.5μa.

Fig 6.1, Photograph of the ICSS-SRT chamber inside the ICSS-Training box, all nose-pokes are lit for the purpose of the photo.



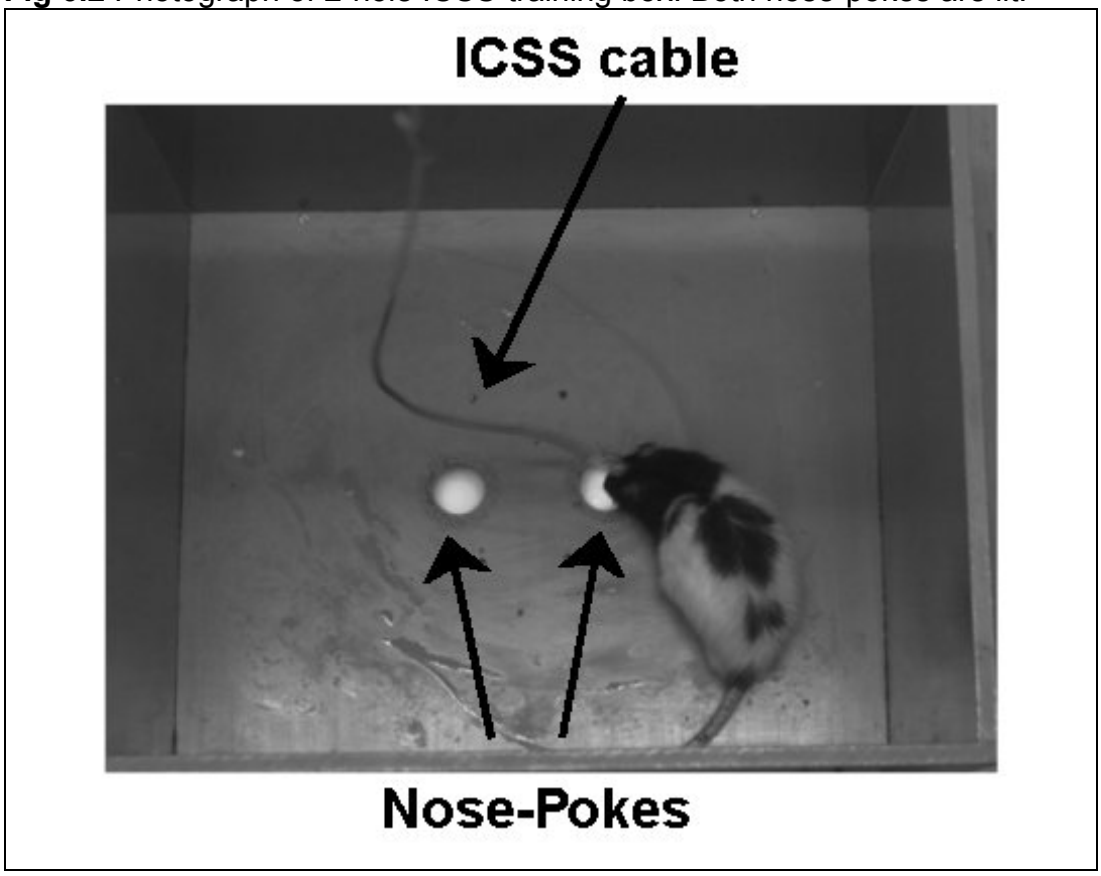
ICSS-Training Box: Initial nose-poke training was conducted in a painted wooden box, 40cm wide by 50cm long and 30cm high, with two nose-pokes (3.5cm diameter) mounted in the floor in the centre of the box, 6.5cm apart (centre to centre; see Fig. 6.2). The nose-pokes were identical to those used in the fan-maze and were also controlled by the MED-PC computer interface. The arm holding the commutator used to deliver ICSS current to the ICSS-SRT chamber was permanently attached to the side of this box and arced up to place the commutator directly over the centre. The box was placed in a corner of a dimly lit room where subjects could not see anything of the room except the ceiling which was featureless. The arm that held the commutator was also mounted with an infra-red camera (Henry's Electronics, Paddington, London; model 208IR B/W board camera) which was connected to a video monitor in the adjacent control room that allowed real-time viewing of behaviour

(in this box and the ICSS-SRT chamber).

Behavioural Procedures

Subjects were initially placed in the ICSS-training box and trained to nose-poke in return for ICSS reward per nose-poke.

Fig 6.2 Photograph of 2-hole ICSS-training box. Both nose-pokes are lit.



Familiarisation: During the first two sessions rats were free to explore the ICSS-training box for 20 minutes, without the ICSS cable attached or any nose-poke lit / active. The second session was identical except the ICSS cable was attached but no current was supplied.

Initial Nose-Poke Training: Initial training sessions in the ICSS-training box involved shaping the subjects to nose-poke at either of the nose-pokes (both were lit throughout the session). An initial current value was selected by stimulating (in 0.5sec. bursts) with very low current when the rat was adjacent to one of the nose-pokes and observing the subject as the current was gradually increased. Once a subject began to display any behavioural response the current was lowered back to the value

immediately prior to this and this value used for the rest of these initial training sessions to shape the nose-poke response. Training continued until the rats were able to quickly and reliably nose-poke either hole, which typically took between three and five sessions. Each session lasted for 20 minutes or 300 trials, whichever came first.

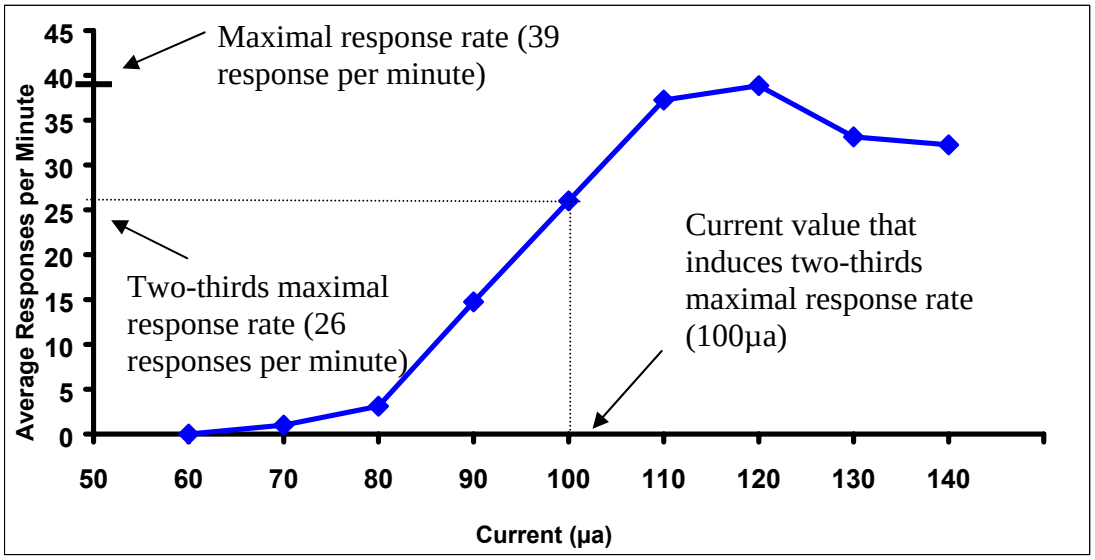
Rate-Response Function: During the next two sessions rate-response functions were generated (see Fig. 6.3) during which current value was systematically altered and the number of responses per minute recorded for each current value. Both holes in the ICSS-training box were lit and a nose-poke in either produced stimulation. The current value used in initial training sessions was the starting point and was incremented / decremented by 0.005μa or 0.01μa as appropriate to the 0.0μa to 0.1μa and 0.1μa to 1.0μa current ranges (respectively) used in the initial nose-poke training.

In the first of these sessions the current value was first gradually decreased until the subject stopped responding, and then gradually increased to beyond the initial starting (i.e. to increase nose-poke response rates) until the response-rate remained stable over five current steps, before being decreased gradually to the starting point again. In this way two response rates were generated at each current value, during the decreasing phases and during the increasing phase of the rate-response function. The second session repeated this process but in the reverse order, the current was firstly gradually increased from the initial value but then decreased to the point the animal stopped responding and then increased again to the initial value. All response rates for a particular current value were averaged and a response-rate plotted (Fig. 6.3). The two-thirds maximal response value was calculated (the current value at which the subject would respond at two-thirds of its maximal response rate) and this value was used for that subject throughout the remainder of the study. Thus an important factor was that all subjects experienced an identical relative stimulation value (66%) and therefore we were able to equate the motivational value of the reinforcer between subjects, something that is practically impossible with food reward.

Discrimination Training: In subsequent sessions only one of the two ICSS-training box holes were lit and only a nose-poke to the lit hole produced stimulation. Immediately after a successful nose-poke the light was turned off and an inter-trial interval (ITI) of 500msec. elapsed before the next hole was lit. Holes were lit in an

entirely random fashion so that the rat had no way of predicting where the light would appear next and had to learn to distinguish between lit and unlit holes. Each discrimination training session lasted for 20 minutes or 300 trials, whichever came first. Once subjects were able to discriminate quickly and accurately (typically between two and three sessions) they were moved onto the ICSS-SRT chamber familiarisation.

Fig. 6.3 Example of a Rate Response Function (values averaged over trials)



ICSS-SRT Familiarisation: Like the previous familiarisation sessions in the ICSS-training box familiarisation to the ICSS-SRT chamber involved two separate sessions. During the first session subjects were placed in an inactive ICSS-SRT chamber (which was inside the ICSS-training box) for 20 minutes without the ICSS cable being attached and left to explore. The second session was identical except the ICSS cable was attached but no current was passed through it.

ICSS-SRT Training, Part 1: Initial training in the ICSS-SRT chamber involved all four holes remaining lit and a nose-poke to any hole resulted in stimulation. Each session lasted for 10 minutes or 200 trials, whichever came first, and rats typically only required one such session.

ICSS-SRT Training, Part 2: Part 2 was identical to Part 1 (all holes were lit at the start of each session / cycle) except that as a hole was nose-poked it was extinguished and any further nose-poke to it did not result in stimulation. Once all four holes had been nose-poked (regardless of order) they were reset (relit) and the cycle began again. Sessions lasted for 20 minutes or 200 trials, whichever came first, and rats typically

became proficient at this within two or three sessions.

SRT Training: Rats were exposed to 100-quasi random trials and had to complete the session within 10 minutes in order to advance onto the SRT proper. A unique 100-trial quasi-random sequence was generated per the rules detailed below and was used for each subject during this session only. After successfully completing this phase subjects were returned to their home cage and did not perform the SRT proper until the next day.

SRT Testing.

All rats underwent a total of 2820 trials in the ICSS-SRT chamber over one or three sessions. Rats in single-session groups completed the 2820 trials within one continuous session, whereas those rats in the triple-session condition completed 2820 trials over three sessions on adjacent days, performing 940 continuous trials per session.

Rats were further divided into three groups within each session-length condition. The purpose was to compare rats that experienced: only random sequences with those that only repeating sequences and with those that experience repeating sequences with a random sequence switch towards the end of the session. In addition the triple-session 'repeating-only' group (see above) received repeating sequences only during sessions one and two but received random sequences at the end of the third session.

Single-Session Conditions.

Group 1: Random-to-Random (no switch). These rats only ever experienced random sequence information throughout the 2820-trial session.

Group2: Repeating-to-Repeating only (no switch). These rats only ever experienced the repeating sequence throughout the 2820-trial session.

Group 3: Repeating-to-Random (switch). These rats received 2580 repeating sequence trials before being switched (uncued) to 240 random trials.

Triple-Session Conditions.

Group 4: Random-to-Random (no switch). These rats only ever experienced random sequences for 940 trials per session during each of the three sessions.

Group 5: Repeating-to-Repeating (plus a switch to random in the last session). This group experienced 940 trials of the repeating sequences in their first two sessions but in the third session received 860 repeating-sequence trials before being switched (uncued) to 80 trials of the random sequence.

Group 6: Repeating-to-Random (switch). This group always experienced 860 trails of the repeating sequence before being switched (uncued) to 80 trials of the random sequence at the end of each of the three sessions.

Group Numbers

Group numbers are presented in Table 6.1 below. Each group initially had eight rats but two groups (Groups 1 and 3) went on to be included in the lesion study (presented in Chapter 7) and had animals added to bring their group size up to 12 per group. These additional animals are included in this study as their SRT procedure was identical to that of other rats in their respective groups.

Table 6.1, Final Group Sizes

	Single-Session Conditions			**Triple-Session Conditions**			
Group Number	**1**	**2**	**3**	**4**	**5**	**6**	**Total**
Number of Rats	13	8	12	8	8	8	57

Repeating and Random Sequences

It was hoped that this short sequence would promote sequence learning in what was still a very novel and largely untested design. If rats could demonstrate a reliable ability to learn this short sequence under these conditions it would then be possible to examine their ability to learn longer sequences. Therefore, the repeating sequence was a novel 4-trial sequence; 3-1-4-2.

An important methodological feature was that random sequences were generated per Johnson and Reed (1994) to ensure that all simple frequency information was identical between random and repeating sequences. Thus such simple frequency information as transition probability and 'reversals' (see 'The Effect of Sequence Structure on SRT Performance' in Chapter 2) did not differ between the random and repeating sequences, ensuring the only difference between sequence types was the presence or absence of the repeating sequence. Four-trial sets of random sequences were combined per Fellows (1967) to create a unique 480-trial block of random sequences and the block examined to ensure it contained no three or four trial sequences identical to those found in the repeating sequence. This 480-trial block of random trials was used for all subjects throughout this part of the study after all sequence to random transitions. In order to minimise any possible learning of these random trials the starting position within the block was randomly varied for each subject (that experienced a switch to random sequences) for each session.

Behavioural Measures

Behaviour was measured by (log transformed) reaction times and error rates. Reaction times were quantified as from the onset of the stimulus (light) to the correct hole being nose-poked (error trials were not discarded). An error was any nose-poke to an unlit hole.

Note: Due to a programming error reaction time data stopped recording during the SRT session for a few animals (two in Group 2 and one in Group 3). However error data recorded normally for these animals and their experience in the task was identical to other animals in their group (i.e. stimulus presentation was not effected). Thus while these rats are not included in the reaction time analysis they are included in the error-rate analysis and hence the degrees-of-freedom values are not identical between the analyses of the two measures. The fault was corrected and all data generated prior to this was checked to ensure reaction times had been recorded properly throughout the entire session.

Histology

Rats were sacrificed with an overdose of sodium pentobarbitone before being perfused with 4% formalin and their brains were removed and fixed in 4% formalin.

Brains were transferred to a long-term-solution (4% sucrose) before being sliced on a freezing microtome at 50μm and stained with cresyl-violet to locate ICSS-electrode tips.

Results

In order to allow comparison between the various session length and sequence type conditions, sessions were divided to produce 36 blocks of trials. In the single-day condition (2820 trials) this involved averaging 20 trials for the first block of trials and 80 trials for all subsequent blocks. In the three-day condition the first block of trials for each session was averaged over 60 trials and all subsequent blocks (for that session) were averaged over 80 trials. This meant that random sequence interference blocks were always averaged over 80 trials regardless of session condition and were thus directly comparable between conditions.

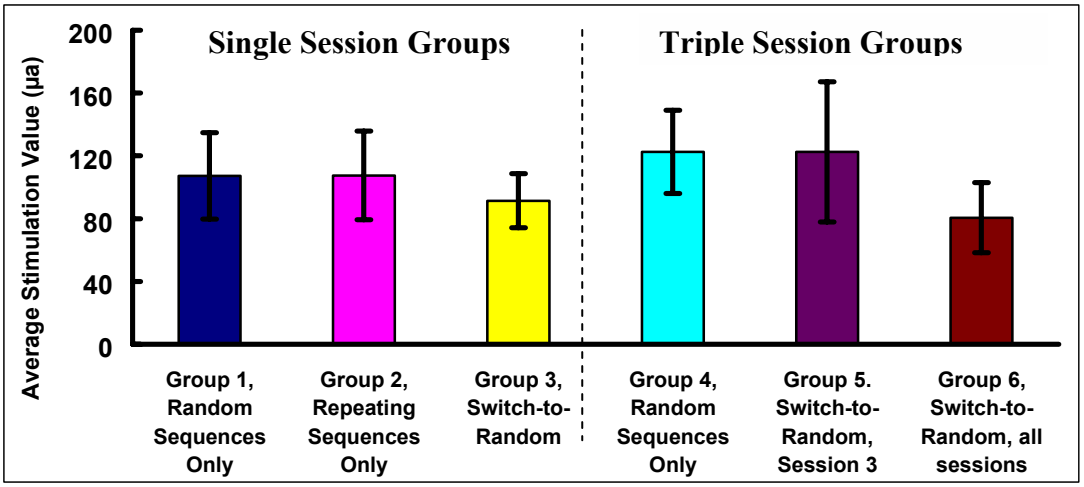
Histology

Placement of electrode tips is indicated in Fig. 6A.1 in the appendix to this chapter. All tips were within the medial forebrain bundle and placements were relatively consistent between groups.

Stimulation Values

A one-way ANOVA comparing the stimulation value used for each rat during SRT sessions across groups was not significant ($F<1$), see Fig 6.4.

Fig. 6.4, Average Stimulation Values (μa) per Group Used in SRT Sessions (Error bars ± 1 SEM).

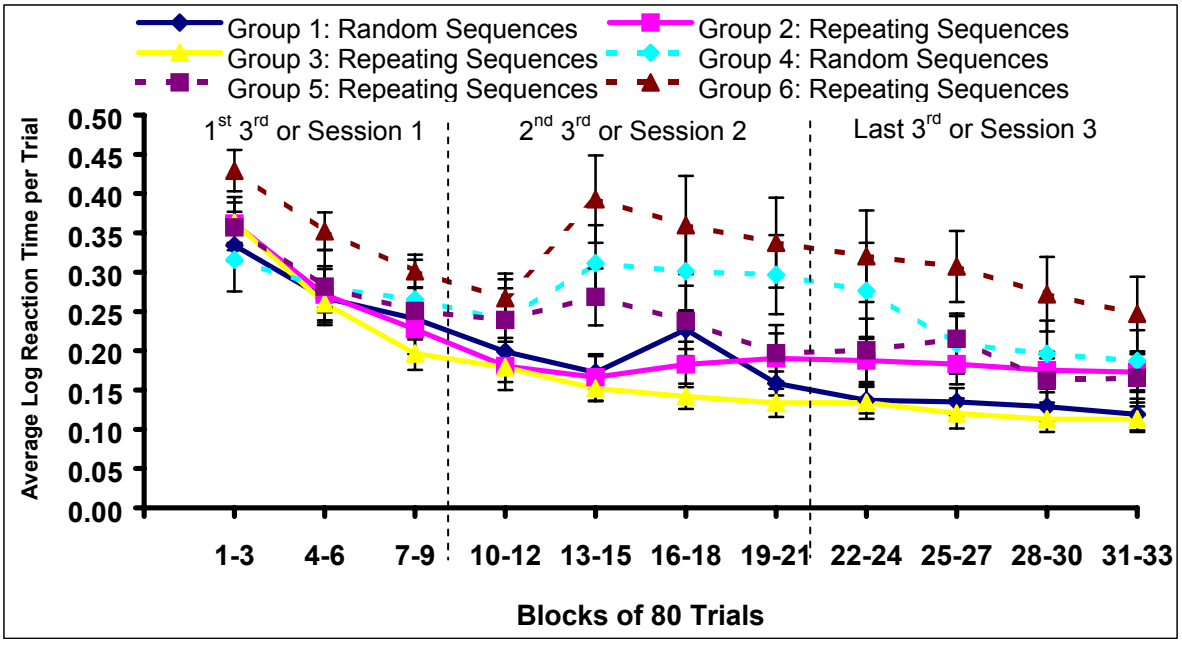


Log-Reaction Time Measure

General Analysis

Analysis was performed on an equal number of trials prior to the point when any interference effect would be introduced (if appropriate to that group). Thus the first 33 'non-interference' blocks (60 trials in the first block, 80 trials per block thereafter) of the total 36-blocks for the single-session conditions were analysed. The triple session conditions each generated one block of 60 trials and 11 blocks of 80 trials per session for a total of 12 blocks per session. The 12th and last block of each session contained 'interference data' as it was at the start of this block that a switch from repeating to random sequences took place (if appropriate to condition). Hence the 12th block from each session was removed and analysed separately and the remaining blocks were collapsed together over sessions to form a continuous set of 33 blocks. See Fig. 6.5.

Fig 6.5, Average Log-Reaction Time per Trial across Non-Interference Blocks (Error bars ± 1 SEM). 'Thirds' refer to the portion of single sessions only. Session numbers are relevant to the triple session conditions only. Session 3 only includes three blocks because the fourth block is the interference effect block (if appropriate to group condition) which is reported separately.



Due to the unequal number of conditions within variables (group type, number of days

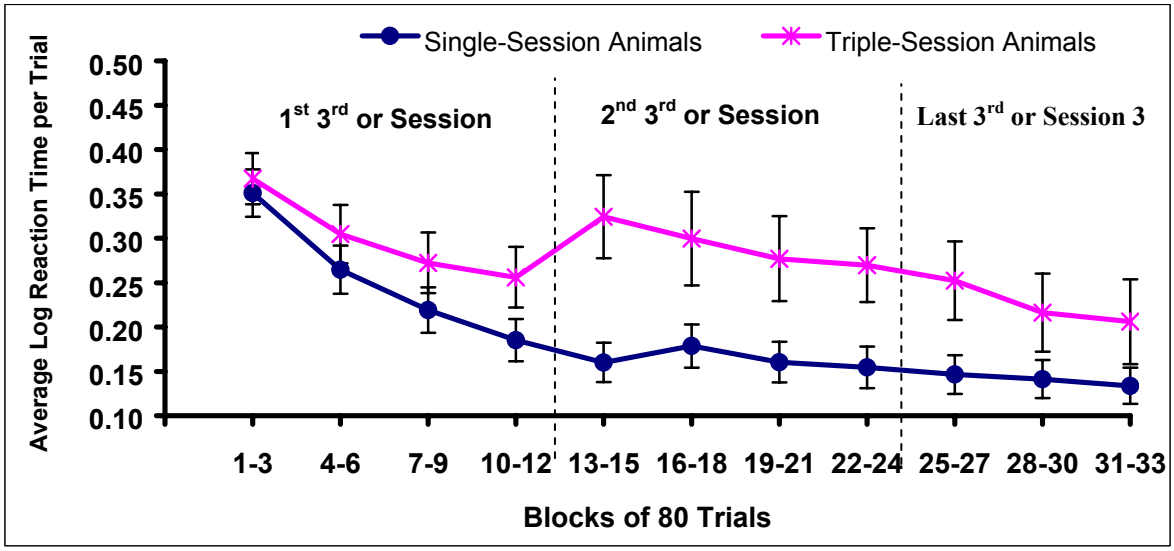
to complete, and sequence type) it was impossible to compare group and either number of days or sequence-type within the same analysis. For example, while there was a single-session, random-only, no-switch-to-random condition (i.e. Group 1) there was no single-session, random-only, switch-to-random condition. Such a condition is nonsensical in terms of the experimental design as to have such a condition would require switching from the random sequence to, either, a 'different' random sequence (which is impossible anyway given that the random sequence was deliberately made identical to the repeating sequence in all ways except for the actual repeating sequence and it is therefore impossible to have a 'different' random sequence) or a repeating sequence. Thus learning effects (across the 33 blocks) were analysed in three separate MANOVAs testing individual variable (number of sessions, sequence type and presence / absence of interference switch) by block effects, with repeated measures on blocks.

Group Analysis: The initial analysis of reaction time data compared the behaviour of the six groups, irrespective of number of sessions and sequence-type conditions, across blocks and was thus a 6 (group) x 33 (block) MANOVA with repeated measure for blocks. This analysis produced a non significant group effect ($F(5,49)=1.32$, NS) but a significant block effect ($F(32,1568)=20.8$, $p<0.00001$) and a significant group x block interaction ($F(160,1568)=1.32$, $p<0. 01$). Thus while groups were not different prior to any sequence switch all rats improved (responded faster) over blocks irrespective of number of sessions and sequence type. The interaction effect most likely stems from the that triple session groups tend to be slower than single session groups *and* groups 4 and 6 diverge from other groups over sessions (Fig 6.5).

Number of Sessions Analysis: Effectively a breakdown of the previous group analysis this analysis simplifies the single and triple session comparison and analyses reaction times irrespective of group and sequence-type, across blocks. It was thus a 2 x 33 MANOVA with repeating measures on the last variable. This analysis produced a significant main effect of number of days ($F(1,53)=4.9$, $p<0.05$) due to the single session rats responded faster than the triple session rats (single-session overall mean(SD) = 0.19(0.004), triple-session overall mean(SD) = 0.27(0.007), see Fig 6.6). Both the block effect and interaction effect for this analysis were significant (block, $F(32,1696)=23.27$, $p<0.00001$; interaction, $F(32,1696)=3.77$, $p<0.00001$). Overall rats

responded faster across blocks irrespective of individual group and sequence type but this improvement was more consistent in the single session animals. The interaction effect is more clearly refects the relatively slower reaction times for the triple-session condition as trials progress and especially the increase in reaction times in this group at the start of second session (blocks 13-15 in Fig. 6.6).

Fig. 6.6, Data aggregated according to Number of Sessions (Error bars ± 1 SEM).). 'Thirds' refer to the portion of single sessions only. Session numbers are relevant to the triple session conditions only.



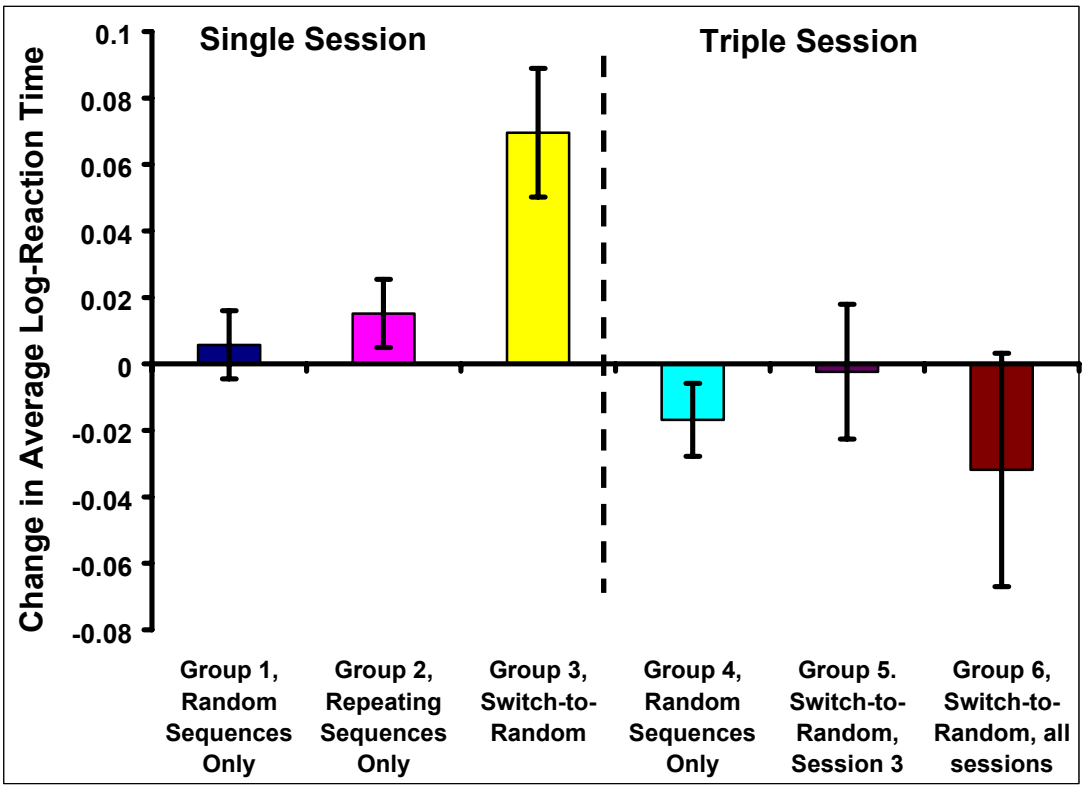
Interference Effect Analysis

To maintain consistency with human SRT procedures the interference effect analysis compared difference scores between groups. Difference scores were generated from subtracting the average of the 80 trials immediately prior to the sequence switch (if any) from the average of the 80 trials immediately after the sequence switch. A one-way ANOVA was performed for each behavioural measure. Dependent measures t-tests were also calculated individually for each group to see if their post-interference behaviour was significantly different to their pre-interference behaviour. For both the ANOVA and t-test analyses interference effects for triple-session groups (Groups 4, 5 & 6) were only calculated on data from the third and last session.

Group Analysis: The first analysis compared reaction time interference effects for the six different groups (see Fig 6.7). The group difference was significant ($F(5,49)=3.65$),

p<0.01) and thus the six groups differed in terms of the strength of their interference effects. *Post hoc* analysis (Newman-Keuls tests) revealed that Group 3 (single-session switch to random) had a significantly stronger interference effect than Groups 4 and 6 (p's <0.05), and differences approached significance when compared to Groups 1, 2 and 5 (p's=0.08, 0.07 & 0.07, respectively). No other group differences were significant or approached significance.

Fig 6.7, Change in Average Log Reaction Time per Trial after switching to a Random Sequence (if appropriate; Error bars ± 1 SEM).



Individual dependent-measures t-tests were carried out for each group to test if their behaviour altered significantly after the point where a sequence switch would take place. The differences between pre- and post-switch blocks was only significant for Group 3 (t(11)=3.12, p<0.01; all other groups p's >0.16).

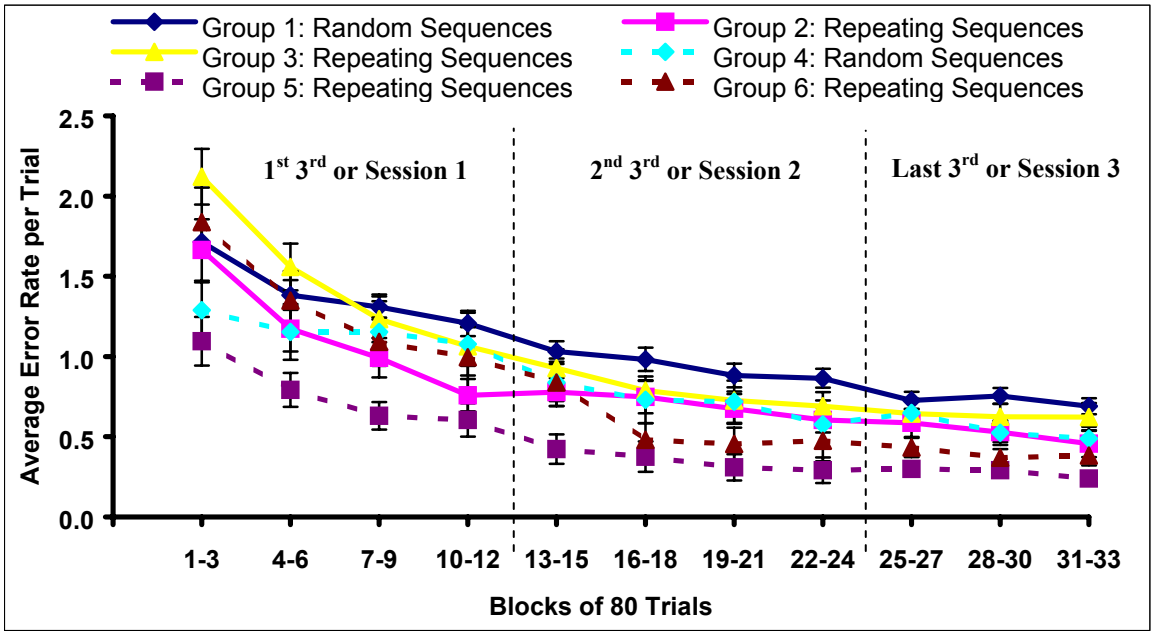
Error Measure

General Analysis

As for the reaction time measure practice effects (across 33 blocks) were calculated for error rates for the three variables (group type, number of days to complete, and sequence type) in separate variable by block MANOVAs with repeated measures on blocks.

Group Analysis: The first variable analysis compared the behaviour of the six groups, irrespective of number of sessions and sequence-type condition, across blocks and was thus a 6 x 33 MANOVA with repeating measures on the last variable. This analysis produced a non significant group effect ($F(5,51)=1.43$, NS; see Fig. 6.8) but a significant block effect ($F(32,1632)=73.86$, $p<0.00001$) and a significant group x block interaction ($F(160,1632)=1.6$, $p<0.00001$). Thus while groups were not different prior to any sequence switch all rats improved (made fewer errors) over blocks irrespective of sequence or number of sessions conditions. The interaction is difficult to interpret as the error data shows no clear pattern except for the variability between groups in the first third / session. In particular Group 5's reaction times are faster than other groups even in the first few blocks, and Group 3's reaction times are initially slower than Group 1's but are faster after block 9. Either of these two effects could produce an interaction effect.

Fig 6.8, Average Number of Errors per Trial across Non-Interference Blocks (Error bars ± 1 SEM). 'Thirds' refer to the portion of single sessions only. Session numbers are relevant to the triple session conditions only. Session 3 only includes three blocks because the fourth block is the interference effect block which is reported separately.



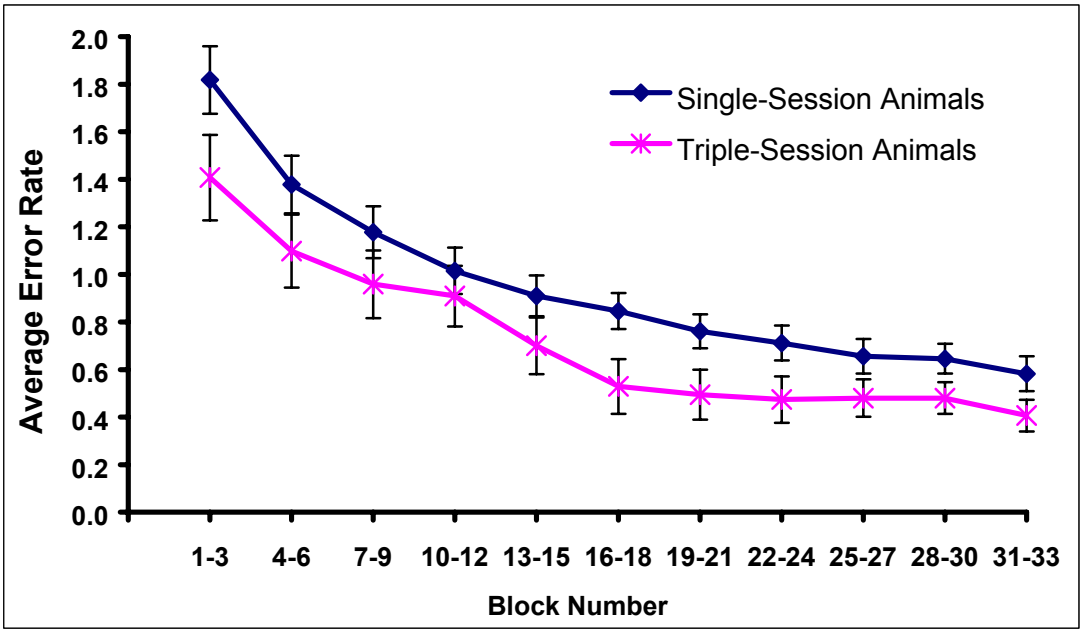
Number of Sessions Analysis: The second variable analysis compared behaviour

between single and triple session conditions, irrespective of group and sequence-type, across blocks. It was thus a 2 x 33 MANOVA with repeating measures on the last variable. This analysis nearly produced a significant main effect of number of days ($F(1,55)=2.93$, $p=0.09$) due to the single session rats making slightly more errors on average than the triple session rats, see Fig 6.9). While the block effect for this analysis were significant ($F(32,1760)=72.54$, $p<0.00001$) the interaction effect was not ($F(32,1760)=1.26$, NS). Thus, overall, rats made fewer errors over blocks irrespective of group or sequence type.

Interference Effect Analysis

Just as for the reaction time analyses the analysis of the interference effects for error rate were carried out via a (one-way) ANOVA to compare between groups Difference scores were again generated from subtracting the average of the 80 trials immediately prior to the sequence switch (if any) from the average of the 80 trials immediately after the sequence switch. A one-way ANOVA was performed for each behavioural measure. Dependent measures t-tests were also calculated individually for each group to see if their post-interference behaviour was significantly different to their pre-interference behaviour. For both the ANOVA and t-test analyses interference effects for triple-session groups (Groups 4, 5 & 6) were only calculated on data from the third and last session.

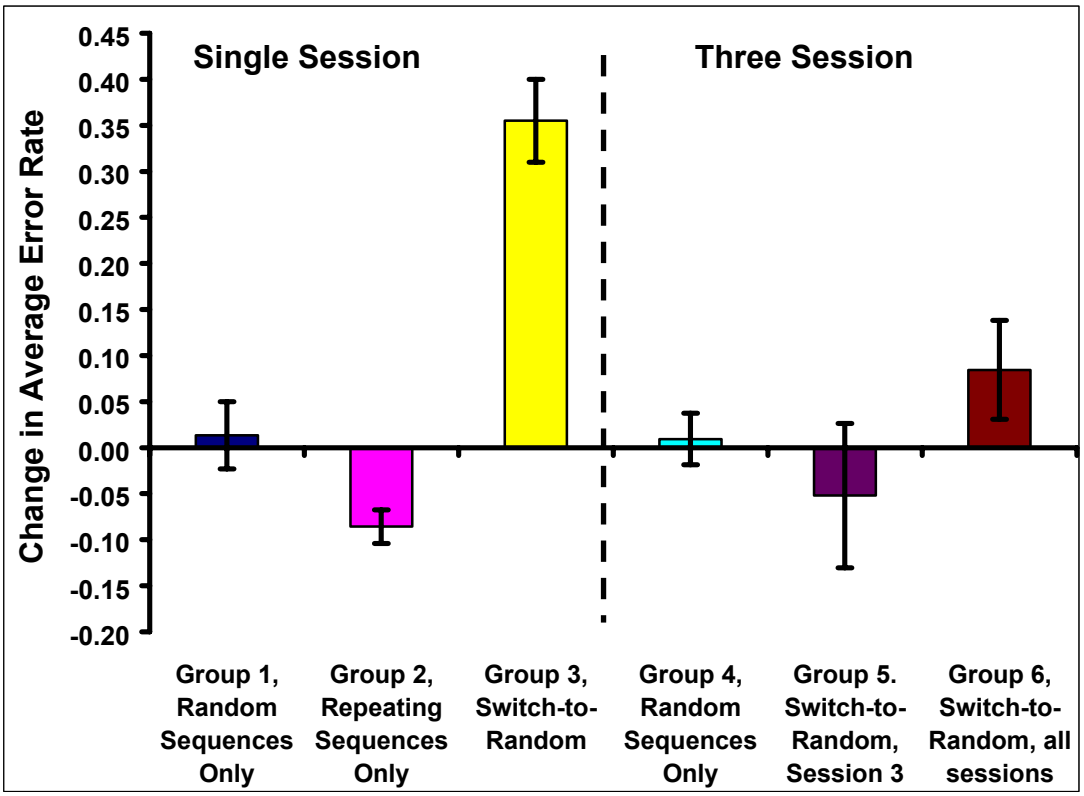
Fig. 6.9, Error Rate Data Aggregated According To Number-Of-Sessions (Error Bars ± 1 SEM).



Group Analysis: The first analysis compared interference effects for the different groups. The analysis was significant ($F(5,51)=8.1$), $p<0.0001$; see Fig. 6.10) and thus the six groups differed between themselves in terms of the strength of their interference effects. *Post hoc* analysis (Newman-Keuls tests) revealed that Group 3 had a significantly stronger interference effect than all other groups (all p's<0.005). No other group differences were significant.

Individual dependent-measures t-tests were carried out for each group to test if their behaviour altered significantly after the point where a sequence switch would take place. The differences between pre- and post-switch blocks was only significant for Group 3 ($t(116.0$, $p<0.00001$ and Group 6 ($t(7)=2.48$, $p<0.05$); all other groups p's >0.16).

Fig 6.10, Change in Average Number of Errors When Switched to a Random Sequence (if appropriate: Error bars ± 1 SEM).



General Discussion

The first conclusion from the data presented above is that rats can learn an embedded repeating sequence in the novel apparatus when rewarded with ICSS. Thus the experimental protocol is valid and useful. These findings show that that the optimal conditions for demonstrating an interference effect in rats is a single session with a switch to random sequences at the end of the session, i.e. Group 3's conditions. Group 3 is the only group to demonstrate a significant change in behaviour when switched from repeating to random sequences (i.e. Group 3 shows a clear interference effect on both measures).

The presence of a strong interference effect in the single-session condition in the repeating-to-random group (Group 3) in contrast to the absence of any such effect in both the random-only and repeating-only single-session groups (Groups 1 and 2 respectively) strongly suggests that rats learn the repeating sequence and apply that knowledge to enhance performance. This pattern of results directly supports the conclusion that it is the switch from repeating to random sequences that causes the deterioration in behaviour at this point seen in Group 3 and not other factors (e.g. fatigue).

There is also a clear advantage of performing the SRT in a single-session. Those rats that experienced the repeating sequence over three days (Groups 5 and 6) displayed no interference effects when switch from a repeating to a random sequence. Furthermore, the negative interference effects for both reaction time and error rates demonstrated by Group 5 (Figs. 6.7 and 6.10) clearly shows that the practice of allowing rats to experience two repeating-only sessions before switching them to random sequences at the end of the third repeating session was not effective. If anything these rats' behaviour improved at this point rather than deteriorated as would be expected if they had learnt the repeating sequence. Group 6 rats, which experienced a switch from repeating to random sequences at the end of each of the three sessions, demonstrated a modest interference effect when switched to random sequences on the error rate measure (Figs. 6.10). Group 6 rats demonstrated if

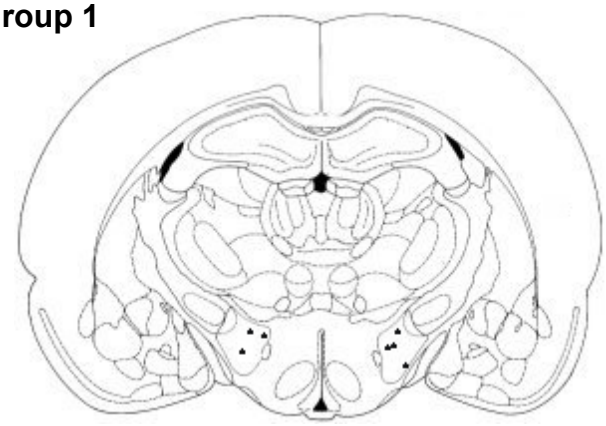
anything a negative interference effect for the reaction time measure (i.e. rats reacted *faster* after being switched to random sequences, see Fig. 6.7).

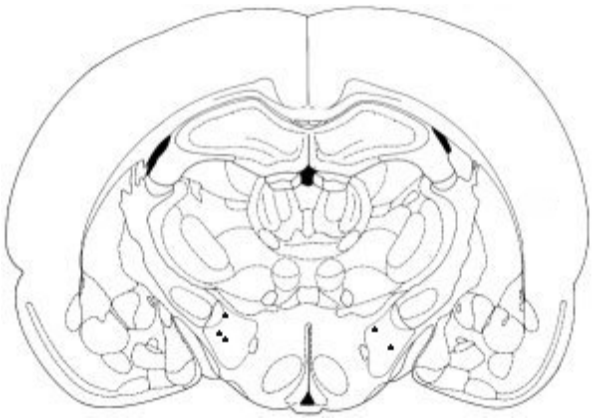
Hence this study has fulfilled its purpose by clearly demonstrated the optimal conditions for generating such behaviour in rats. Single-session repeated-sequence performance produces a far stronger interference effect than triple-session repeated-sequence performance. For this reason these conditions were used in the SRT-lesion study reported in the next chapter. Thus Group 1 (single-session random-only sequences) was used in the SRT-lesion study as the initial control for the presence of a repeating sequence.

Appendix to Chapter 6.

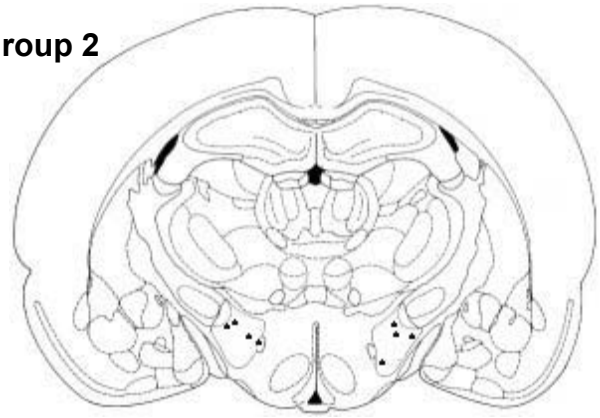
Fig. 6A.1, Schematics of representative ICSS electrode tip placements in the rat brain. All brain images are 2.6mm posterior to Bregma. Brain sections are adapted from Paxinos and Watson (1986).

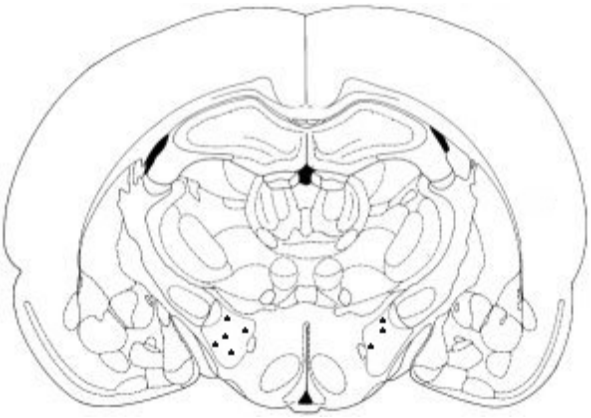
Group 1



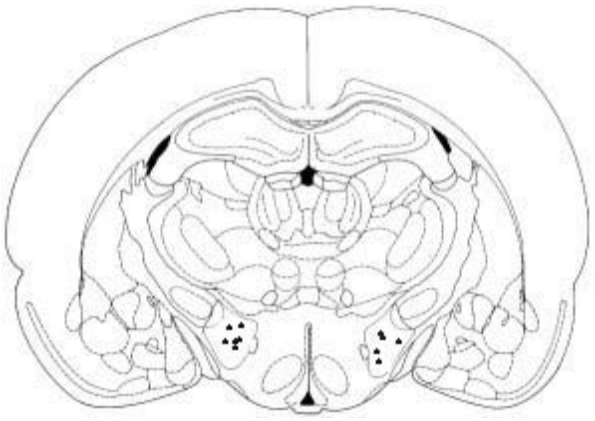


Group 2

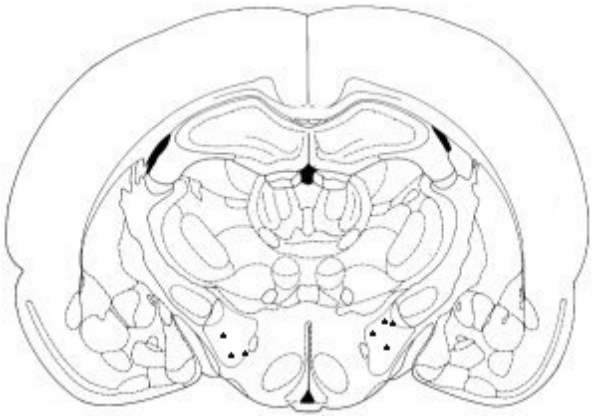




Group 4



Group 5



Chapter 7

The Intracranial Self-Stimulation Serial Reaction Time Task: A Lesion Group Study

General Introduction

The first aim of the animal empirical work presented in this thesis was to demonstrate that the rat-SRT is a valid and reliable animal-analogue of the human SRT task. This aim has been achieved with the ICSS-derived findings in Chapter 7, that rats perform the SRT task in manner broadly similar to human subjects and in particular show a strong interference effect when switching from a repeating to random sequence when tested in a single session. The second aim of the animal empirical work was to determine the neural substrates responsible for rat-SRT and hence address the anomalies that arose from the meta-analysis of the human SRT data. Therefore the first experiment presented in this chapter will contrast control rats with rats with caudate or hippocampal lesions in order to determined whether basal ganglia or limbic system dysfunction impairs SRT behaviour in rats. If caudate lesions, but not hippocampal lesions, impair a rat's ability to perform the SRT relative to control (neurologically intact) rats this would provide convincing evidence that the rat-SRT is a valid and reliable animal-analogue of the human SRT.

It can be expected that caudate lesions will impair rat-SRT behaviour based on the studies presented in Chapter 4 that conclude caudate lesions impair reference / procedural memory tasks in rats (e.g. Packard and White, 1989 and 1990; Kesner, Bolland and Dakis, 1993), and, if the rat-SRT is a valid analogue of the human SRT, then caudate lesions should disrupt the rat-SRT (Knopman and Nissen, 1991;

Willingham and Korroshetz, 1993; Ferraro, Balota and Connor, 1993). Based on the same evidence it can also be expected that hippocampal rats should not show a SRT impairment. However, the meta-analysis found that subjects with amnesia due to limbic system dysfunction do show a SRT impairment suggesting that hippocampal rats may also be impaired in the SRT. Thus the inclusion of a hippocampal lesion group in experiment one will address this issue, which of course reinforces the particular utility of the rat-SRT.

One of the primary reasons for contrasting procedural / reference memory tasks with spatial working memory tasks in animals is that procedural / reference memory tasks are impaired by basal ganglia dysfunction (Cohen and Eichenbaum, 1993). These tasks are assumed to be analogous to human non-declarative memory tasks by virtue of sharing a similar neuroanatomy. As concluded in Chapter 4 however these procedural / reference tasks, while dissociable for allocentric spatial memory tasks, bear very little relationship to human non-declarative memory tasks apart from being dependent on similar neural substrates. They are therefore of limited utility for modelling *human* non-declarative memory functions in animals.

The previous chapter demonstrated that rats are capable of learning a relatively short (4-trial) repeating sequence in the SRT chamber while being rewarded with ICSS during a single session. Given the success of that study it became possible to examine the ability of rats to learn longer sequences. Furthermore, based on the relative success of the 12-trial fan-maze (Chapter 5) it is reasonable to expect that rats in the ICSS-SRT task will be able to learn longer (than 4-trial) repeating sequences. For this reasons this experiment will include a parametric test of the ability of rats to learn 4, 8, and 12-trial repeating sequences (with a separate session for each sequence length). It is also reasonable to suggest that learning a longer repeating sequence requires more effort on the part of the non-declarative system. Thus a longer sequence may result in an impairment in lesioned and / or intact rats that is not evident at shorter sequences lengths, in much the same way longer delays in the radial-maze produce greater working memory impairments in hippocampally lesioned rats.

The first experiment attempted to demonstrate a dissociation between the

effect of neural lesion (caudate nucleus and hippocampus) on SRT performance. If it was successful it will provide strong evidence of the independence of declarative and non-declarative memory in animals. In particular it would be the first demonstration of a neuroanatomical dissociation for non-declarative memory using a memory task that is a valid animal-analogue of its human counterpart.

As noted in Chapters 1 and 2 demonstrations of single dissociations, while useful, are less empirically compelling than double-dissociations. Therefore, in experiment 2 the ability of rats to perform the rat-SRT was contrasted with their ability to perform an allocentric spatial working memory task. In order to take advantage of the ICSS protocol employed with these rats a well accepted spatial memory task, the 16-hole hole board, was adapted for use with ICSS reward. Precisely the opposite pattern of results to that in the rat-SRT was expected in the hole board. caudate lesions should not impair spatial working memory whereas hippocampal lesioned rats should suffer an impairment.

Experiment 1

Method

Subjects.

58 Norwegian hooded rats aged between 120 & 150 days at the time of electrode implantation for intracranial self-stimulation were used. Subjects were housed individually with food & water available *ad libitum* and trained / tested during the dark portion of a reversed 12hr light / dark cycle (lights on from 6pm).

Lesions and ICSS Implant Surgery.

Subjects were prepared with a presurgical injection of atropine sulphate (Phoenix Pharmaceutical Distributors, Auckland, New Zealand) injected IP (concentration=0.13mg/ml, dose=0.18mg/kg) 20 minutes before being anaesthetised with sodium pentobarbital injected IP (concentration=50mg/ml, dose=100mg/kg). Immediately prior to ICSS electrode implant subjects received bilateral radiofrequency lesions of either the dorsal caudate or the dorsal hippocampus. Stereotaxic coordinates were taken from the Paxinos and Watson (1986) atlas with the skull flat.

The coordinates used for the bilateral caudate lesion were: from Bregma A/P +0.3mm, L ±3.2mm, and from dura –4.5mm ventral. Bilateral hippocampal lesions were made at two locations, an anterior and a posterior site. Stereotaxic coordinates for the anterior site were: from Bregma A/P –3.0mm, L ±2.0mm, and from dura –3.3mm ventral. Posterior site coordinates were: from Bregma A/P –3.7mm, L ±2.0mm, and from dura –3.4mm ventral. The lesions were created with a Radionics radiofrequency generator and a 0.7mm diameter electrode with a 2mm uninsulated tip (Radionics Inc. Burlington, Mass. USA; model RFG-4). For the caudate lesion the bilateral sites underwent 60 seconds of current with the electrode held at 60°C. The anterior hippocampal lesions were created with 60secs. of current with the electrode tip held at 56°C and the posterior hippocampal lesions with 60 secs. of current with the electrode tip held at 57°C. During the same surgery rats received bilateral implants of a twisted wire electrode (MS305/1, Plastics One, Roanoke, VA) into the medial forebrain bundle (mfb) at the level of the lateral hypothalamus. Stereotaxic coordinates and surgical procedures (for ICSS electrode implantation) were identical to those used in Chapter 6.

Intracranial Self-Stimulation (ICSS).

ICSS parameters, procedures and conditions were identical to those used in the previous chapter (Chapter 6) except that the best of the two electrodes implanted in each animal was determined (typically the electrode that required the least current of the two to produce reliable responding) during early training and only used thereafter throughout the experiment. Note: the additional controls (five additional rats were added to both groups 1 & 3) were also given double electrode implants but were not different to their single electrode counterparts.

Table 7.1, Summary of Surgery Details

Surgery Type	**Flat-Skull Coordinates; from Bregma**		**From Dura**	**Lesion Parameters**
	A / P	**L**	**D / V**	
ICSS Electrode Implantation (all rats)	**-2.6**	**±1.8**	**-8.6**	**NA**
Caudate Lesions	**+0.3**	**±3.2**	**-4.5**	**60ºC, 60sec**
Hippocampal Lesions: Anterior site	**-3.0**	**±2.0**	**-3.3**	**56ºC, 60 sec**
Posterior site	**-3.7**		**-3.4**	**57ºC, 60 sec**

Group Numbers

Final group numbers are presented in Table 7.2 below. Two additional caudate subjects were removed due to having small and / or unilateral lesions. Three additional hippocampal animals were removed for the same reason. Because of the small number of Group 1 rats in the SRT12 condition their data are not included in any analysis of this condition, although their data are provided in figures for comparative purposes.

Table 7.2, Group Sizes for Experiment 1

Group Type / Test Type	Random-Only Controls : Group 1	Repeating and Random Controls: Group 3	Caudate Lesions	Hippocampal Lesions	Total
Initial (post surgical) Numbers	13	13	14	18	**58**
Number of Rats in the SRT4	13	13	12	15	**53**
Number of Rats in the SRT8	12	11	12	15	**50**
Number of Rats in the SRT12	4[a]	11	13[b]	15	**43**

[a] Eight Group 1 rats were unfortunately euthanased before they could perform the SRT12

[b] The apparent 'appearance' of a caudate rat in the ST12 is due to the data from a (different) rat in each of the SRT4 and SRT8 conditions being lost. Although the experience of these rats in the session was identical to that of their group-mates a computer failure corrupted the data files after they were recorded but before they could be analysed.

Apparatus.

The SRT equipment was identical to those used in Chapter 6.

Serial Reaction Time Task.

Task performance was broken up into three parts. Part 1, a 4-trial repeating sequence, Part 2, an 8-trial repeating sequence, and Part 3, a 12-trial repeating sequence.

Part 1 employed the same 4-trial repeating sequence as used in Chapter 6 (3-1-4-2). Part 2 employed a novel 8-trial (2-4-2-1-4-3-1-3). Part 3 employed a novel 12-trial repeating sequence (4-1-3-2-4-3-2-4-1-2-1-3). All random sequences were generated to ensure only actual sequence information varied between the random and repeating sequences. The 480-trial block of random sequences used in Chapter 6 (appropriate to the 4-trial repeating sequence) was used again in this study in Part 1, and two new sets of 480-trials of random sequences appropriate to the 8- and 12-trial condition were generated and used in Parts 2 and 3 respectively. All random sequence blocks were used in an identical manner to that of Chapter 6.

Procedure

Lesion rats initially completed ICSS and SRT training as in Chapter 6. After SRT training all rats went on to perform Part 1 of the SRT task (4-trial repeating sequence). 48-hours after this they completed Part 2 (8-trial repeating sequence) and then completed Part 3 (12-trial repeating sequence) 48 hours later. Five days after completing Part 3 all subjects (i.e. both control and lesion animals) started training in experiment 2.

SRT Training.

All basic training procedures were identical to those used in Chapter 6.

SRT Testing.

All subjects underwent a total of 2820 trials in the ICSS-SRT chamber over a single session irrespective of sequence length (4, 8, or 12). The repeating sequence used in each session was appropriate to the sequence-length condition. Groups 1 & 3 (i.e. unlesioned rats) from the previous study (see Chapter 6) were used as control subject data for the lesioned rats. Group 1 only ever experienced 2820 random trials whereas Group 3 experienced 2560 repeating sequence trials before switching to 240 random sequence trials. Both lesions groups experienced the same experimental conditions as Group 3: 2560 repeating sequence trials before switching to 240 random sequence trials in a single session.

Behavioural Measures.

The main measure of human-SRT behaviour task is reaction time. As in Chapter 6, this was measured from the onset of the hole being illuminated until it is successfully nose-poked. All reaction-time data were log-transformed per Knopman & Nissen, 1987. For the rat-SRT, the number of errors (nose-poking an unlit hole) is also an important measure.

Histology.

Subjects were sacrificed with an overdose of sodium pentobarbitone before being perfused with 4% formalin and their brains were removed and fixed in 4% formalin. Brains were transferred to a long-term-solution (4% formalin and sucrose) before being sliced on a freezing microtome at 50μm and stained with cresyl-violet to

locate ICSS electrode tips and to determine lesion extent.

Results

Histology

Placements of ICSS electrode tips and position and extent of lesions are presented in Figs. 7A.1 ands 7A.2, respectively in the appendix to this chapter.

Analysis

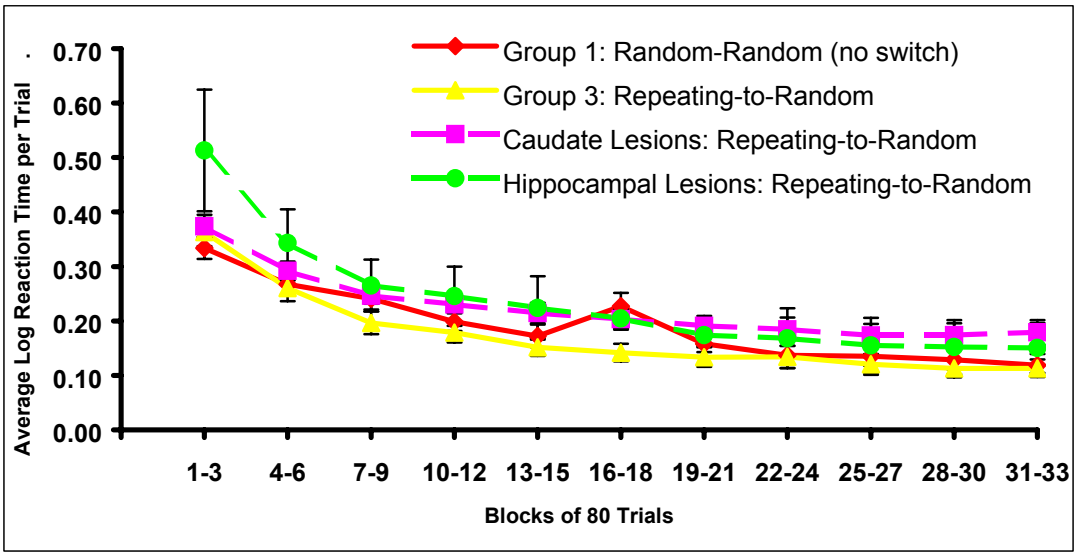
The analysis for each part (sequence-length) was conducted separately by measure (reaction times and error rates) and by condition (training and interference effect). Training effects are analysed via group by block MANOVAs with repeated measures on blocks. Interference effects between groups were analysed via one-way ANOVAs (using difference scores). The change in behaviour over the switch point is also analysed independently for each group via pre- post-switch dependent means t-tests.

Part 1: The SRT4

Reaction Time Analysis: Training Data for SRT4

The main effect of group in MANOVA comparing groups across pre-interference blocks (see Fig. 7.1) was not significant ($F(3,48)=1.69$, NS) and neither was the interaction effect ($F(96, 1546)=1.2$, NS). However, the main effect of block was significant ($F(32,1536)=34.17$, $p<0.00001$), due to the fact that all rats reacted faster over blocks.

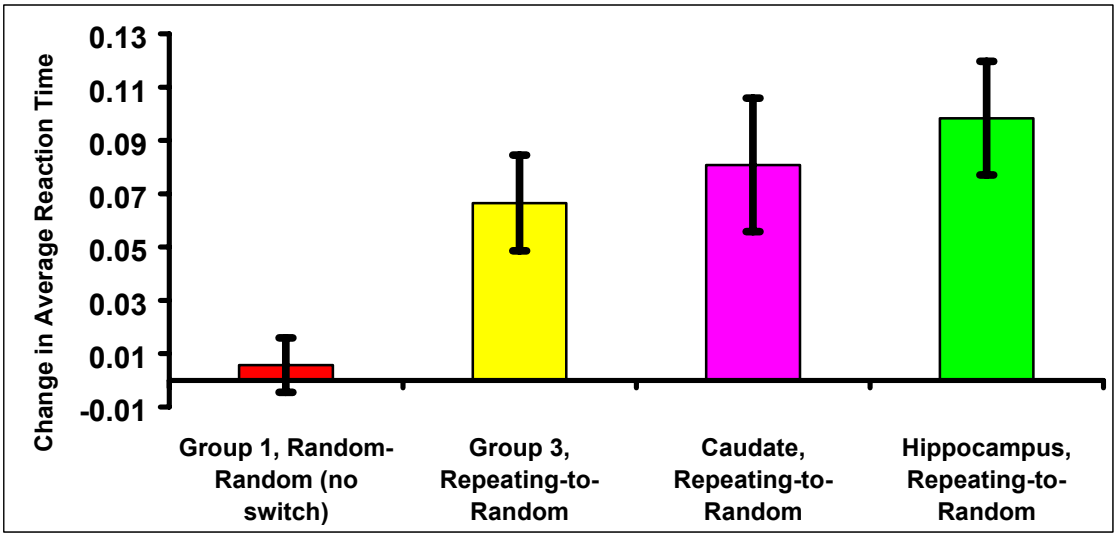
Fig. 7.1, SRT4; Average Log Reaction Time per Trail Across Blocks (Error bars ± 1 SEM).



Reaction Time Analysis: Interference Effect for SRT4

The ANOVA of this interference effect between groups was significant ($F(3,48)=4.4$, $p<0.01$; see Fig. 7.2). *Post-hoc* testing revealed that Group 1 (random-only controls) had a significantly weaker interference effect than the other three groups (Newman-Keuls: p's<0.05), but the other three groups were not different to each other. Furthermore, sequence control rats (Group 3), caudate lesions (CN), and hippocampal lesions (Hip) all had significant pre / post interference effects (Group 3, $t(11)=3.7$; CN, $t(11)=3.2$; Hip, $t(14)=4.6$, all p's<0.01) whereas the interference effect for random control rats (Group 1) was not significant ($t(12)=0.6$, $p=0.58$). Thus all groups that experienced repeating sequences demonstrated the capacity for generating a robust interference effect (i.e. reaction times slowed after being switched from a repeating to a random sequence). However, the group that only experienced random sequences failed to demonstrate any interference effect as expected.

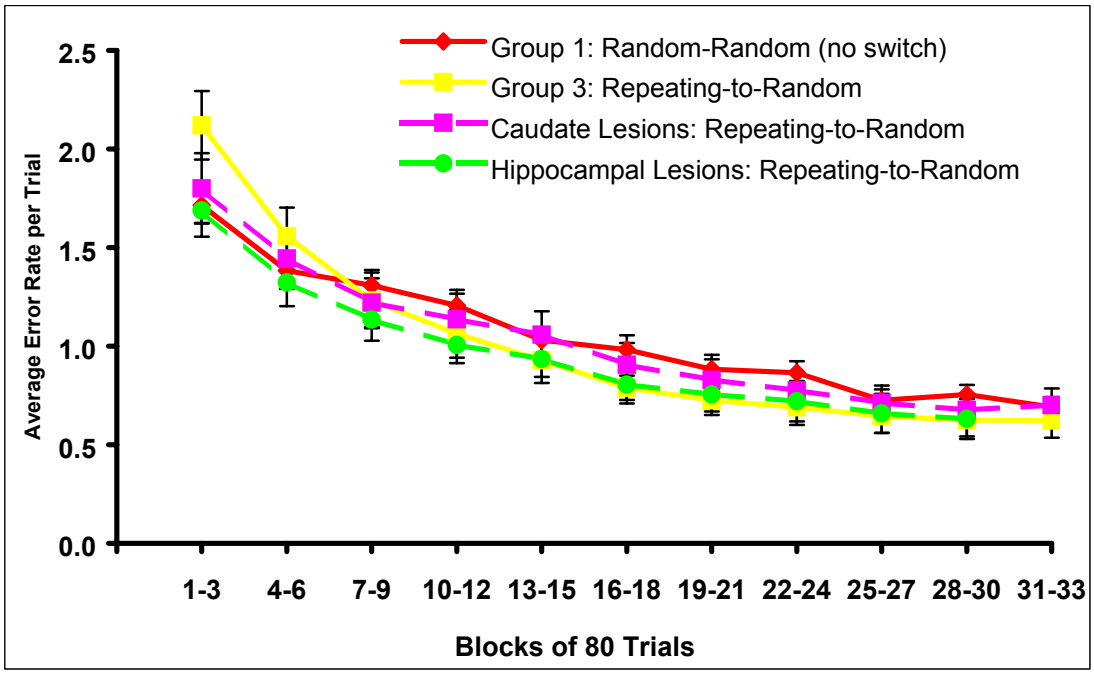
Fig. 7.2, SRT4; Change in Average Reaction Time When Switched to a Random Sequence (Error bars ± 1 SEM).



Error Rate Analysis: Training Data for SRT4

The main effect of group in the error rate MANOVA comparing groups across pre-interference blocks (see Fig. 7.3) was not significant ($F<1$). However, both the main effect of block and the interaction effect were significant (block, $F(32,1536)=75.09$, $p<0.00001$; interaction, $F(96, 1536)=1.82$, $p<0.00001$). Thus all rats made fewer errors over blocks. Examination of figure 7.3 suggests that the interaction effect probably arose from Group 3 initially making more errors than the other groups but them improving by the end of block 18 to the point where it made slightly fewer errors than all other groups over the second half of testing. In contrast the hippocampal rats made fewer errors across the initial sessions. Importantly there are no group differences at the end of training.

Fig. 7.3, SRT4; Average Error Rate per Trial Across Blocks (Error bars ± 1 SEM).



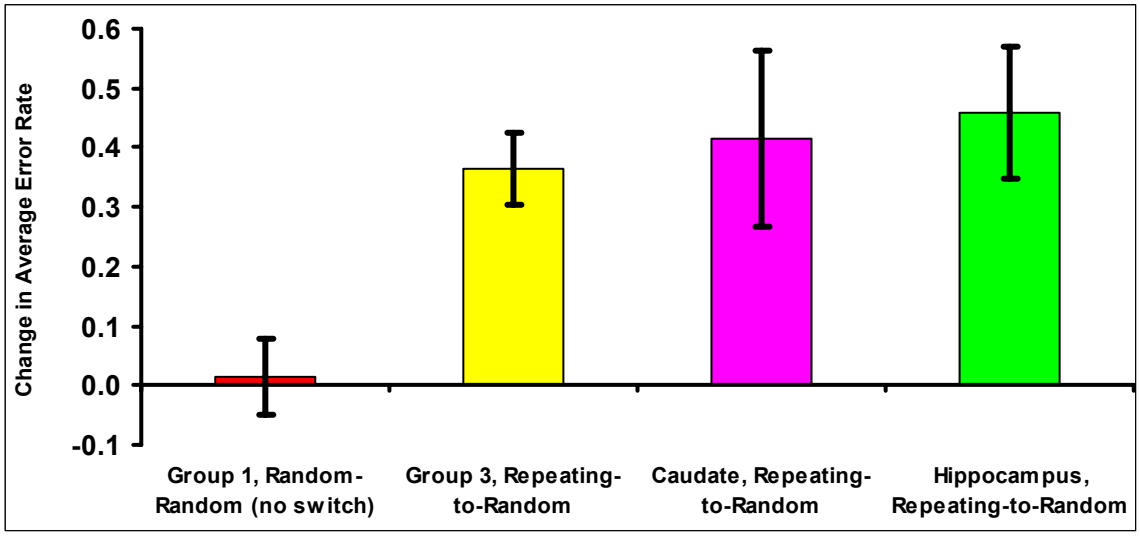
Error Rate Analysis: Interference Effect for SRT4

The ANOVA between groups was significant ($F(3,48)=3.25$, $p<0.05$; see Fig. 7.4). *Post-hoc* testing revealed that the random control group (Group 1) had a significantly weaker interference effect (a much smaller difference score) than the other three groups (Newman-Keuls: p's<0.05), but the other three groups were not different to each other. Furthermore, the sequence control group (Group 3), caudate lesions (CN), and hippocampal lesions (Hip) all had significant pre / post interference effects (Group 3 $t(11)=6.0$; CN $t(11)=2.8$; Hip $t(14)=4.1$, all p's<0.025) but once again Group 1's interference effect was not significant ($t(12)=0.2$, $p=0.84$). Thus all groups that experienced repeating sequences demonstrated the capacity for generating a robust interference effect (i.e. their error rate increased markedly when switched from a repeating to a random sequence). However, the group that only experienced random sequences failed to demonstrate any interference effect for the this measure.

Summary of Part 1: The 4-Trial SRT

Rats showed evidence of learning an embedded repeating sequence. Rats that only experienced random sequence did not display an interference effect. This pattern of interference effects is identical to human SRT performance. However, at a 4-trial sequence, neither caudate nor dorsal hippocampal lesions impaired interference effects.

Fig. 7.4, SRT4; Change in Average error Rate When Switched to a Random Sequence (Error bars ± 1 SEM).



Part 2: The SRT8

Reaction Time Analysis: Training Data for SRT8

The main effect of group in MANOVA comparing groups across pre-interference blocks (see Fig. 7.5) was almost significant ($F(3,48)=2.36$, $p=0.08$). This was due to the caudate lesion group reacting more slowly across blocks. However, both the main effect of block and the interaction effects were significant (block ($F(32, 1440)=43.78$, $p<0.00001$; interaction $F(96, 1440)=1.4$, $p<0.01$). Thus rats as a whole react faster over blocks. The interaction effect was most likely due to caudate lesions rats initially reacting as quickly as the other rats but their reaction times did not improve to the same degree as other rats across blocks. The reaction time of the caudate group did, however, reach about the same speed as they had shown towards the end of the SRT-4 session. The reaction times for the other three groups was faster than they had shown in the SRT4 task.

Reaction Time Analysis: Interference Effect for SRT8

The ANOVA for this measure between groups was significant ($F(3,46)=5.32$, $p<0.005$; see Fig. 7.6). In this instance, however, *Post-hoc* testing revealed that the caudate lesions (repeating-to-random conditions) had a significantly weaker interference effect than the other three groups (Newman-Keuls: p's<0.05), but the other three groups (Group 1, Group 3 and hippocampal lesions) were not different to

each other. Group 1, Group 3 and the hippocampal lesion group each had significant pre / post differences (G1 $t(11)=4.2$, $p<0.01$; G3 $t(10)=5.5$, $p<0.001$; Hip. $t(14)=4.3$, $p<0.001$) whereas the caudate lesion group did not (CN $t(11)=1.4$, $p=0.3$). Thus although the caudate lesion rats enjoyed the same experimental conditions as Group 3 and the hippocampal lesion rats the caudate rats were unable to learn the 8-trial repeating sequence as demonstrated by their failure to generate an interference effect.

Fig. 7.5, SRT8; Average Log Reaction Time per Trial Across Blocks (Error bars ± 1 SEM).

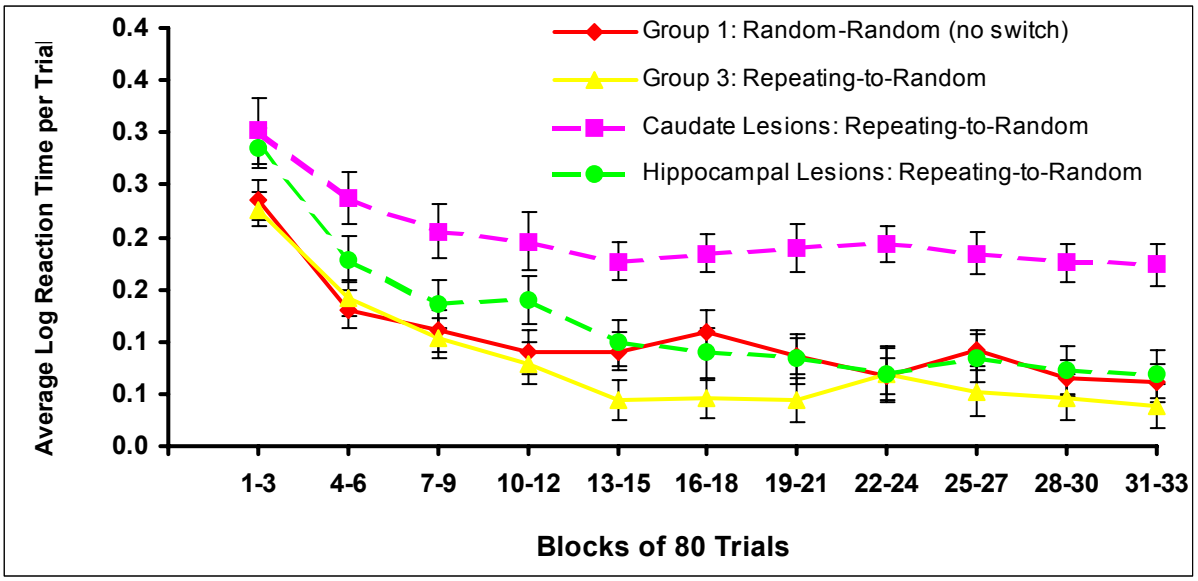
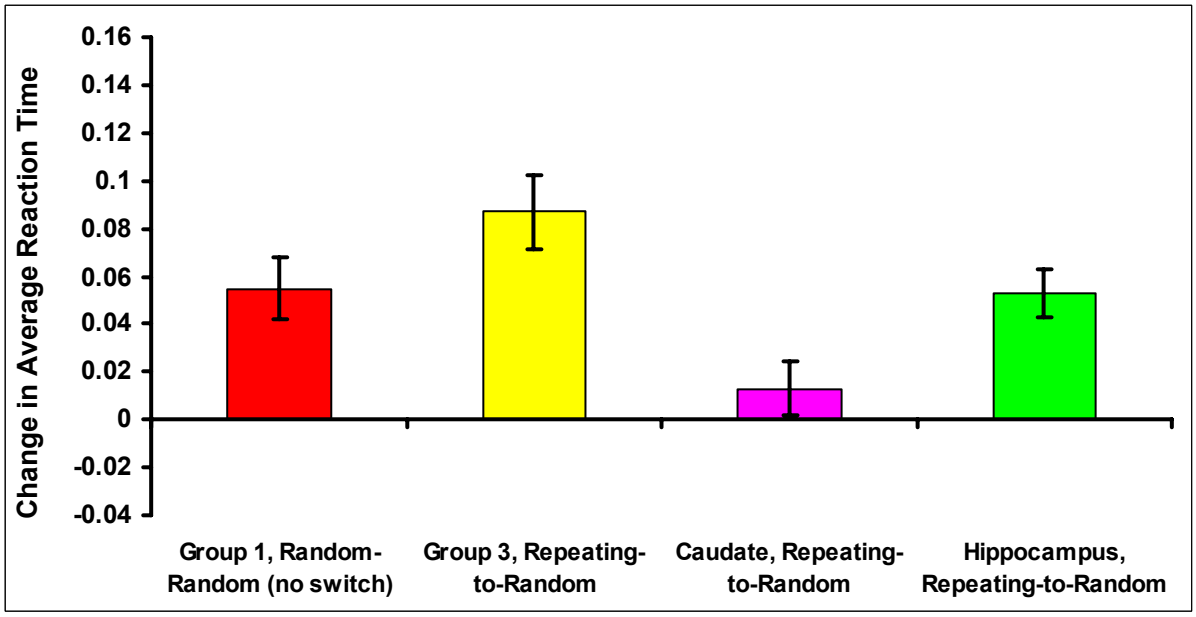
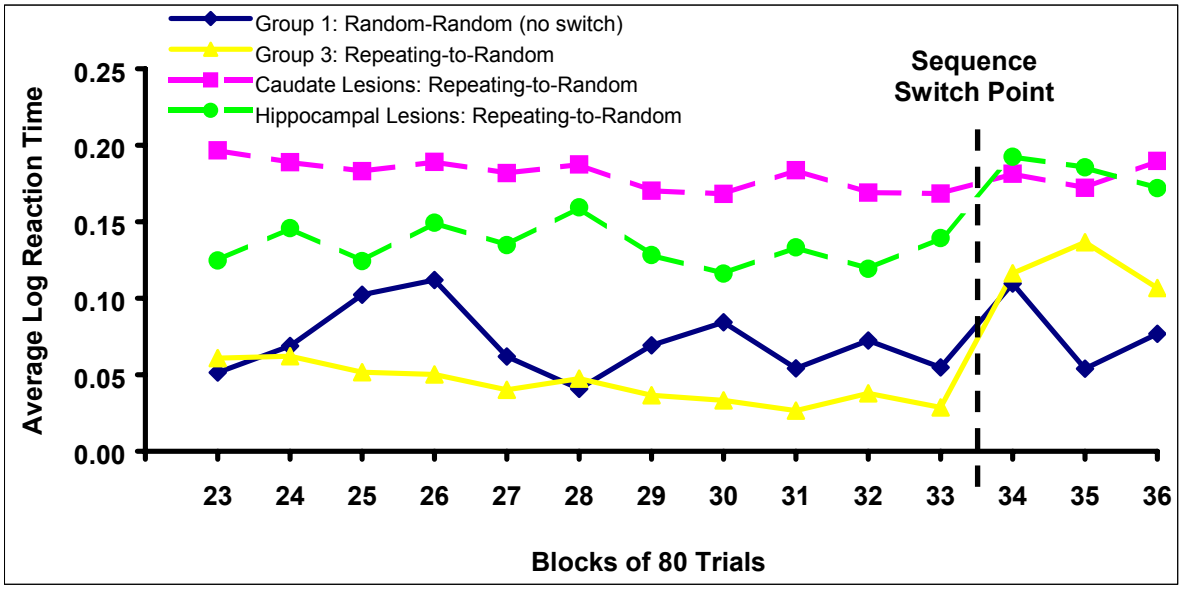


Fig. 7.6, SRT8; Change in Average Reaction Time When Switched to a Random Sequence (Error bars ± 1 SEM).



It is important to note that the apparent interference effect demonstrated by the random controls (Group 1) was an anomaly, due to the consistently high variation between blocks in this group. As Group 1 only ever experienced random sequences there is therefore nothing for these rats to learn and therefore no sequence learning to disrupt. To explain this anomaly Fig. 7.7 presents the last 14 blocks (including the, final, 3 random sequence blocks) of the session for all groups. As can be seen the behaviour of the three repeating sequence groups (repeating controls, caudate and hippocampal lesion groups) is quite stable prior to the switch point (between block 33 and 34). The repeating controls (Group 3), the caudate lesion group and the hippocampal lesion group show relatively little between block variation prior to the sequence switch, whereas Group 1 displays considerable between block variation prior to the switch point.

Fig. 7.7, Average Log Reaction Times for the Last 14 Blocks of the Session.

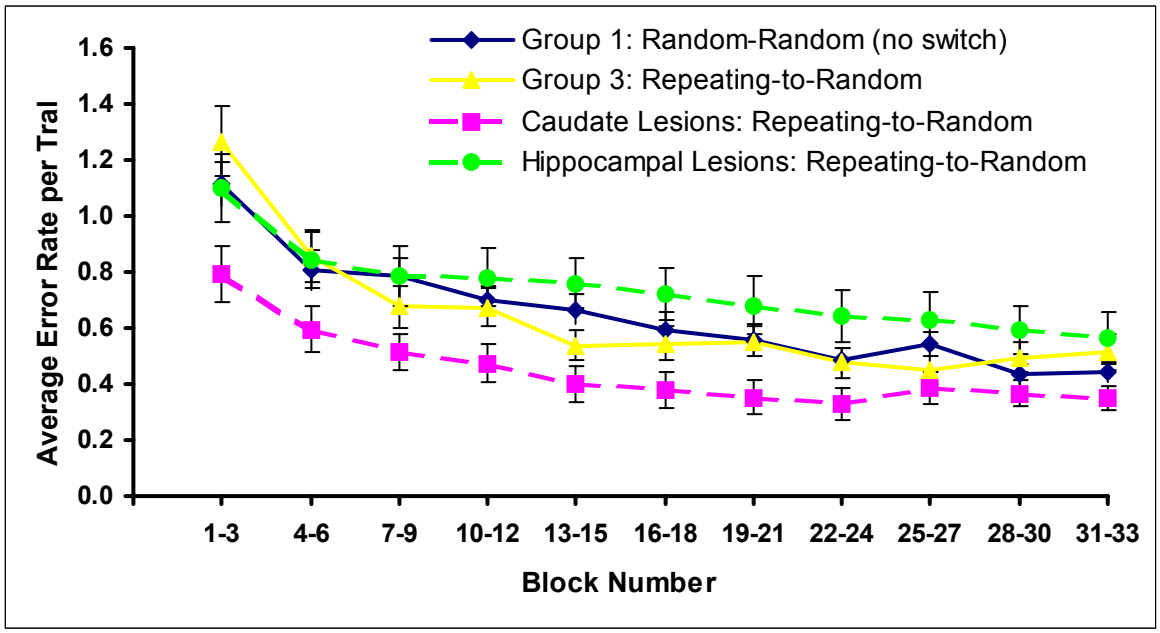


Secondly, those groups that do demonstrate a reliable interference effect (Group 3 and the hippocampal lesion group) not only demonstrate a marked deterioration in performance after being switched to random sequences but their performance remains poor over the next few blocks relative to their pre-interference performance. In contrast performance for Group 1 deteriorates markedly at the switch point but them promptly returns to its pre-interference level in the next block (Block #35). Hence it was merely coincidental that Group 1's reaction times deteriorated at this point (blocks 33 versus 34 were used to generate the data shown in Fig 7.6).

Error Rate Analysis: Training Data for SRT8

The main effect of group in MANOVA comparing groups across pre-interference blocks (see Fig. 7.8) was not significant ($F(3,46)=1.54$, NS) and neither was the interaction effect ($F(96, 1472)=1.09$, NS). However, the main effect of block was significant (block ($F(32, 1472)=38.85$, $p<0.00001$). Thus rats as a whole made fewer errors over blocks.

Fig. 7.8, SRT8; Average Error Rate per Trial Across Blocks (Error bars ± 1 SEM).



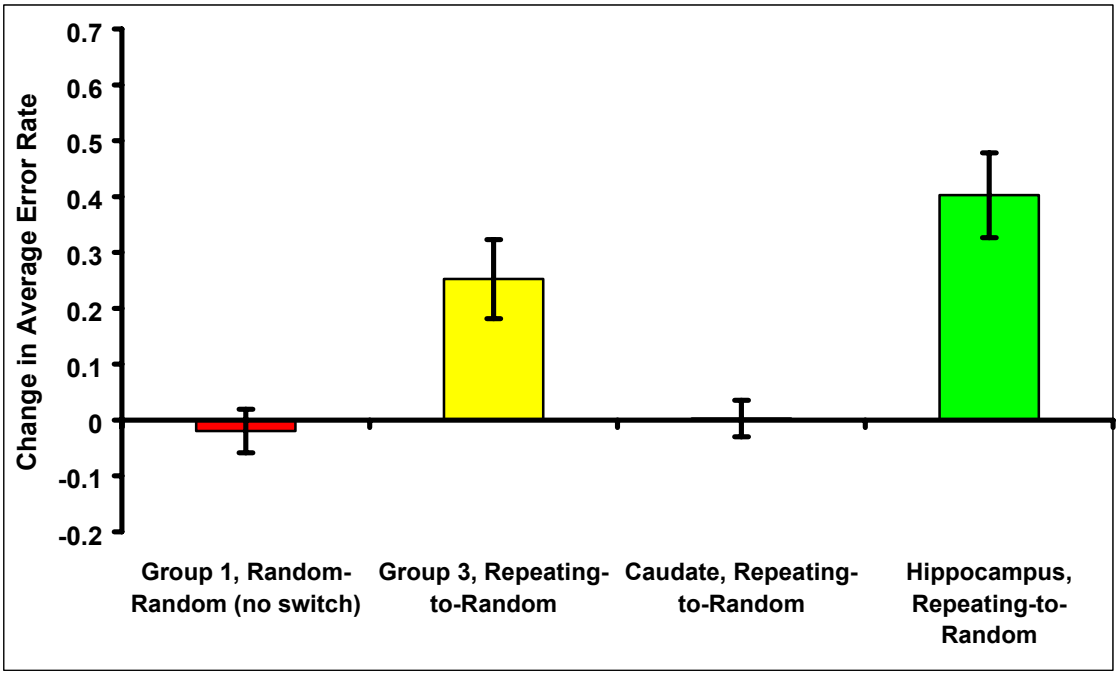
Error Rate Analysis: Interference Effect for SRT8

The ANOVA for this measure between groups was significant ($F(3,46)=12.0$, $p<0.00001$; see Fig. 7.9). *Post-hoc* tests revealed that the caudate lesion group had a significantly weaker interference effect than both the repeating controls (Group 3) and the hippocampal lesion group (both p's <0.01) but was not different to the random controls (Group 1). Similarly Group 1 had a significantly weaker interference effect than either Group 3 or the hippocampal lesion group (both p's <0.01). Furthermore, Group 3 and the hippocampal lesion group (Hip) both had significant pre / post interference effects (Group 3, $t(11)=3.56$; Hip, $t(14)=5.3$, both p's<0.01) whereas both Group 1 and the caudate lesion group did not demonstrate significant interference effects across the point where Group 3, the caudate lesion group and the hippocampal lesion group were switched to random sequences (Group 1, $t(12)=0.5$; caudate lesion group, $t(11)=0.09$). Thus caudate lesions impaired SRT behaviour as measured by error rate.

Summary of Part 2: The 8-trial SRT

Rats with caudate lesions are markedly impaired (i.e. show no interference effect) relative to both control and hippocampal lesion rats. This indicates that performance of the rat-SRT relies on the basal ganglia in a similar fashion to the way in which the human SRT relies on the basal ganglia. Furthermore, intact and hippocampal rats who experienced the repeating sequence can perform the SRT at this sequence length. Thus the caudate lesion impairment is not due to an 'excessive' sequence length.

Fig. 7.9, SRT8; Change in Average Error rate When Switched to a Random Sequence (Error bars ± 1 SEM).



Part 3: The 12-Trial SRT

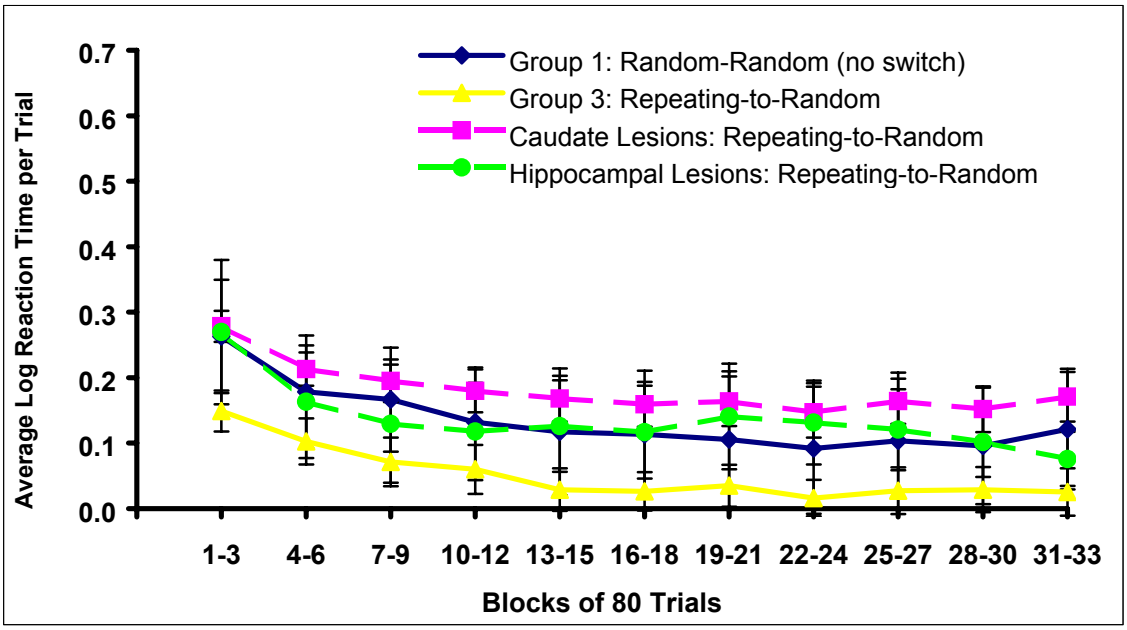
Note, although the aggregate data for Group 1 is included in all figures in this section Group 1 is not included in any of the analyses in this section due to its small sample size (N=4).

Reaction Time Analysis: Training Data for SRT12

The main effect of group in MANOVA comparing groups across pre-interference blocks (see Fig. 7.10) was significant ($F(2,36)=3.36$, $p<0.05$). *Post hoc* analysis revealed that the reaction times of the caudate group was slower overall in comparison to Group 3 (Newman Keuls; $p<0.05$), while the random controls and the

hippocampal lesion group showed non-significant intermediate reaction times. While the main effect of block was significant ($F(20, 720)=11.29$, $P<0.00001$) the interaction effect was not ($F<1$). Thus rats as a whole reacted faster over blocks. Reactions times for each group were generally consistent with those seen in the SRT8 task.

Fig. 7.10, SRT12; Average Log Reaction Time per Trial Across Blocks (Error bars ± 1 SEM).



Reaction Time Analysis: Interference Effect for SRT12

The ANOVA for the difference scores between groups was not significant ($F(2,36)=1.42$, NS), see Fig. 7.11. Thus interference effects did not differ across groups as a whole. The pre / post t-tests performed separately for each group were not significant (Group 3 $t(10)=0.36$; caudate lesion group $t(1)=0.94$) except for the hippocampal lesion group which *did* demonstrate a significant change in behaviour when switched to the random sequence ($t(14)=2.76$, $p<0.025$).

Error Rate Analysis: Training Data for SRT12

The main effect of group in MANOVA comparing groups across pre-interference blocks (see Fig. 7.12) was not significant ($F(2,36)=1.85$, NS). However, both the main effect of block and the interaction effect were significant (block ($F(20,720)=17.96$, $p<0.00001$; interaction ($F(40,720)=1.53$, $p<0.025$). Thus rats as a whole made fewer errors over blocks. The interaction effect is likely due to the fact that caudate lesioned rats show a greater change across blocks than other groups

and hippocampal lesioned rats show a more marked initial reduction in error rate than the two control groups.

Fig. 7.11, SRT12; Change in Average Reaction Time When Switched to a Random Sequence (Error bars ± 1 SEM).

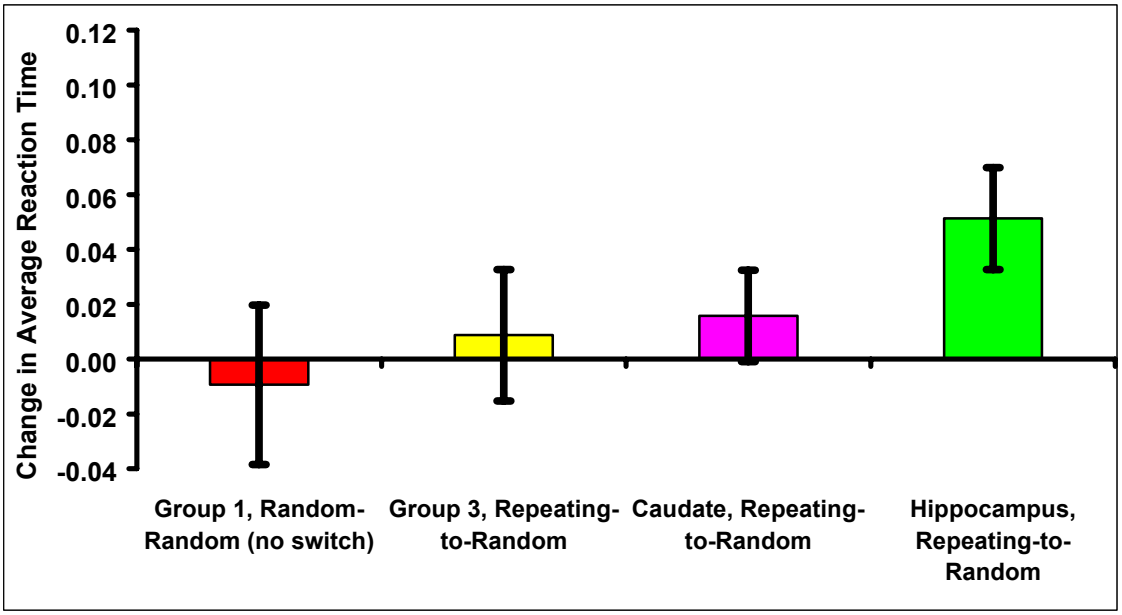
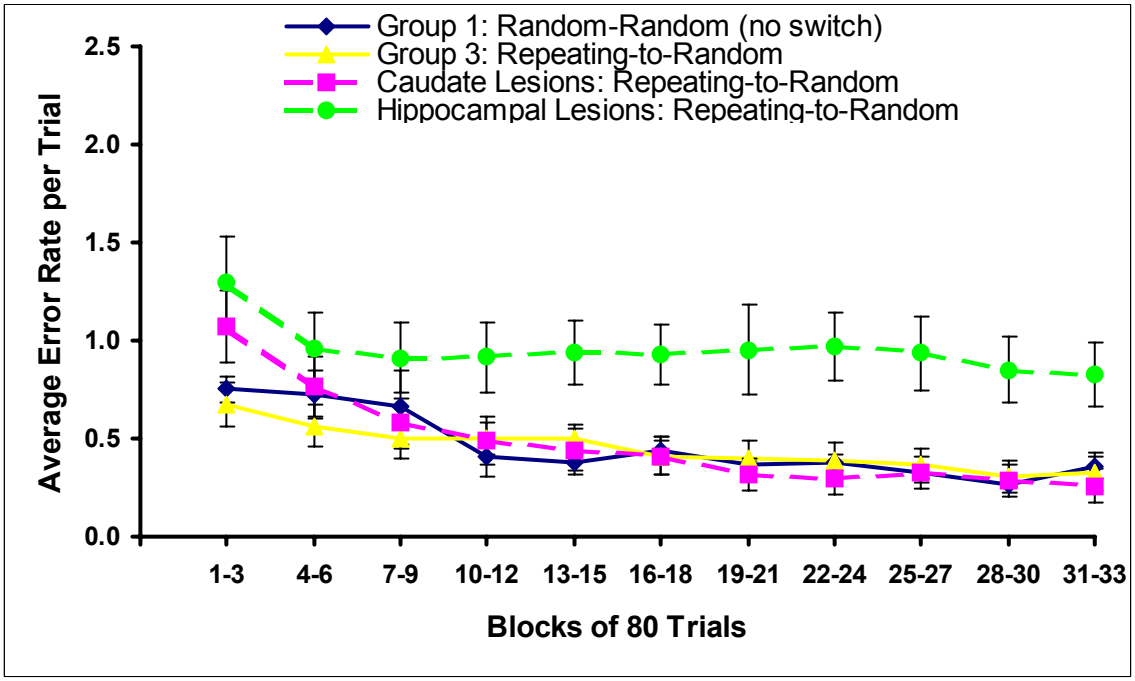


Fig. 7.12, SRT12; Average Error Rate per Trial Across Blocks (Error bars ± 1 SEM).

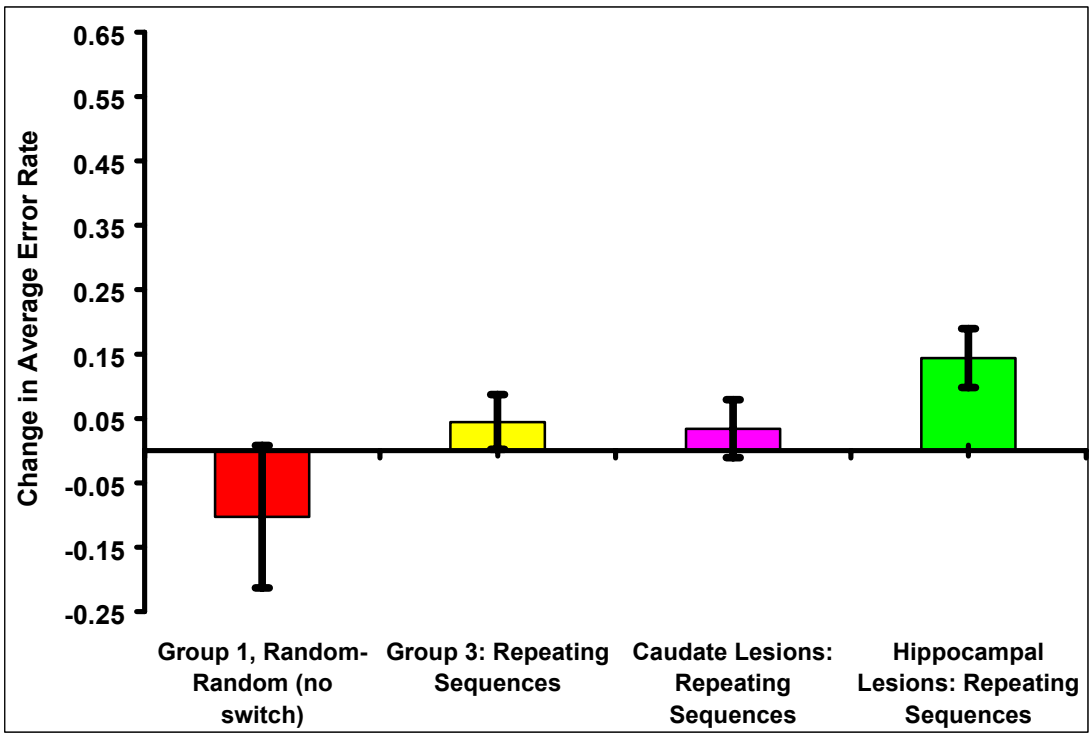


Error Rate Analysis: Interference Effect for SRT12

The ANOVA for the difference scores between groups was not significant ($F<1$, see Fig. 7.13). Thus overall groups did not differ in terms of the strength of their

interference effects. While the pre / post tests for Group 3 and the caudate lesion group were not significant (Group 3, $t(10)=1.04$, NS; caudate lesion group, $t(12)=1.15$, NS) the hippocampal lesion group did demonstrate a significant change in pre / post interference behaviour ($t(14)=3.13$, $p<0.01$). Thus just as for the reaction time measure the hippocampal lesion groups showed a modest ability to learn this very long (12-trial) repeating sequence, unlike the neurologically repeating-sequence control group (Group 3).

Fig. 7.13, SRT12; Change in Average Error rate When Switched to a Random Sequence (if appropriate: Error bars ± 1 SEM).



Summary of Part 3: The 12-trial SRT

Neurologically intact control rats who experienced the 12-trial repeating-sequence displayed no evidence they could learn it although being capable of learning shorter sequences. However, hippocampal rats displayed some evidence of sequence learning at this sequence-length. Thus a hippocampal lesion in some way facilitates SRT performance.

General Discussion of Experiment One

The first point to make from the findings presented above is that all rats that

experienced a repeating sequence demonstrated some ability to learn a repeating sequence (albeit a short, 4-trial sequence, in the case of the caudate lesion rats). This provides further evidence that rats, irrespective of neurologic status, can perform the SRT task under these conditions. Because caudate lesioned rats were not impaired in this task the impairment they demonstrated at longer sequence-lengths was indicative of impaired sequence learning and not simply an inability to perform the underlying requirements of the task. In contrast both the neurologically intact, and hippocampally lesioned, rats who experienced the same sequences were capable of learning the longer sequence. This, therefore, is unequivocal evidence that rats rely on the basal ganglia to perform the SRT.

As predicted hippocampal lesions did not impair SRT behaviour. In fact there is a strong suggestion in Part 3 (SRT12) that hippocampal lesions may actually facilitate behaviour. The hippocampal rats were the only group do show any evidence of sequence learning at this, longer, sequence length. Furthermore, the hippocampal interference effect in the SRT12 task was consistent across both behavioural measures (reaction times and error rates) which provides a degree of convergent validity for the finding. It is especially interesting to note that the neurologically intact repeating control rats (Group 3) showed no evidence of learning the 12-trial sequence.

An important point to make about the hippocampal SRT facilitation is that it while it is consistent with the multiple-memory systems theory it directly contradicts the results of the meta-analysis as regards the ability of limbic system amnesics to perform the SRT. Although this matter with be discussed in some detail in the following chapter it is worthwhile to briefly address the main points here. In contrast to the constituent studies aggregated into the Limbic System Neuropathology (LSN) group the meta-analysis itself concluded that limbic system amnesics *were* impaired relative to controls in the SRT. In particular that limbic system amnesics had a *weaker* interference effect than neurologically intact controls. The inclusion of a hippocampal lesion group in the present study directly addresses this issue. If limbic system neuropathology that (mildly) impairs SRT behaviour then the hippocampal lesion rats should be similarly (mildly) impaired in this experiment. The finding that the hippocampal lesion group not only were *not* impaired but even demonstrated a mild facilitation effect strongly suggests that it is not limbic system dysfunction *'per se'* that

produces the apparent SRT deficit in LSN subjects seen in the meta-analysis. A possible alternative is that the frontal pathology often evident in limbic system syndromes may be responsible for their apparent SRT impairment. This is consistent with the finding in the meta-analysis that subjects with pre-Frontal cortex injury (pFC) and moderately severe traumatic brain injury (TBI; which can be assumed to be primarily frontal) were impaired in the SRT (Pascual-Leone *et al*, 1993; Mutter, Howard and Howard, 1994) to a similar degree as LSN subjects (Part 2; pFC d=0.64, moderately severe TBI d=0.67, and LSN d=0.68).

The use of the finding that hippocampal lesion subjects are not impaired in the rat-SRT to address the apparent SRT impairment of LSN subjects reported in the meta-analysis is a clear and obvious example of the utility of the rat-SRT task. Rhe point that it is precisely this sort of work that can be carried out in animal subjects provides a perfect example of just how useful the rat-SRT will be, and has already been, for addressing theoretically and empirically important issues in memory research.

Experiment 2 : The ICSS 16-Hole Board Task

In an initial attempt to develop a spatial working memory protocol to contrast with the rat-SRT the 16-hole hole board task was adapted for use with ICSS. First developed by Oades and Isaacson (1978) the hole board task requires a rat to locate a fixed set of four food rewards hidden amongst an open field containing 16 holes. The particular advantage of this task is that two forms of spatial memory can be measured simultaneously: working and reference memory. As the four target holes are fixed (invariant) across trials for individual rats the discrimination between target and non-target holes is considered be the domain of reference memory. In contrast within trial information i.e. whether or not a target has already been visited in that particular trial, is the domain of working memory.

Van der Staay, van Nies, and Raajimakers (1990) used a 16-hole hole board to examine the effects of age in a ‘spatial… discrimination task’ and concluded not only that age was inversely related to both working and reference memory performance but that the two forms of memory measure ‘represent distinct aspects of spatial memory’.

A similar result of age was found by Raajimakers, Blockland and van der Staay (1993) who tested young and old rats in a 16-hole hole board task, a water maze, and a modified conefield task. Van der Staay (1999) also used a 16-hole hole board to examine any differences in spatial working memory behaviour between albino WAG rats and pigmented Brown Norway rats and that working memory performance was generally poorer in Brown Norway rats than WAG rats, but the reverse was true of reference memory performance. He concluded as a result that the two forms of memory measure are independent of each other.

In relation to hippocampal injury and hole board performance Oades (1981a) reported that only rats with hippocampal or ventral tegmental area lesions were impaired in the hole board. In particular both lesion types severely impaired both working and reference memory. Similarly Oades (1981b) reported that, unsurprisingly, treatment with haloperidol did not effect either the (marked) working or reference memory impairments rats produced by hippocampal lesions. However, haloperidol treatment did reduce the number of working memory errors (but not reference memory errors) in rats with neocortical lesions.

Thus the hole board task appears to be a sensitive measure of allocentric spatial learning in rats, which (allocentric spatial learning) is considered the primary animal-analogue of human declarative memory (Aggleton, Vann and Good, 2000; Aggleton and Brown, 1999; and Gaffan, 1998). Therefore lesions of the hippocampus should impair hole board performance (i.e. rats with hippocampal lesions should make more working and / or reference memory errors), but caudate lesions should not impair hole board behaviour (but see Packard and White, 1990).

Method

Subjects

A total of 36 rats who had completed all parts of experiment one were then included in experiment two. Control animals in the holeboard sessions are a mixture of Group 1 (four animals) and Group 3 animals (nine animals) from experiment 1, selected on the basis of still being available when holeboard training started. Two subjects in the caudate group failed to learn the holeboard task and are not included in

that part of the study. Two other caudate subjects died during holeboard training and are not included for that reason

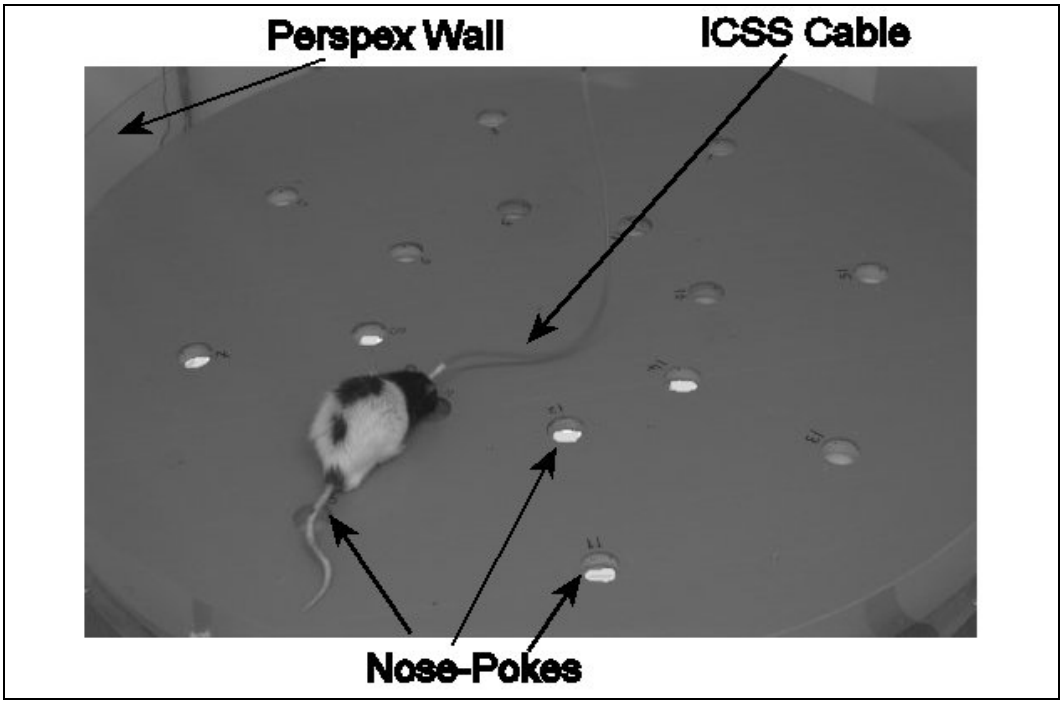
Table 7.3. Group Sizes for Experiment 2

Group Type / Test Type	Random-Only Controls : Group 1	Repeating and Random Controls: Group 3	Caudate Lesions	Hippocampal Lesions	Total
Number of Rats in the Holeboard Sessions.	13		8	15	**36**

Apparatus

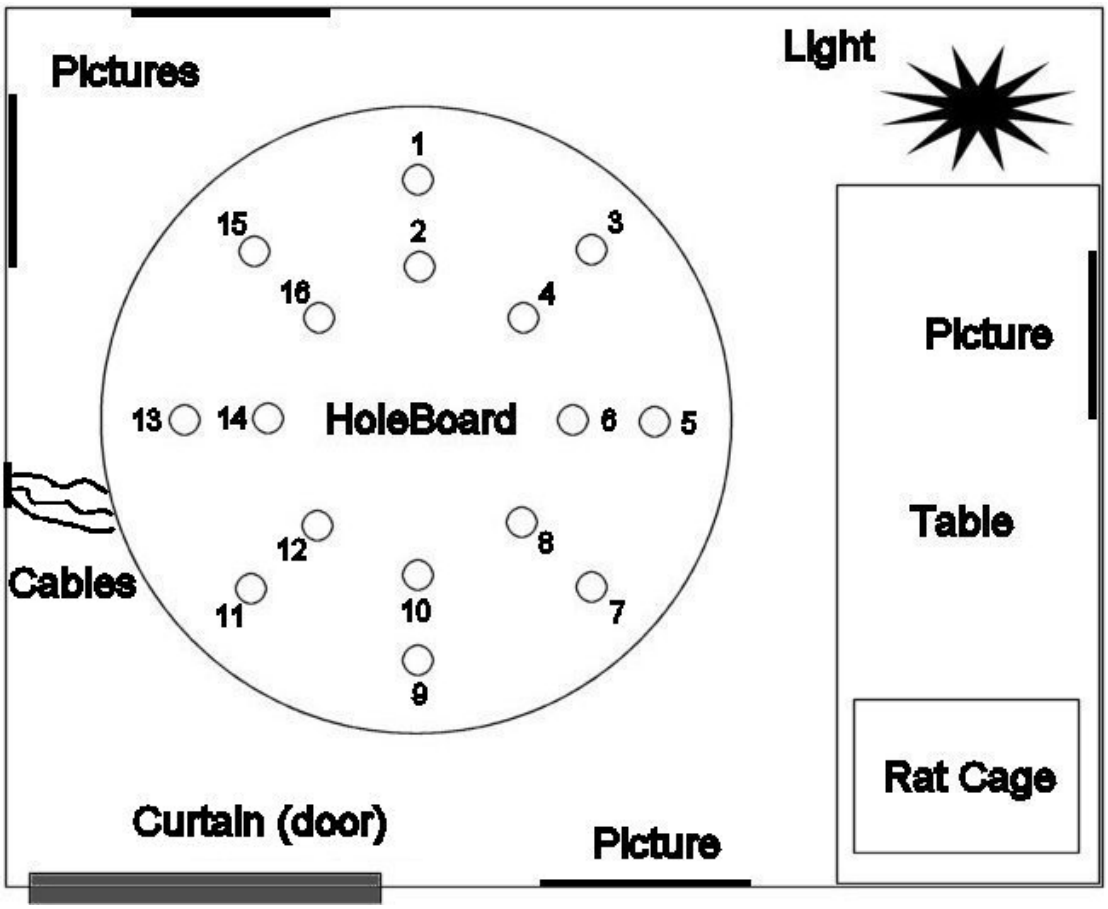
The holeboard was a novel apparatus, consisting of a 1.2m diameter grey Perspex circular platform elevated 75cm off the ground with 16 nose-pokes (capable of being independently illuminated) mounted into the body of the board (see Fig. 7.14). The nose-pokes were arranged in two concentric rings (inner and outer) of eight nose-pokes each and placed at the cardinal and demi-cardinal compass positions (i.e. North, North-East, East etc.). The nose-pokes were identical to those used in the SRT equipment except for being slightly larger at 4cm diameter. The holeboard was surrounded by a low clear Perspex wall (10cm high) which was continuous around the periphery of the holeboard. The same model commutator as used for the SRT equipment was mounted directly above the centre of the holeboard and supplied the ICSS current to the animal. The commutator fitting also held the same model infra-red camera as used in the SRT equipment which allowed real-time viewing of the animals during holeboard training / testing.

Fig. 7.14. The 16-hole holeboard with behaving rat. Note: the brighter nose-pokes are still illuminated whereas the dimmer nose-pokes are unlit having been extinguished after a successful nose-poke.



The holeboard was placed in a small (2m wide by 3.2m long) room painted white. The room contained a number of obvious spatial cues including: different sized pictures on all of the walls, a curtain door at one end, a table at the other end of the room where cages were kept during behaviour, cables which exited from half way up the wall and ran down the wall and under the holeboard, and a low intensity light which was placed on the floor (i.e. below the level of the holeboard) next to the table (see Fig. 7.15). Care was taken to ensure the spatial environment was consistent across sessions. Nose-pokes and ICSS was controlled by the same Med-Associated computer interface as controlled the SRT equipment.

Fig. 7.15. A schematic of the arrangement of the holeboard room (not to scale).



Holeboard Training

All sessions were performed across consecutive days.

Familiarisation. As rats had already learned to nose-poke in the ICSS-SRT task there was no need to train them to nose-poke in the hole board. Thus familiarisation consisted of one session during which the body of the holeboard was completely covered by a wooden cover. Animals were left to acclimatise to the room for 20 minutes without the ICSS cable attached. The second familiarisation session was similar except the holeboard was now uncovered and the ICSS cable attached (but no current passed through it). Rats were left to explore the holeboard and the (unlit) nose-pokes for 20 minutes.

Initial training. The first phase of initial training consisted of all 16 nose-pokes being lit and an animal being rewarded with ICSS for any response in any nose-poke. However, after five nose-pokes to any one hole the light for that hole was extinguished and no further stimulation was received for any further nose-poke. After all 16 holes had been nose-poked (for a total of 80 responses) all nose-pokes were relit and the cycle began again, for a maximum of 160 nose-pokes or for 20 minutes, whichever

came first. Animals continued in this procedure until they learnt to nose-poke all 16 holes and learnt to actively explore the holeboard for any they may have missed.

After the first phase of initial training (which typically lasted two or three sessions) animals were changed to a training condition in which only a single nose-poke to each hole was rewarded. After a successful nose-poke that hole was unlit and no further reward was given. Unlit nose-pokes were relit once a nose-poke had been made to all 16 holes. Animals typically learnt to move around the holeboard responding quickly, and only once, to each hole within two to three sessions.

Holeboard Task Training. Thereafter animals experienced one session where nose-poking only 12 of the 16 holes gave ICSS reward (always the same 12 holes for each animal). Furthermore those holes that had been successfully nose-poked *remained* illuminated and any subsequent response to such holes did not produce ICSS reward. Animals were required to nose-poke all 12 target holes at which point all 16 holes were unlit and a 10-second ITI started. After the ITI expired the 16 holes were relit and the cycle began again. Animals had to complete 10 sets of trials to complete the session. Immediately thereafter (i.e. within the same session) animals completed another session of 10 sets of trials which was identical except that only eight holes now gave ICSS reward (always the same eight). Immediately thereafter (i.e. within the same session again) animals progressed to the first actual testing session (see below).

Holeboard Testing

The first testing session was identical to the immediately preceding training sessions except that only four holes (of the 16) now gave ICSS reward. There were six sets of four holes and each animal was assigned one set which was used throughout holeboard testing for that animal. Each set of four holes was counterbalanced for side (two holes on the left and two on the right), for quadrant (one hole from each of the four quadrants), and for ring (two inner ring and two outer ring holes). Note: A quadrant consisted of four holes, e.g. holes one, two, three and four made up the “North-East’ quadrant.

As in the final training sessions all 16 holes were lit and the first response to a

target hole within a trial resulted in ICSS reward. However, the light for any hole was not extinguished after a successful response and any subsequent responses to it within the trial was recorded as a working memory error. Responses to non-target holes (which were always the same 12 holes for that animal for each session) were not rewarded (the hole remained illuminated) and were counted as reference memory errors.

After all four target holes had been nose-poked (one trial) all holes were unlit and a 10 second ITI started. After the ITI expired all 16 holes were lit again and the process started again. The initial testing session consisted of 10 trials. The next seven sessions all consisted of 20 trials per session.

During testing sessions animals had to perform within two criteria. Firstly, that had to locate and nose-poke all four target holes within five minutes. Secondly, they could not make more than 24 error responses (being twice the number of non-target holes). If either of these criteria were exceeded all non-target holes and all target holes that had already been nose-poked within that trial were unlit leaving only those non-nose-poked target holes. Once non-target and already nose-poked holes were unlit (i.e. after making more than 24 errors or taking longer than 5 minutes) within a trial no animal ever failed to go on and nose-poke the remaining lit holes within the time available to it.

Procedures

Five days after completing Part 3 of experiment one all subjects (i.e. both control and lesion animals) began training in the 16-hole holeboard. After holeboard training subjects performed one 10-trial test session in the holeboard and then seven 20-trial test sessions (all sessions performed on separate, and consecutive, days) for a total of 150 trials.

Behavioural Measures

Four measures of behaviour were recorded / generated from hole board behaviour in much the same way as in normal hole board studies (van der Staay, van Nies, and Raajimakers, 1990; Raajimakers, Blockland, and van der Staay, 1993; van der Staay, 1999) and are defined as follows:

Working Memory Errors: Returning to nose-poke a target hole already nose-poked in that trial.

Reference Memory Errors: Nose-poking a non-target hole

Total Number of Errors: The combination of both working and reference memory errors.

Trial Time: Time taken to complete the trial, from all holes being lit (starting the trial) until the fourth target hole was nose-poked (ending the trial).

Results

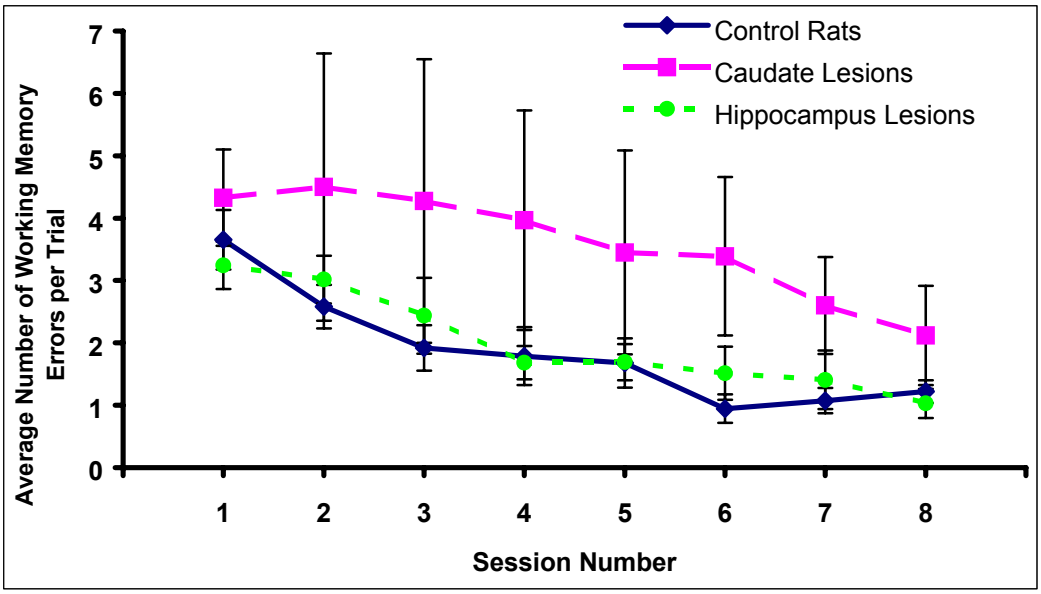
Analyses of hole board data were MANOVAs performed between groups and across block with repeated measures on blocks for each measure. Data from each block of five trials was collapsed (averaged) to form a single data point which produced 30 blocks of data over the total 150 trails. The first session generated two blocks of data (being only 10 trials long) whereas all subsequent sessions generated four blocks of data (each being 20 trials long).

Note, for ease of presentation the data in figures has been further aggregated from that data used in the analysis to one data point per session.

Working Memory Errors Analysis

The main group effect of the MANOVA between groups was not significant ($F(2,30)=2.0$, NS) nor was the interaction effect ($F(58,870)=1.12$, NS) Thus there was no difference between groups for this measure even though the caudate lesion animals appear to make more errors on average (see Fig. 7.16). There was however a significant effect of block ($F(28,870)=7.16$, $p<0.00001$) indicating that overall animals made fewer errors across blocks.

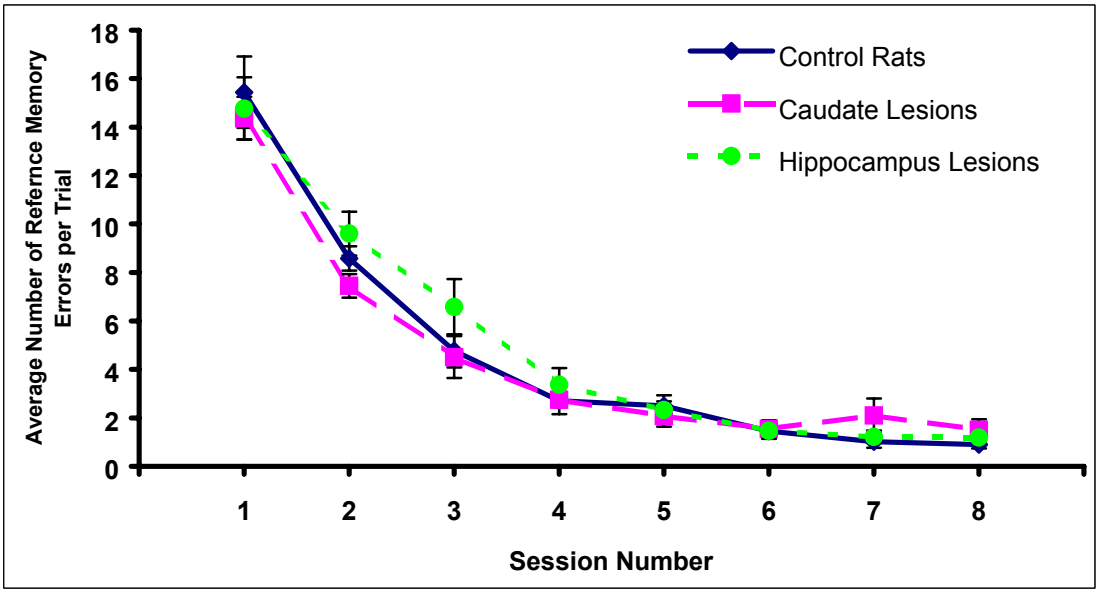
Fig. 7.16, Average Number of Working Memory Errors Across blocks (Error bars ± 1 SEM)



Reference Memory Errors Analysis

Although the main effect of group was once again not significant ($F<1$) both the main effect of block and the interaction effects were block, $F(29,870)=112.12$, $p<0.00001$; interaction, $F(58,870)=1.6$, $p<0.005$). once again rats made fewer errors over sessions irrespective of group. The interaction effect is more difficult to ascribe but may be due to the hippocampal lesion rats improving slightly more slowly than other rats in sessions two and three. See Fig. 7.17

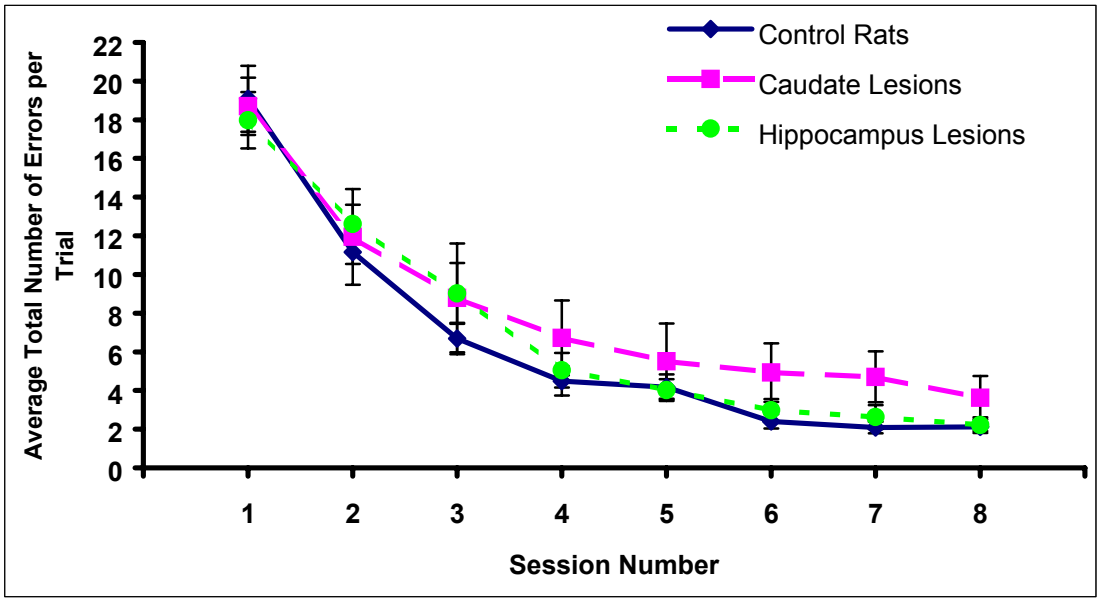
Fig. 7.17, Average Number of Reference Memory Errors Across blocks (Error bars ± 1 SEM)



Total Number of Errors Analysis

Neither the main effect of group nor the interaction effect were significant for this analysis (both F's <1). However, the main effect of block was significant ($F(29,870)=77.41$, $p<0.00001$), rats made fewer errors over sessions. See Fig. 7.18

Fig. 7.18, The Mean Total Number of Errors Made per Session (Error bars ± 1 SEM)



Average Trial Time Analysiss

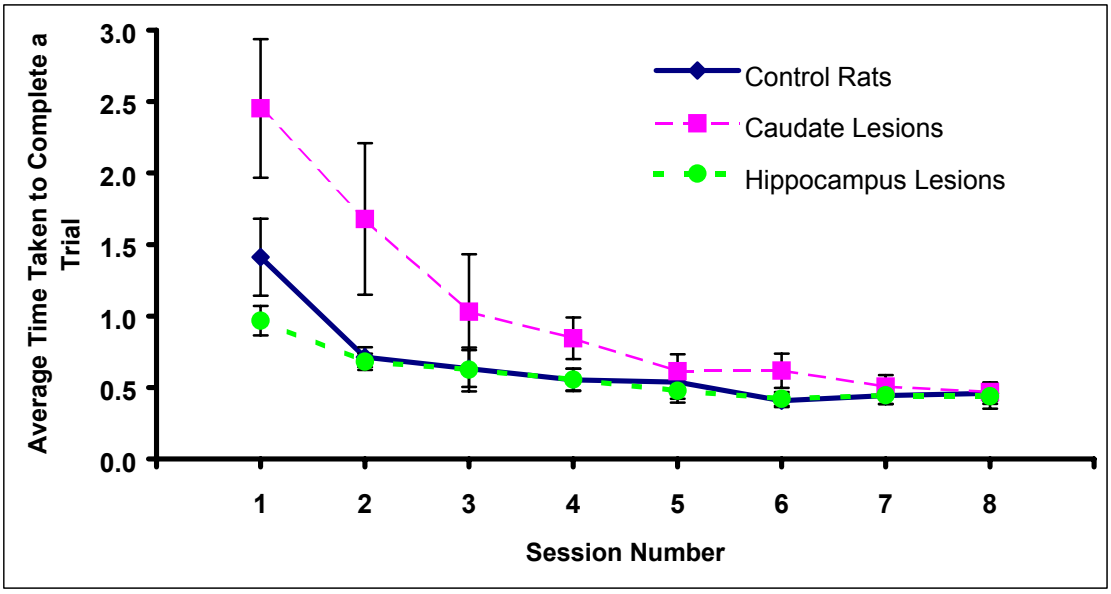
The main effect of group was significant ($F(2,30)=5.09$, $p<0.025$; see Fig. 7.19). *Post hoc* analysis revealed that the caudate lesion group took significantly longer on average to complete a trail than either of the other two groups (Newman Keuls, both p's<0.025). Similarly the main effect of block was also significant ($F(29,870)=14.72$, $p<0.00001$) as was the interaction effect ($F(58,870)=1.82$, $p<0.001$. Overall rats completed a 4-hole trial faster across sessions. The interaction effect is due to the fact that the caudate lesion animals were much slower than the other animals during the first four blocks, but on average completed a trial in a very similar time to the other animals in later blocks.

Summary of The Hole Board Analysis

In contrast to expectations the hippocampal lesioned animals were not impaired

in the holeboard task. In fact the only impairment evident in the task was that of caudate lesioned animals which, in the first four blocks, took longer (on average) to complete a 4-hole trial than either the neurologically intact controls or the hippocampal lesion animals.

Fig. 7.19, Average Time Taken to Complete a (4-hole) Trial (Error bars ± 1 SEM)



This lack of any behavioural impairment due to a hippocampus lesion is unexpected as the hole board task is presumed to be a spatial memory task, and thus should reveal an impairment in rats with limbic system dysfunction. Other spatial memory tasks (e.g. the radial-maze and the water-maze) are impaired by hippocampal lesions (Squire, 1992; Cohen and Eichenbaum, 1993; Kesner, Bolland and Dakis, 1993; Devan, Goad and Petri, 1996; Floresco, Seamans and Phillips, 1997; Aggleton and Brown, 1999) and several authors have concluded that the hole board is also a sensitive test of animal spatial memory (van der Staay, van Nies, and Raajimakers, 1990; Raajimakers, Blockland, and van der Staay, 1993; van der Staay, 1999).

However, closer examination of the procedures used in normal (i.e. food rewarded) hole board studies reveal several conceptually important differences between those protocols and the protocols used in the current study. The usual practice in hole board tasks is to place the animal in a new starting position within the maze on every session, if not every trial. This is identical to the procedure used in the water maze task to prevent the use of stereotyped response strategies. If hippocampal

lesioned rats are placed in the same starting position every time in water maze task they soon learn to locate the hidden platform and do not display a behavioural deficit (McDonald and White, 1994; Devan, Goad and Petri, 1996; Setlow and McGaugh, 1999). However, if instead the rat is placed in a new starting position then its attempts to use a stereotyped response are not effective and it displays a behavioural deficit. Unfortunately our hole board procedure did not do this. Not only were the rats *not* removed from the maze between trials but the starting position at the beginning of each session was always the same. On closer examination of the data it was discovered that all rats regardless of neurologic status adopted a stereotypical pattern of behaviour. After initially orienting themselves in the hole board they nose-poked their closest target hole and then ran around the maze in a circular pattern nose-poking target holes as they went. Furthermore, because rats were always placed in the same starting position there was little requirement for spatial cues once they had learned the position of the closest target hole and could simply employ a stereotyped pattern of behaviour straight away

Why the usual procedures were not employed in the ICSS version of the hole board is largely due to the success of using ICSS in SRT task. Using ICSS in the SRT enabled the use of far more trials of (essentially) non-interrupted behaviour than normal. Because the success of the initial ICSS-SRT study was so recent at the time when the hole board task was being designed it was decided to apply these advantages to the hole board task as well. Thus rats would be allowed to behave continuously and perform far more trials per sessions than normal. This however was a serious error. By obviating the need for a rat to constantly employ spatial cues to reorientate itself in the maze (as removing it between trials and replacing it in a different start position for each trial would do) the task requirements were fundamentally altered, such that deficits in spatial memory would no longer produce impairments in the task. Instead, if anything, the task could be performed via more procedural / non-declarative functions (once the target holes were learnt) and this may explain why the caudate lesion rats were initially slower to complete trials.

It is also quite common in hole board tasks for the hole board to wiped down between trials in order to destroy odour trails between target holes as rats can simply follow their odour trail on subsequent trials. Once again because of the use of ICSS

and a relatively short ITI this was not done. Thus it was possible for rats to follow their own odour trail on subsequence trials after first successfully locating their particular target holes.

In summary the failure to remove rats from the hole board between trials, the failure to use different starting positions within the maze at the start of each session / trial, and the failure to prevent rats following odour trails *substantially* reduces the spatial memory components of the task. Rats are instead able to rely on stereotyped behaviour which is not impaired by hippocampal lesions, and for this reason hippocampal rats were not impaired in the hole board. Therefore, the present version of the ICSS hole board task is invalidated as a test of allocentric spatial memory and can offer no useful evidence for contrasting with SRT task performance and thus can not be used to demonstrate a double-dissociation between memory task and neural substrate.

However, while the hole board task in the form used in this experiment is invalid it has at least demonstrated that it is possible to use ICSS reward with the task. Thus, an obvious avenue for future research will be to repeat the hole board portion of the study while employing appropriate methodological procedures. Under these conditions it is expected that hippocampal lesions will produce a spatial memory deficits in the hole board.

General Conclusions of the Chapter

The first aim of the animal empirical work presented in this thesis was to demonstrate that the rat-SRT is a valid and reliable analogue of the human SRT. This aim was largely realised in the control study Chapter 6. However, the current study also provides some evidence of this type. The finding that rats with neurological lesions can perform the rat-SRT provides further evidence that the rat-SRT is a valid animal-analogue of the human SRT. Furthermore, the fact that rats with caudate lesions could perform the task at the short trial length is a particularly compelling example of the SRT's validity in light of the caudate rat's impairment at the longer sequence length. It is not difficulties with attending to or responding to stimuli that produces the SRT impairment displayed by caudate lesion animals, but rather an inability to learn a (longer) repeating sequence.

The second aim of the animal empirical work presented in this thesis was to demonstrate that the rat-SRT task was reliant on the same neural substrates as the human SRT which was addressed by experiment 1. Although not impaired in the short sequence conditions (Part 1; SRT4) caudate lesion rats were impaired on the 8-trial repeating sequence (Part 2) in contrast to neurologically intact and hippocampal lesion rats. This is clear evidence that rats rely on the basal ganglia to perform the SRT in the same way humans do. Not only did caudate lesion animals have markedly weaker interference effects than the control and hippocampal lesion rats that experienced the repeating sequence but there was also some suggestion that caudate rats reacted more slowly overall than these other rats (see reaction time learning effect in Part 2). In conclusion basal ganglia dysfunction produces a clear and reliable impairment in the SRT that is not related to an inability to perform the underlying requirements of the task.

In summary this chapter presented strong neuroanatomical evidence for the analogical validity of the rat-SRT task as a model of a human non-declarative memory task. In combination with the strong theoretical and behavioural evidence from Chapters 2 & 4 and Chapters 5, 6 & 7 (respectively) this means that there are now multiple grounds for claiming that the rat-SRT is a reliable, valid and useful animal-analogue of the human SRT task.

As a consequence of having achieved both of the primary aims of the thesis the following chapter (8) will present the general discussion and conclusions of the thesis. Chapter 8 will also discuss the consequences of the results of the analytic studies presented in this thesis (both human and animal) in relation to the primary theories and issues presented in the thesis. In particular it will be shown how the findings of both the meta-analysis and the rat-SRT studies apply to the general multiple-memory systems theory, the declarative / non-declarative memory dissociation, and the validity and utility of the SRT task as a test of non-declarative memory. Limitations of the thesis and suggestions for future research will also be discussed.

Chapter 8

General Discussion and Conclusions

General Contributions

The primary aim of this thesis was to examine the contributions of the SRT task to the multiple memory systems theory and to develop an animal model of the human SRT task.. In the first instance a qualitative review of the human SRT literature (Chapter 2) was presented in which the validity of the SRT task as a test of non-declarative memory was discussed. Although a number of limitations of the task and issues of conceptualisation were raised the ultimate conclusion of the chapter was that the SRT task, when used appropriately, is a sensitive measure of non-declarative memory. Hence the SRT is a useful candidate for demonstrating a dissociation between declarative and non-declarative memory and consequentially providing evidence for the multiple memory systems theory.

Both humans and animals have declarative and non-declarative memory systems. The human evidence presented in Chapters 1, 2 and 3 is generally difficult to reconcile with the idea of a unitary memory system. It is possible to demonstrate a double-dissociation of memory task and neural substrate in animals which are considered to be strong evidence of multiple memory systems (in both animals and humans). Nevertheless, because the 'other' animal memory tasks that are typically dissociated from spatial learning tasks are not valid analogues of human non-declarative tasks it is difficult to meaningfully relate this dissociation to humans. In summary the work presented in this chapter will discuss the specific contributions made, and revisit briefly some of the main issues addressed in the thesis, and suggest some directions for future research.

In contrast to the contributions of the human SRT to the multiple memory systems theory the animal experimental contributions to non-declarative memory functions are more problematic. The thesis provided a succinct summary of the animal memory literature and found that animals show good evidence of multiple systems. It was also concluded that, irrespective of the demonstration of dissociable memory systems in animals, problems of validity and the lack of explicit animal analogues of a human non-declarative memory task made it difficult to draw firm conclusions about human non-declarative memory from animal non-declarative memory tasks. Prior to completion of this thesis only two pilot animal SRT studies appeared using monkeys (but see DeCoteau and Kesner, 2000, for a recent radial maze rat "SRT" task based on the animal learning a sequence of places). This thesis has provided two new examples of rat SRT tasks, the fan-maze and ICSS task. It has been shown both that animals can perform a SRT task, and that rats with caudate lesions show an impairment on the ICSS-SRT task, the same neural substrate appears to be especially important for humans when they experience the SRT. It was also shown that damage to the brain area known to be responsible for the animal analogue of human declarative memory, the hippocampus, did not disrupt the animal SRT task. Instead, hippocampal lesions even facilitated SRT performance. Thus the rat-SRT is a valid animal-analogue of the human SRT and provides new evidence of multiple memory systems in rats which is conceptually close to the evidence that generated the hypothesis of: human multiple memory systems.

Theoretical Contributions of the Thesis

This thesis has reported results consistent with the multiple memory systems theory. Rats show evidence of non-declarative sequence learning which, in conjunction with the animal memory research presented in Chapter 4, strongly suggest that rats have at least two memory systems. Furthermore, rat-SRT performance demonstrates a dissociation between injury to different neural structures which provides further evidence of the independence of these memory systems, which in turn provides additional support for the general multiple memory systems theory.

The empirical evidence presented in this thesis provides further support for

Reber's principle of commonality, that evolutionarily earlier forms and functions will be displayed across species. By providing good evidence that rats perform the SRT in much the same way as humans, and that rats rely on the same neural substrate for SRT performance as humans, the thesis provides empirical support for the theory that humans and animals have similar (multiple) memory systems. In consequence therefore the empirical evidence presented in the thesis also supports Reber's axiom that consciousness "is a late arrival on the evolutionary scene", and hence must be largely independent of the phylogenetically older non-declarative

Not-Declarative Memory vs. Non-declarative Memory

As some authors (Rovee-Collier, Hayne and Colombo 2001) have noted non-declarative memory is often (implicitly) defined as those memories that are not declarative i.e. definition by dissociation rather than association. This characterisation neatly encapsulates a point that was made in Chapter 4. All too often the declarative memory system in animals is dissociated from 'another' memory system which is *not-declarative* rather than non-declarative. Although there is good evidence to suggest that animal declarative memory is dissociable and independent from a memory system that is not-declarative, there is currently little evidence that the declarative memory in animals is dissociable from non-declarative memory in particular. Largely because the 'not-declarative' memory tasks that are used in animals do not make good conceptual or behavioural contact with human non-declarative memory tasks. Thus the rat-SRT provides an ideal opportunity to demonstrate a dissociation between declarative and non-declarative memory in animals, which is far more generalisable to human memory than the current declarative / not-declarative dissociation.

Contributions of the Meta Analysis

A surprising but important finding in the thesis was that a meta-analysis of the human SRT data showed that subjects with limbic system amnesia suffer a mild SRT impairment. As discussed in Chapter 3 this contradicts both the multiple memory systems theory and the conclusions of virtually all the amnesic studies included in the meta analysis. If this finding is an accurate reflection of the behaviour of limbic systems amnesics, *and* is a consequence of the same dysfunction that produces the amnesia, then this poses a serious challenge for the independence of memory

systems. There seems little doubt that this finding is valid. All but two of the ten studies that made up the limbic system neuropathological group contained indications that the amnesic subjects were not as unimpaired relative to controls as the studies had concluded. As shown by the power-analysis, this disparity between the studies' conclusions and the meta-analysis' conclusions is largely due to the low power enjoyed by these studies and consequent insensitivity to group differences. Combining these studies in a meta-analysis enabled us to perform a more sensitive test of group differences than that of the individual studies which meant that the meta-analysis provided a far more accurate and reliable estimate of group differences than any of the individual studies. Using such an approach under conditions of low power greatly increases the risk of making a type-II error, in this case failing to reject the null hypothesis of no difference between groups. One of these SRT studies employed mildly dementing AD patients (Ferraro, Balota and Connor, 1993) and reported that these subjects were indeed impaired relative to controls. The fact that these subjects were mildly dementing confounds the interpretation of their SRT deficit. However, the test of homogeneity for the limbic system neuropathology (LSN) group was not significant for either the group as a whole or for its constituent aetiologies. The effect sizes for the three aetiologies collapsed into the general LSN group were very similar between groups (AD d=0.6, MA d=0.77, and KS d=0.79) and very similar to the LSN group as a whole (LSN d=0.68). In particular it is useful to note that studies with 'pure' amnesics (Reber and Squire, 1994 & 1998) suggest the same degree of impairment that is suggested by studies with other limbic system disorders. While the LSN SRT literature is confounded by the wide variety and extent on neural pathology in these groups, and the presence of other forms of cognitive dysfunction in some of the aetiologies (e.g. dementia in AD) we can be confident that the three constituent LSN groups are very consistent between themselves and demonstrate a very similar degree of impairment. Thus we can conclude that the conclusion that there is a SRT impairment in LSN subjects is reliable. Given that the LSN groups all suffer a very similar degree of SRT impairment it is tempting to conclude also that there may be something common to these three aetiologies that produces their SRT impairment.

Not only are LSN subjects impaired in the SRT but so to are subjects with neither limbic system or basal ganglia dysfunction. The 'ON' group in the meta-analysis were markedly impaired in the SRT task, even more so than BGN subjects in

places. The widespread nature of the brain damage in the ON aetiologies suggests a number of different regions are important for SRT behaviour. However most of the individual aetiologies that make up the ON group produce frontal brain damage (with the obvious exception of the subjects with cerebellar lesions), and this may produce the SRT impairment in such subjects. This also suggests that the impairment seen in LSN subjects may be a consequence of the non-specific frontal neuropathology common in such disorders. The LSN SRT impairment in particular provides a strong rationale for exploring the LSN SRT impairment in animal subjects as animal studies using focused brain lesions should be capable of determining why LSN subjects suffer a SRT impairment. The thesis supported this point by presenting empirical data that suggests that the SRT impairment in LSN subjects is not a consequence of limbic system pathology. The fact that rats with hippocampal lesions were not impaired in the SRT and even demonstrated a mild facilitation of SRT performance suggests that it is not limbic system dysfunction *'per se'* that produces the SRT impairment in LSN subjects (the hippocampus being a major component of the limbic system). As noted in the discussion of Chapter 3 many of the aetiologies that produce amnesia also produce other forms of 'non-specific' neuropathology (AD in particular). Hence it may be that this additional, non-limbic, neuropathology is what produces the mild SRT impairment in LSN subjects. This possibility is strengthened by the fact that many of the aetiologies included in the LSN group are known to produce some disruption to the frontal area of the brain (see Aggleton and Brown, 1999).

The meta-analysis also concluded that subjects with basal ganglia damage are substantially impaired in the SRT. This corresponds with the multiple memory systems literature as a whole and the SRT literature in particular. The finding that basal ganglia patients are more impaired in the SRT provides evidence of a (partial) dissociation between different the limbic system and the basal ganglia in SRT performance.

Behavioural Contributions of the Current Research

The double (and triple) dissociations between memory task and neural substrate discussed in Chapter 4 provided clear evidence that animals have multiple memory systems. Furthermore, such dissociations are superficially similar to the human memory dissociations discussed in Chapters 1 & 2. Based on the similarity in

human and animal memory work therefore, and in particular the similarities in their memory behaviour, there are strong *a priori* grounds for expecting animals to use an animal analogue of human non-declarative sequence learning when they (the animals) perform a SRT task. Moreover, the studies discussed in Chapter 4 provide similarly strong grounds for expecting that the animal non-declarative memory system used to perform the rat-SRT would be dependent on the basal ganglia.

As established in Chapter 4, however, prior to the development of the current rat-SRT there was no good animal analogue of a human non-declarative memory task. Although many studies had demonstrated reliable dissociations between allocentric spatial learning and reference / procedural memory tasks these latter tasks have poor conceptual and behavioural connections with human non-declarative memory tasks. As discussed in Chapter 1 brain / behaviour relations in animals are often different (in varying degrees) to human brain / behaviour relations (Schacter and Tulving, 1994) and as a result it would seem theoretically and behaviourally useful to ensure that the tasks used to test and compare memory systems in animals and humans are as similar as possible. Thus there was a need for a reliable animal analogue of a human non-declarative memory task in order to help validate the generalisation of the results of memory studies from animals to humans (and vice versa).

The rat-SRT extended the exploratory SRT work recently reported with non-human primates (Nixon and Passingham, 2000; Procyk *et al,* 2000). Critically rats that experienced a repeating sequence demonstrate an interference effect when switched from the repeating- to a random-sequence. This was first demonstrated in the fan-maze (Chapter 5) in which rats demonstrated behaviour consistent with the presence of absence of a sequence switch. Specifically, rats demonstrated an interference effect when switched from repeating to random sequence and, just as importantly, demonstrated no interference effect at the same point in the fan-maze session when the repeating sequence was not switched to random. Thus the interference effect across days / sessions was not an anticipatory response.

Examination of the contribution of the rat-SRT to multiple memory systems was somewhat more difficult than of the contribution of the human SRT, given that at the

start of this project there was no rat-SRT. However, this thesis has now demonstrated three critical strands of evidence necessary to validate the rat-SRT as an animal analogue of the human task: conceptual / theoretical (Chapters 2 & 4), behavioural (Chapters 5, 6 & 7) and neuroanatomical (Chapter 8).

In contrast to using food to reward behaviour, and in contrast to the limitations of the fan-maze, the ICSS-SRT task has a number of advantages. Rats rewarded with ICSS experience far less behavioural interruption during performance and can thus respond rapidly and continually. The ICSS-SRT procedure is substantially more similar to the human SRT and has far fewer spatial cues than the fan-maze. Under the right experimental conditions the rats in the ICSS-SRT task demonstrated robust and reliable sequence earning behaviour deteriorated markedly when switched from a repeating to a random sequence. Those rats that only ever saw random sequences demonstrated no evidence of an interference effect which means that any interference effect demonstrated as a consequence of a switch from repeating to random sequence was not a general behavioural artefact (e.g. due to fatigue).

The SRT control study (Chapter 6) clearly demonstrated that spaced learning is not an effective technique for sequence learning in the SRT. This contrasts with many memory tasks in rats that report a clear advantage of spaced learning (e.g. DeCoteau and Kesner, 2000. Why learning a repeating sequence over several long sessions should not produce any sequence learning (Groups 5 & 6 in Chapter 6) is uncertain. Irrespective of the fact that learning is broken down into three sessions each session still contains far more trials (940) than is usual in rodent behavioural tasks. Nevertheless, spaced learning was not a viable technique in the ICSS-SRT task. Instead the clearest indication of sequence learning came from a single session of massed repeating-sequence trials.

A useful metric for the validity of a behaviour is the convergence between different measures of the same phenomenon. Thus it is important to note that reaction time and error-rate measures produced the same pattern of findings. In fact there is some evidence that, irrespective of the much higher error-rates in rats than in humans, error-rates made be a slightly more sensitive measure of sequence learning in rats (compare Figs. 6.7 & 6.10, and Figs. 7.6 & 7.9 in particular).

Intra Cranial Self Stimulation

Although ICSS had been used as a behavioural reward in the past (e.g. Stellar and Gallistel, 1975) it had only been used either to contrast ICSS reward with food reward systems and / or to examine the parametric functions of the ICSS phenomenon itself. The novel use of ICSS as a behavioural reward for complex behaviour clearly indicates its utility and advantages as a reward delivery system for such behaviours. Not only is ICSS minimally disruptive during behaviour but it also promotes fast and continual responding. The other great advantage of ICSS is that it is non-satiating which enabled the use of many more trials within a session than would be possible with food reward. The non-disruptive and non-satiating features of ICSS in particular made it ideal for use with the SRT task which requires rapid and continuous responding over a large number of trials. Although rats can learn a SRT when motivated by food reward (the fan-maze) the limitations of the food reward system (i.e. behaviourally disruptive, variably motivation, and satiation) introduce serious conceptual and empirical confounds into a SRT task in particular.

Neurobiological Contributions of the Current Research

The fan-maze and SRT control study tasks used different sequence lengths, 12 and 4-trial, respectively. The use of the short sequence length raises the issues of whether or not rats were able to acquire declarative knowledge of the sequence, as humans most certainly would. If rats do develop declarative sequence knowledge then their behaviour may not be indicative of non-declarative sequence learning, defeating the point of the model. However, there are two strands of evidence that suggest rats did not acquire declarative sequence knowledge. Firstly neither of the groups in the SRT-control study (Chapter 5) that experienced repeating sequences over multiple sessions (Groups 5 and 6) demonstrated any sequence learning. If rats can declaratively acquire the short repeating sequence it seems reasonable to suggest that they should be able to do within even one session, let alone across three, given that declarative memory acquires information very quickly (e.g. even after a single exposure). Thus the fact Groups 5 & 6 demonstrated no sequence learning at the end of session three (Group 6, switched to random at the end of each of the three sessions, also displayed no interference effect in any session) suggests they did not acquire declarative knowledge of the repeating sequence. Secondly, and critically,

caudate lesioned rats demonstrated a SRT impairment at both a moderate (8-trial) and long (12-trial) sequence length but not at a short (4-trial) length (but note that even neurologically intact repeating-sequence control rats could not learn the 12-trial repeating sequence). As the caudate is the neural correlate of non-declarative, but not declarative, memory caudate rats should not have suffered any SRT impairment if they learnt the sequence declaratively. Furthermore, as the hippocampus is the neural correlate of declarative memory SRT performance that relies on declarative memory should be impaired by a hippocampal lesion. Instead the hippocampal lesion actually facilitated SRT performance. Given this pattern of results there is every reason to conclude that rats learned the repeating sequence non-declaratively.

The hippocampal facilitation effect is not a complete surprise given that the hippocampus is typically thought of as responsible for declarative memory. Although most theorists adopt the position that each memory systems is solely responsible for the memory tasks associated with it, it is undoubtedly the case that, except in extreme instances, memory tasks are not process pure. The obvious speculation therefore as to why hippocampal lesions facilitated SRT performance is that an intact declarative memory system actually interferes with SRT behaviour. The suggestion is that the declarative memory system interferes with non-declarative memory when the organism attempts to use declarative memory to solve the problems posed by the task but the information required is only available to the non-declarative memory system. Thus the declarative memory system constantly chases spurious relationships between stimuli which interferes, to a fairly limited extent, with the non-declarative memory system. As a result an impaired declarative system (i.e. as a consequence of a hippocampal lesion) may interfere to a far lesser extent than an intact system would, thus facilitating non-declarative memory performance. Previous theoretical formulations (e.g. Eichenbaum, 1999) have also suggested that any learning situation will use a combination of declarative and non-declarative memory and that instances of facilitated performance after hippocampal lesions may reflect a shift in this balance.

As noted in the discussion to the previous chapter the dissociation of the effect of caudate lesions and hippocampal lesions on rat-SRT behaviour is verification that the rat SRT relies on at least one neural substrate for SRT performance in common with humans. Hence this is good neuroanatomical evidence that the rat-SRT is a valid

animal-analogue of the human SRT and, furthermore, that rodent non-declarative memory is dissociable from rodent declarative memory.

Unresolved issues and Future Research

Sequence Length

There is a disparity between the parametric analysis of the effect of sequence length on SRT performance in the rat-SRT task and studies in the human SRT literature. While neurologically intact rats can learn both a 4- and an 8-trial sequence they are incapable of learning a 12-trial sequence (but note that hippocampal lesion rats demonstrate some evidence of sequence learning at this trial length). In contrast Stadler (1992) and Stadler and Neely (1997) concluded that human SRT performance is independent of sequence length. Stadler (ibid) found that, if anything, SRT performance actually *improved* at the longer (16-trial) sequence lengths. However, two points must be kept in mind when comparing these results to those of the rat-SRT. Firstly, it is not so much that the SRT performance of neurologically intact rats show a length dependent deterioration in SRT behaviour, but more that they demonstrate very similar interference effects in both the 4- and 8-trial tasks and then no interference effect at all in the 12-trial task. It may be therefore that there is simply an all-or-nothing effect with non-declarative sequence learning and Stadler *et al*, simply did not use long enough sequence lengths to observe this in humans. Note: it I also possible than the subjects simply learnt fragments of the repeating sequence and thus treated the task as if it were a shorter repeating sequence and thus generated an interference effect.. Given that humans are, by definition, cognitively superior to rats the notion that rats and humans perform the SRT in similar ways but that rats can not learn sequences as long as those humans can learn seems reasonable. Secondly, there is contrasting evidence of a sequence length effect in humans. Pascual-Leone *et al* (1993) demonstrated progressively weaker interference effects in humans over 8, 10, and 12-trial sequence lengths. Furthermore, this progressive weakening in interference effect is seen in both control and PD subjects, although it was more marked in the PD subjects.

Thus there is some disagreement as to the role sequence length plays in SRT performance. Unfortunately it appears that the utility of the rat-SRT task to address this issue is exhausted as it is difficult to use a sequence length between 8 and 12

trials in the 4-hole apparatus without introducing frequency of stimulus presentation confounds (i.e. as with the 10-trial repeating sequence in humans). It may however be possible to test a 10-trial repeating sequence in rats if a 5-hole apparatus was employed, or even a 9-trial sequence with a 3-hole apparatus, although altering the number of stimulus positions may introduce problems of its own.

The Lack of a Double-Dissociation in the Current Research

The most serious unresolved issue of this thesis is the failure to demonstrate a double-dissociation between memory task and neural substrate. There is every reason to expect that if the hole board had been run correctly it would have provided evidence of a spatial working memory impairment in hippocampal lesion rats but not in caudate lesion rats, and thus a double dissociation between memory task and site of neural injury. While the absence of double-dissociation does not threaten the empirical and theoretical contributions of the thesis these contributions would have been even more compelling had the double-dissociation been demonstrated. A repetition of the hole board study in light of the lesions learned in this thesis is an obvious, and useful, possibility for future research.

Pharmacological Studies

Packard and White, (1991) and White, Packard and Seamans (1993) demonstrated that dopamine plays a critical role in memory in both the hippocampus and caudate. The administration of dopamine (ant)agonists into the caudate and hippocampus during SRT performance would help establish the role of dopamine in the SRT and thus non-declarative memory. This would be useful given that human basal ganglia dysfunction often results from dopamine degeneration (i.e. Parkinson's disease). It may even be worthwhile to target dopamine receptor subtypes (e.g. DA1 and DA2) to see if different population of dopamine receptors play different roles in non-declarative memory. A similar technique for increasing the specificity of the neural substrate under scrutiny would to infuse a local anaesthetic (e.g. lidocaine) into the caudate during SRT behaviour and observe the effect. It would of course be especially useful to demonstrate that temporary inactivation of the caudate (either in its entirety or restricted sub-receptor populations) disrupted SRT performance but that once the inactivation wore off normal performance was reinstated.

Pre-Frontal Cortex Lesions

Given the finding in the meta-analysis that pFC lesions (and other aetiologies that may affect the frontal region of the brain, e.g. AD and close head injury) seriously disrupt human SRT performance, and that (unconscious) attention is an important component of non-declarative memory it would be useful to examine the effect of pFC lesions in the rat-SRT. Such research could help to tease out the role of attention and also (non-specific) frontal neuropathology in human non-declarative memory.

Limbic System / Hippocampal Lesions

While hippocampal rats demonstrated some facilitation of SRT performance the lesions were relatively small and largely restricted to the CA1 and dentate gyrus (see Fig. 7A.2). Thus an examination of larger hippocampal lesions and / or lesions to other limbic system structures might help to discover why amnesics are impaired in the SRT. It may be, for example, that the small lesions used in Chapter 7 were insufficiently disruptive to produce any impairment. If rats with larger hippocampal lesions and / or other limbic system lesions were still unimpaired in the SRT this would provide strong empirical support for the hypothesis discussed in Chapter 3 and above. That the amnesic SRT impairment seen in the meta-analysis is not due to limbic system dysfunction but rather to non-specific neuropathology of the type common in the various amnesic aetiologies. Oh the other hand, if rats with larger hippocampal lesions or other limbic system lesions *were* impaired in the SRT this would suggest that the hippocampal lesion used in this thesis were too small. If such rats were impaired in the SRT it would also suggest that the amnesic SRT impairment demonstrated in the meta-analysis was due to limbic system dysfunction, with appropriately serious consequences for the multiple memory systems theory.

Future Research in the Human SRT

An unexpected finding in the meta-analysis was the lack of relationship between SRT performance and dementia. By definition dementia produces cognitive dysfunction, and more severe dementia produces commensurately greater degrees of cognitive impairment which may eventually include non-declarative memory. Alzheimer's disease results in progressively worsening dementia as a direct consequence of progressive brain damage. However, brain

damage in AD is not limited to the limbic system (except in the very early stages), but includes damage to the widespread cortical association areas and, ultimately, subcortical areas including the basal ganglia. Thus it is reasonable to assume that dementia will produce a SRT impairment related to the severity of the dementia. Empirical evidence for this is provided by Ferraro, Balota and Connor (1993) who found that very mildly dementing AD subjects were not impaired in the SRT but mildly dementing subjects were. Although, the meta-analysis found that there was no relationship between severity of dementia and SRT performance there is some evidence that this conclusion may be incorrect. Firstly, even after combining studies in the meta-analysis there are still relatively few subjects in each of the groups and thus the moderator analysis of severity of dementia may be insufficiently sensitive to any group differences by virtue of low power. Secondly, when the two dementia groups were analysed separately from the non-dementing subjects there was a marginally non-significant difference between groups, and the lack of significance may again be due to low power. Thus there is some suggestion that severity of dementia may be related to SRT impairment. Finally it must be remembered that only very-mildly and mildly dementing subjects were included in the analysis and it seems likely that more severely dementing subjects would produce a very clear SRT impairment.

Due to the uncertainty surrounding the relationship between dementia and the SRT a study of limbic system it would be useful to examine the effect of relatively pure limbic system pathology. The problem is of course, as discussed in the meta-analysis, it can be difficult to obtain sufficiently large sample sizes of ‘pure amnesics’, and thus assuring adequate power. A possible population to examine instead might be those subjects with ‘age associated memory impairments’. This diagnosis is a very reliable predictor of AD (~80% of people diagnosed with it go on to develop AD) but is characterised as a *pre-dementing* phase to the disorder (Bartes-Faz *et al,* 2001). Hence, subjects with this diagnosis have relatively pure limbic system pathology. it may be that even though these subjects suffer amnesia they would not be impaired in the SRT as they suffer amnesia in the absence of dementia (by virtue of their pre-AD diagnosis). If this is the case it may be more appropriate to compare these subjects with controls in the SRT rather than diagnosed AD patients (even the very-mildly dementing AD patients in Ferraro, Balota and Connor, 1993). If subjects with an age associated memory impairment diagnosis were not impaired in the SRT relative to controls this would suggest that it is the addition / non-specific neuropathology

associated with the more severe amnesia that produce the mild SRT deficits in LSN subjects seen in the meta-analysis. If, on the other hand, these subjects are impaired in the SRT this would suggest that the SRT does rely on the limbic system to some extent. However, given that the very-mildly dementing AD patients in Ferraro, Balota and Connor, (1993) were not impaired relative to controls, and given that the hippocampal lesioned rats in Chapter 7 showed a mild facilitation of SRT performance, it seems unlikely that subjects with an age associated memory impairments diagnosis would not be impaired in the SRT.

Conclusions

In conclusion the thesis has demonstrated that human subjects with limbic system amnesia are impaired in the SRT in contradiction to the various studies in the literature. The failure of SRT studies with amnesic patients to report any difference between amnesic and control groups is due to SRT studies generally having poor power. However, the animal evidence presented by this thesis also demonstrated that this SRT impairment in amnesic subjects is most likely not a consequence of limbic system dysfunction, and suggested that it may instead be due to the additional (frontal) neuropathology common in such disorders.

The thesis has also presented empirical evidence that rats posses a non-declarative system that is behaviourally and neuroanatomically analogous to human non-declarative memory, at least as measured by the SRT task. Thus rats are likely to posses multiple memory systems related to those found in humans.

While the failure to demonstrate a double-dissociation of memory system and brain structure is regrettable it does not detract from the ultimate conclusion that the ICSS-SRT task is a valid analogue of the human SRT task. Hence the rat-SRT is likely to be useful for: examining the neurology of non-declarative memory, the pattern of spared / impaired non-declarative memory in different neuropathologies, and the multiple memory systems theory as a whole.

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