RECOVERY FOLLOWING

ANTERIOR THALAMIC LESIONS

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Abstract

Extensive neural connections between the anterior thalamic nuclei (ATN) and the hippocampal system may explain the overlapping amnesic syndromes associated with diencephalic and medial temporal lobe brain injury. Despite the debilitating nature of the diencephalic amnesia, treatments for this condition are lacking. In rats, lesions to the ATN or hippocampus generally produce similar memory deficits, which further implicate these structures in a single functional memory system. First evidence is presented here that seemingly permanent and robust spatial working memory deficits seen after lesions to the ATN in rats are ameliorated by environmental intervention and pharmacological treatment. Post-operative housing of ATN-lesioned rats for 30 days in enriched environment resulted in marked improvements in performance on the spatial working memory task in the cross-maze irrespective of whether rats were exposed to enrichment immediately after surgery or enrichment was delayed by 40 days post-surgery. Long-term beneficial effects of enrichment were also demonstrated. Behavioural improvements were observed when Cerebrolysin - a neurotrophic compound - was injected intraperitoneally for 30 days post-surgery. The combination of enrichment and Cerebrolysin treatment was more effective in inducing recovery on a delayed memory test in the cross-maze task. The influence of enrichment and Cerebrolysin on the neural changes produced by ATN lesions was examined utilising an immediate early gene marker c-*fos*. Replicating previous studies, ATN lesions produced marked hypoactivity in the retrosplenial cortex, but this effect was not reversed by either enrichment or Cerebrolysin. Unexpectedly, enrichment produced further hypoactivation in this region. Although lesion-induced deficits in a radial-arm maze spatial discrimination task were not improved by enrichment, a related study in our laboratory showed that spatial reference memory can also be improved by enrichment in ATN rats. The current research provides strong support for potential opportunities for therapeutic intervention in the human domain.

Chapter 1

Introduction

1.1 General Introduction

Memory is the ability to retain information and utilize it for adaptive purposes (Fuster, 1995). Efficient memory requires intact functioning of many brain regions. Converging evidence from functional imaging studies, neuropsychological investigations and laboratory research all points to the importance of the medial temporal lobes structures (Eichenbaum & Cohen, 2001) in normal learning and retention of information. Structures in the medial diencephalon have also been implicated in memory and learning (Aggleton, 2008; Aggleton & Brown 1999; Kopelman, 2002), although less is known about the contribution of these regions. Pathology in both medial temporal lobe and medial diencephalon is consistently associated with the presence of anterograde amnesia or inability to learn new information following an injury to the brain (Aggleton, 2008; Aggleton & Sahgal, 1993; Gold & Squire, 2006; Harding, Halliday, Caine & Kril, 2000; Kopelman 2002; Mair, 1994; Markowitsch, 1982; Vann & Aggleton, 2004; Victor, Adams, & Collins, 1971). Anterograde amnesia that develops as a consequence of damage to the diencephalon (or diencephalic amnesia) is most frequently observed in humans who develop alcoholic Korsakoff's syndrome, but can also occur in cases of tumors, cysts involving the third ventricle, infarcts, direct penetrating injury or in rare conditions such as Creutzfeld-Jakob disease (Harding et al., 2000; Kopelman, 1995, 2002; Kapur, Abbott, Lowman, & Will, 2003; Markowitsch, 1982; Schmahmann, 2003; Tsivilis, et al., 2008; Victor, et al., 1989; Van der Werf, Jolles, Witter & Uylings, 2003). This introductory chapter provides a brief initial synopsis of the more detailed information presented in subsequent chapters.

Although diencephalic amnesia was first investigated before temporal lobe amnesia its neural basis remains less certain. Classical neuropsychological studies have

struggled to provide clear evidence concerning the neural basis of diencephalic amnesia, as human cases of diencephalic amnesia are generally associated with a high degree of variability in both presentation and degree of damage sustained to the diencephalic and surrounding structures. Fortunately, valuable insights can be gained from functional neuroimaging studies and animal models of pathology. Within the diencephalon the structures that have been postulated to be involved in memory processes include the mammillary bodies and various thalamic nuclei including the anterior thalamic nuclei, nucleus medialis dorsalis, nucleus parataenialis, nucleus lateralis dorsalis and intralaminar nuclei (Aggleton, 2008; Aggleton & Brown, 1999; Gaffan & Parker 2000; Mair, 1994; Victor et al., 1989; Van der Werf, Witter, Uylings, Jolles, 2000; Van der Werf et al., 2003). More recently, the anterior thalamic nuclei (ATN) have received increasing attention as an important structure in supporting learning and memory processes.

A decade ago, Aggleton & Brown's (1999) influential review highlighted the similarity of the syndrome produced by the damage to the medial temporal lobe structures and the diencephalic structures and suggested that both structures work in parallel. After reviewing neuropsychological evidence and the effects of lesions to different parts of the hippocampal complex, and the thalamic structures with which they are associated, Aggleton & Brown (1999) postulated a model of hippocampal-medial diencephalic interactions. The model gives prominence to the anterior thalamic nuclei which together with the hippocampus, fornix and mammillary bodies form an "extended hippocampal system" vital for encoding and subsequent recall of episodic information. Since the model's conception, substantial amount of research has accumulated (reviewed in Chapter 3) which supports the link between anterior thalamic nuclei and diencephalic amnesia. The anterior thalamic nuclei have strongly been implicated in the pathology of Korsakoff's syndrome (Harding et al., 2000) and both anterior thalamic nuclei, the mammillary bodies and their neural connections (the mammillothalamic tract) have been associated with the severity of memory deficits seen following thalamic vascular accidents (Van der Werf et al., 2000; Van der Werf et al., 2003) (see Chapter 2). Laboratory investigations utilizing precise lesion techniques provided evidence that

damage to the anterior thalamus results in robust and permanent deficits in spatial memory (generally considered to be an animal equivalent of episodic memory in humans) and some aspects of non-spatial memory such as memory for temporal events in rats (Aggleton, Keith, & Sahgal, 1991; Aggleton, Neave, Nagle, & Sahgal, 1995b; Byatt & Dalrymple-Alford, 1996; Gibb, Wolff, & Dalrymple-Alford, 2006; Mitchell & Dalrymple-Alford, 2005; Mitchell & Dalrymple-Alford, 2006; Mitchell, Dalrymple-Alford, & Christie, 2002; Moran & Dalrymple-Alford, 2003; Warburton, Baird, & Aggleton, 1997; Warburton, Baird, Morgan, Muir, & Aggleton, 2000; Warburton, Morgan, Baird, Muir, & Aggleton, 1999; 2001; Wolff, Gibb, Cassel, & Dalrymple-Alford, 2008; Wolff, Gibb, Dalrymple-Alford, 2006). More recently, Aggelton (2008) suggested that the severity of the memory deficits observed after diencephalic damage and the similarity of the amnesia syndrome following hippocampal and anterior thalamic lesions may be explained by the presence of lesion induced pathology in another region, the retrosplenial cortex. The retrosplenial cortex has reciprocal connections with both hippocampus and the anterior thalamic nuclei and lesions in either of these sites lead to marked neuronal hypoactivity in the otherwise intact retrosplenial cortex regions (Albasser, Poirier, Warburton, & Aggleton, 2007; Jenkins, Dias, Amin, Brown, & Aggleton, 2002b; Jenkins, Vann, Amin, & Aggleton, 2004). Evidence that impaired ATN functioning can also negatively influence other ostensibly “healthy” brain regions offers exciting prospects particularly for the field of therapeutic intervention, where functional gains my be obtained via reactivation of these regions of covert pathology.

Despite the advances made in the understanding of the neuronal basis of diencephalic amnesia very little is known about the possibility of ameliorating the memory deficits. The degree of functional impairment demonstrated by diencephalic amnesia sufferers is often profound and in some cases, such as in Korsakoff’s syndrome can result in the individual living in a time zone of about three to five minutes, having little or no ready access to prior events. Finding effective therapeutic approaches which can to some extent reverse these memory deficits remains a challenge. Traditionally two modes of treatment have been associated with promoting recovery after brain insults in

animal models, namely, environmental experience, and pharmacological treatment (Nilsson, Perfilieva, Johansson, Orwar, & Eriksson, 1999).

Environmental enrichment has been extensively utilized in the last 60 years in the field of animal research to promote recovery of function in animal models of various neurodegenerative disorders and following acute brain damage (Will, Galani, Kelche, & Rosenzweig, 2004). Environmental enrichment generally involves housing large groups of animals in large cages with a variety of stimulus objects. Exposure to such living conditions has been demonstrated to enhance learning abilities in normal rats on a variety of spatial and non-spatial memory tasks (Cheal, 1987; Frick, Stearns, Pan, & Berger-Sweeney, 2003; Rockman, Borowski, & Glavin, 1986; Schrijiver, Bahr, Weiss, & Wurbel, 2002; van Praag, Kempermann, & Gage, 2000; Wong & Jamieson, 1968) and has been shown to attenuate or reverse the sequale of the central nervous system insults such as seizures, ischemia, infarcts, lesions and traumatic brain injury (Hamm, Temple, Pike, O'Dell, Buck, & Lyeth, 1996; Johansson, 1996; Johansson & Ohlsson, 1996; Kolb & Gibb, 1991). The effects of lesions to the structures in the medial temporal lobe, and in particular the hippocampus, is also sometimes ameliorated by exposure to enriched environments, with the animals demonstrating improved performance on the spatial memory tasks (Einon, Morgan, & Will, 1980; Galani, Jarrard, Will, & Kelche, 1997). However, no evidence is yet available on the potential of enrichment to induce recovery of function after lesions to diencephalic structures.

How effectively the brain can respond to injury and undergo structural repair has become one of the most exciting areas of contemporary basic and translational neuroscience research. Although there are no clinical treatments yet available to enhance repair of the damaged brain, a number of potential therapies are being investigated. New drugs are designed to provide some degree of neuroprotection by preventing injured or vulnerable nerve cells from dying, or they are given in hope of stimulating regenerative processes that could lead to the restoration or the formation of new cells. One such potential drug candidate is a porcine brain-derived peptide Cerebrolysin, postulated to

have general neuroprotective properties and neurotrophic effects (Satou, Itoh, Tamai, Ohde, Anderson, & Hashimoto, 2000; Windisch, 2000). Cerebrolysin has been most extensively used with Alzheimer's disease patients with some success in terms of delaying cognitive decline (Bae, et al., 2000; Muresanu, Rainer, & Moessler, 2002; Panniset, Gauthier, Moessler, & Windisch, 2002; Ruether, Ritter, & Apecechea, 1994; Ruether, et al., 2001; Ruther, Ritter, Apecechea, Freytag, Gmeinbauer, & Windisch, 2000). In the animal field, Cerebrolsyin was demonstrated to enhance memory and learning following fornix lesions (Francis-Turner & Valouskova, 1996), as well as in animal models of ischemia (Frey, 2002; Schwab, Antonow-Schlorke, Zwiener, & Bauer, 1998) and Alzheimer's disease (Rockenstein, et al., 2006). In comparison to other available neurotrophic factors Cerebrolsyin has the added and unique benefit of being able to cross the Blood Brain Barrier in pharmacodynamically active amounts, which permits systemic administration. The ease of Cerebrolysin administration, its low toxicity and postulated neurotophic properties make it an appealing choice when investigating recovery of functioning in diencephalic amnesia.

Sufficient evidence has now been accumulated that implicates diencephalic structures in memory functioning, with anterior thalamic nuclei playing an essential role in learning and memory for "episodic-like" information. It has been repeatedly demonstrated that the spatial/context dependent-type memory deficits after anterior thalamic lesions are profound and permanent, and yet no attempts so far have been made to examine whether these deficits can be reversed or at least attenuated. Examining the possible effectiveness of environmental and pharmacological type treatments after lesions to the anterior thalamus represents a reasonable first step towards developing better treatment models for sufferers of diencephalic amnesia.

1.2 Aims of the Present Study

The main aim of the present study was to focus on two different modes of treatment – Environmental Enrichment and the drug Cerebrolysin, and compare and contrast the effects of both of these interventions on recovery of functioning after neurotoxic anterior

thalamic lesions in rats. These objectives were accomplished in a series of four experiments. In each of the experiments highly selective N-methyl-D-asparate acid lesions to anterior thalamic nuclei were performed with an aim to minimize damage to any surrounding structures and fibers of passage and ensure reliable assessment of behavioral and memory changes associated with anterior thalamic damage.

The aim of the first experiment was to examine whether exposure to enriched environment introduced 5 days after anterior thalamic lesions can produce improvements in memory function. The animals were housed in enriched or standard cages for a 30-day continuous period post-surgery and then tested on an array of behavioral memory tasks. The primary task of interest was a spatial working memory task in a cross-maze, on which anterior thalamic lesioned rats ordinarily demonstrate seemingly permanent and severe deficits. A novel spatial memory task, which previously has not been used with animals with anterior thalamic lesions, was also employed to examine the animal's ability to discriminate between fixed locations in a radial-arm maze, using problems with different spatial pattern separations. The potential effects of anterior thalamic lesions and enrichment were also examined on a non-spatial memory task for reward value.

The encouraging results obtained following the first experiment prompted further investigations on the robustness of the enrichment effects. The second experiment focused on examining whether delaying the introduction of enrichment 30 days after surgery can still result in behavioral improvements on the spatial working memory task in a cross-maze. The animals' performance was also re-assessed some 4 months after surgery and when no further enrichment was occurring, to examine the long-term effects of the treatment intervention.

The goal of the third experiment was to evaluate whether administration of the drug Cerebrolyin following lesions to anterior thalamus can induce behavioral change. The drug was administered daily via intraperitoneal injections for 30 days post-surgery

and the spatial working memory abilities of the animals were subsequently assessed on the cross-maze.

The fourth and final study aimed to compare and contrast the therapeutic effectiveness of Cerebroslyin and enrichment and to examine whether combination of both interventions can result in further behavioral gains. Animals with anterior thalamic lesions were exposed to a 30-day period of enrichment, injections with Cerebrolysin or both soon after surgery and then tested on a spatial working memory task in a cross-maze. The potential beneficial effects of treatment administration were also evaluated on a neuronal level by examining the pattern of behavior induced changes in the immediate early gene product c-*fos*. This protein is a metabolic marker for neuronal activation which has been linked to mnemonic processes, including spatial memory (Herdegen & Leah, 1998; Tischmeyer & Grimm, 1999). The pattern of c-*fos* activation was examined following completion of behavioral testing post-surgery and post-treatment.

It was envisaged that the research would provide first available evidence on the potential for functional recovery following anterior thalamic lesions.

1.3 Outline of thesis

The following chapter provides an introduction on the involvement of anterior thalamic nuclei in diencephalic amnesia by examining human clinical data. The subsequent chapter discusses the animal models of memory deficits providing recent experimental evidence on the involvement of anterior thalamic nuclei in memory function. Chapter 4 gives an introduction to the field of recovery of function with a specific focus on environmental enrichment as a mode of intervention. Chapter 5 examines in detail the evidence from both human and animal literature on the effectiveness of Cerebrolysin in improving memory functioning following brain insults.

Subsequent four Chapters (6-9) provide details concerning the four experiments conducted that are mentioned above.

A general discussion of the findings is provided in the last Chapter (10). The experimental and theoretical relevance of the current research towards understanding of the possibility of recovery of function after thalamic injury is reviewed. Suggestions are also made for future directions in research.

Chapter 2

Human amnesia and the diencephalon

2.1 Introduction

In this chapter the case is made for anterior thalamic involvement in human anterograde amnesia, that is, a failure to learn new information following a brain injury. The potential role of diencephalic structures in amnestic deficits present in various human neuropathological conditions is discussed. As the anterior thalamus provides a focus of this thesis and plays a prominent role in many cases of diencephalic amnesia the chapter focuses primarily on the evidence pertaining to the involvement of anterior thalamus in amnesia and begins with the overview of the anatomy of this region. Brief introductory comments are also made on the contribution to memory of other brain regions, particularly structures in the temporal lobe.

2.2 Anterior Thalamus

The thalamus belongs to a group of brain structures that develop as the major part of the diencephalon (additional diencephalic regions include the hypothalamus and sub-thalamic nuclei). The thalamus consists of a number of large nuclei. The anterior thalamic nuclei constitute the region of particular interest in the current study, (see Fig 1).

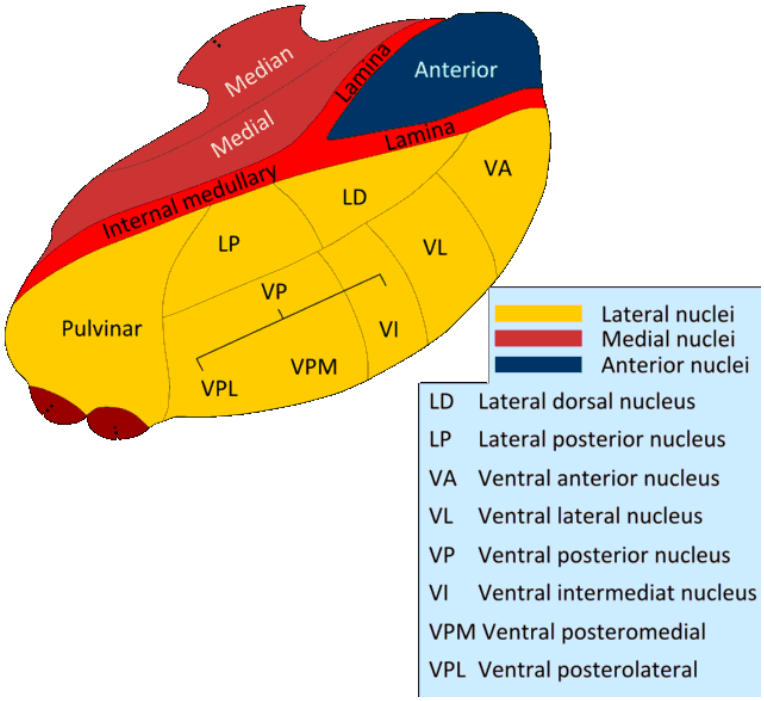

Fig 1. Schematic representation on the thalamic nuclei. From: http://en.wikipedia.org/wiki/File:Thalmus.png

The ATN are located in the anterior thalamus and consist of three subdivisions: anteroventral (AV) nuclei, anteromedial (AM) nuclei and anterodorsal (AD) nuclei. The ATN form part of the circuit first identified by Papez (1937 and cited in Aggleton & Brown, 1999) which also includes the hippocampus, mammillary bodies, and cingulate/retrosplenial cortices. The circuit was originally thought to be responsible for processing emotional responses, but this suggestion has been revised in the last decade and it is now postulated that the circuit is mainly involved in memory (Aggleton & Brown, 1999). The anatomical details of the circuit have also been reviewed. The ATN receive bilateral input from the hippocampal formation either directly via the subicular complex through the fornix (Aggleton, 1986) or indirectly via mammillary bodies and the mammillothalamic tract (Allen & Hopkins, 1988; Shibata, 1992; Vann, Saunders, & Aggleton, 2007). The ANT also have reciprocal connections with the retrosplenial cortex. This cortical region provides a route for indirect projections from the medial diencephalon to the medial temporal lobe (Amaral & Price, 1984). The ATN project directly back upon the hippocampal formation (Amaral & Cowan, 1980; De Vito, 1980). Aggleton and Brown (1999) suggested that within this "extended hippocampal system"

the ATN play a major role in diencephalic amnesia and that they function in a reciprocal fashion with the hippocampus.

Prior to Aggleton and Brown's (1999) influential review article relatively little research had investigated the memory role of the ATN in contrast to the medial temporal lobe regions of the brain, especially the hippocampus (Eichenbaum & Cohen 2001). This situation was due to the long held idea that thalamus functions mainly as a relay station which transmits information from the sub-cortical regions to the cortex (Jones, 1985). Memory deficits after thalamic lesions may arise from the disconnection of the information flow between the brain regions involved in memory (Macchi & Jones, 1997). More recently, however, it has been suggested (Aggleton, 2008; Aggleton & Brown, 1999) that the anterior thalamus contributes to episodic memory operation and is not just merely passively processing hippocampal outputs.

The following section provides an introduction to the main findings concerning the involvement of the temporal lobe and diencephalic structures in anterograde amnesia. Given Aggleton & Brown's (1999) idea that the anterior thalamus provides a nodal point within an extended hippocampal system, a brief summary of some of the relevant human cases of temporal lobe amnesia is presented prior to addressing the issue of human diencephalic amnesia.

2.3 Temporal lobe amnesia

The accepted definition of anterograde amnesia emphasizes the presence of a severe and permanent deficit for the recall of recent events that contrasts with intact short-term memory, IQ, semantic memory, skill learning, and priming (Parkin, 1991). The most pronounced effect is the loss of episodic memory, which is the capacity to remember specific personal experiences that occur in a unique spatial and temporal context.

The classic example of the anterograde amnesia deficit comes from the recently deceased patient, HM, who underwent surgery in 1953 to remove substantial portion of his medial temporal lobes bilaterally in order to alleviate severe epileptic seizure activity

(Corkin, Amaral, Gonzalez, Johnson, & Hyman, 1997). Following the surgery, HM demonstrated global inability to acquire new episodic and factual knowledge (for example stories, words, faces, verbal associates, geometric designs, supraspan digits, new vocabulary words, block diagrams, songs, common objects and object locations) and showed extremely poor recall and recognition. He also had difficulties recalling events that happened prior to the onset of his amnesia, with the retrograde deficits spanning 11 years for some events. However, his more remote memories were intact and he was able to recall his childhood and general knowledge acquired earlier in life. Despite this severe memory impairment, HM retained normal implicit memory and intellectual capacities. He was also able to retain information in his immediate memory for a brief period of time and as long as he was not distracted (Baddeley & Warrington, 1973). He could acquire new perceptual motor skills, such as mirror drawing, and could retain these skills over several months (Corkin, 2002), but without any awareness of having learnt the skills. HM's case provided evidence that the hippocampus and the medial temporal lobes are crucial for acquisition and formation of new memories, but are not involved in retrieval of remote long-term memories, immediate memory or motor and perceptual functions. The presence of severe and permanent deficits for the recall of events in contrast to the intact short-term memory, skill learning and perceptual learning has provided important insights into the distinctions between long-term and short-term memory (Baddeley, 1990) and between explicit and implicit memory (Schacter, 1987).

Cases like HM emphasized the involvement of temporal lobes in anterograde amnesia. It is now almost universally agreed that the hippocampal formation is the most critical region for temporal lobe amnesia (Spiers, Maguire & Burgess, 2001; Squire, Stark & Clark, 2004). Clinical evidence of selective damage in the hippocampus points to the significant involvement of this region in memory processing. Rempel-Clower and colleagues (1996) reported three cases of enduring anterograde and temporally graded retrograde amnesia impairments after bilateral damage limited to the hippocampal formation (Rempel-Clower, Zola, Squire & Amaral, 1996). A more recent report of five patients with limited hippocampal damage found marked deficits in episodic as well as semantic memory (e.g. knowledge for events in the news) that occurred after the onset of

amnesia (Manns, Hopkins, Reed, Kitchener & Squire, 2003) and was also evident for events that occurred sometime before the onset of amnesia. Other studies suggested that unilateral damage to either the left or right hippocampus produces material specific memory impairments to either verbal or non-verbal components (Eichenbaum & Cohen, 2001). However, rigorous debate continues over the extent and nature of the contributions from pathology in adjacent parahippocampal regions (Witter & Woutrlood, 2002) to the temporal lobe amnesia, especially the perirhinal cortex. The evidence that perirhinal cortex supports primary memory functions comes from studies in monkeys which demonstrate visual and tactile "explicit" memory impairments at long retention delays after perirhinal cortex lesions (Buffalo, Ramus, Clark, Teng, Squire & Zola, 1999). However patients with damage to the perirhinal cortex do not demonstrate impairments on the visual discrimination tasks similar to those used with monkeys (Stark & Squire, 2000).

Additional support for temporal lobe involvement in episodic memory comes from studies in laboratory animals, mainly rats. In rodent models, spatial memory tasks are often used, as these tasks are considered to be analogous to testing aspects of human episodic memory (see Chapter 3 for a detailed discussion). Since the discovery of hippocampal place cells in the rodent (O'Keefe & Dostrovsky, 1971) an influential idea has been that the hippocampus creates and uses spatial maps and its predominant function is to support spatial memory. Indeed, hippocampal lesions in rats produce spatial memory impairments on the radial maze (O'Keefe & Nadel 1978) T and Y mazes (O'Keefe & Nadel 1978; Olton, Becker & Handelmann, 1979; Olton & Papas, 1979) as well as the water maze (Eichenbaum, Stewart & Morris, 1990; Morris Garrud, Rawlins & O'Keefe, 1982). Other evidence that hippocampal lesions primarily affect memory for the environmental context has been provided in conditional fear paradigms. Learning of a conditioned fear response to the background context was impaired by a hippocampal lesion, but associating a fear response to a specific cue was unaffected (Phillips & LeDoux, 1992). In animals, performance on the delayed non-matching to sample tasks, that tax recognition memory for items/objects, remains relatively intact after selective hippocampal lesions (Aggleton, Hunt & Rawlins, 1986; Alvarez, Zola-Morgan & Squire,

1995). Deficits on these recognition tasks are only observed when large aspiration lesions are performed that also involve the rhinal cortices (Gaffan & Lim, 1991). Taken together the animal data indicate that hippocampus may play a limited role in some aspects of recognition memory, but may be crucial for normal spatial and contextual memory in the rat.

Pathology in other brain regions such as the diencephalon, which comprises the thalamus and the hypothalamus has also been implicated in memory processes. As mentioned, the diencephalic amnesia was first investigated long before temporal lobe amnesia, but its neural basis remains more uncertain. While early reports suggested that the temporal lobe damage and diencephalic damage might produce qualitatively different kinds of memory impairment (Huppert & Piercy, 1978; Parkin, 1984) the current view is that damage to either region produces comparable memory impairments (Aggleton & Brown, 1999; McKee & Squire, 1992). Comparisons of patients with temporal lobe damage and diencephalic damage showed that damage to either region results in a well-circumscribed amnesic syndrome characterized a common impairment in anterograde and temporally limited retrograde declarative memory but intact non-declarative (procedural) memory (Gold & Squire, 2006). Diencephalic amnesia is most readily observed in cases of the alcoholic Korsakoff's syndrome (KS), as well as in other cases of medial thalamic damage produced by tumors, cysts and thalamic infarcts. The following section discusses the contribution of diencephalic structures to anterograde amnesia observed in various human neuropathological conditions.

2.4 Diencephalic amnesia

Neuropsychological studies have struggled to provide definitive evidence concerning the neural basis of diencephalic amnesia. Such evidence relies on finding patients with a well-characterized amnesia that is associated with pathology in one distinct structure confirmed at post-mortem. Given the proximity of various thalamic nuclei (see Fig 1) to each other and the presence of numerous white matter tracts running through the diencephalon such evidence has been difficult to obtain. Nevertheless, strong clues can still be drawn from the neuropsychological studies of patients with diencephalic damage.

2.4.1 Korsakoff Syndrome

Behavioral and Cognitive Impairments: Korsakoff syndrome (KS) is a disorder characterized by disproportionate memory impairment relative to the other aspects of cognitive functioning, as a result of alcohol abuse and associated thiamine deficiency (Kopelman, 1995). KS is usually preceded by an acute Wernicke's encephalopathy, which can be treated by thiamine replacement if identified sufficiently early. KS is characterized by both retrograde and anterograde amnesia deficits although these patients also often have impairments in executive function. While various other disorders can produce Wernicke's encephalopathy, Korsakoff's is almost always caused by prolonged alcohol abuse (Kopelman, 1995).

Patients with Korsakoff's syndrome live in a time zone of about three to five minutes, having little or no ready access to events or learning experiences in which they have participated prior to the temporal window provided by their short-term memory. Butters and colleagues (1985) implicated defective encoding of new information as the main feature of the KS memory disorder (Butters, Wolfe, Martone, Granholm & Cermak, 1985). Defective encoding results in patients retaining access to much of the immediate experience of the past two-three minutes, with little or no ability to utilize whatever might have been stored in the recent memory. It was suggested that the same deficits also lead to inconsistent and poorly organized retrieval of remote memory which would contribute to the temporally graded retrograde amnesia observed in KS. The learning deficits demonstrated by KS patients are not modality specific and extend to all kinds of material (Butters, et al., 1985). What little learning ability the patients demonstrate on recall may be vulnerable to proactive inhibition (Leng & Parkin, 1989), although they benefit from long rehearsal times (Butters, 1984). The patients show little, if any, learning curve on repeated recall trails and have difficultly learning and recalling information in a temporal sequence (Shimamura, Janowsky & Squire, 1990). Short-term recall does not differ greatly from that of normal subjects (Kopelman, 1985) although some contradictory findings exist (Leng & Parkin, 1989). On recognition tasks, KS patients demonstrate some learning, particularly if given long exposure times (Butters, 1984) and generally

perform better on recognition formats for previously learnt material (Kopelman, 1989). Their retrograde memory deficit, which is evident for both past personal or public information (McCarthy & Warrington, 1990), follows a standard temporal gradient, with poorest recall on the most recent events and improvements as the time since memory acquisition becomes more remote.

Most early studies of KS patients concentrated on the memory deficits. Relatively little attention was paid to other functions, due to the observation that KS patients' scores on other usual tests of cognitive function were virtually identical to those of alcoholics (Kapur & Butters, 1977). Korsakoff's patients tend to perform well on structured, untimed tests of familiar material, although take abnormally long time to identify visually presented material due to their impaired visual processing capacities (Oscar-Berman, 1980). On clinical examination most KS patients demonstrate adequate span of attention but are unlikely to resume interrupted activities (Oscar-Berman, 1980), being vulnerable to distraction. They also suffer from working memory impairments, struggling to maintain and manipulate information in mind (Piekema, Fernández, Postma, Hendriks, Wester, Kessels, 2007; Pitel, et al., 2007; van Asselen, Kessels, Wester, Postma, 2005). Affective processing can be impaired in Korsakoff's patients as they find it difficult to interpret the meaning of affective prosody (Snitz, Hellinger, & Daum, 2002) and make affective judgments on tasks comprising negative, neutral and positive words (Brand, Fujiwara, Kalbe, Steingass, Kessler & Markowitsch, 2003). It was thought that the affective difficulties arise due to the injury of the diencephalic structures that process emotional responses (Grossman & Butters, 1986) or to the damage sustained to the prefrontal cortex (Brand, et al., 2003). Snitz and colleagues (2002), however, speculated that it is the involvement of the basolateral circuits, across limbic, anterior temporal and prefrontal structures that impairs the processing of affective prosody. Executive functioning impairments such as premature responding, diminished ability to profit from mistakes, diminished ability to perceive and use cues, and poor problem-solving have also been documented (Butters, et al., 1985; Oscar-Berman, 1984), and these deficits may contribute to the poor memory performance.

Comparison of the KS patients and with temporal lobe amnesics suggested that both groups show similar impairments on the tasks of recognition and recall (Kopelman & Stanhope, 1998), but may differ in the degree of executive functioning impairment demonstrated and in some aspects of spatial memory performance. While temporal lobe patients tend to perform close to the normal subject on tests of executive functioning (Eichenbaum & Cohen, 2001), the KS patients often show marked impairments as evident in the increased perseveration tendencies on the Wisconsin Card Sorting Test (Downes, Mayes, MacDonald, & Hunkin, 2002). Differences between KS and temporal lobe memory patients in the type of spatial memory strategies utilized were also documented (Holdstock, et al., 1999). In the Holdstock and colleagues (1999) study two patients with medial temporal lobe damage, seven Korsakoff amnesics and fourteen healthy control subjects were tested on three conditions of a spatial memory test ('short delay', 'allocentric' and 'egocentric'). The task required subjects to recall the position of a single spot of light presented on a board after various delays. The 'short delay' condition tested memory over very short, unfilled intervals. The other two conditions used longer, filled delays. The allocentric condition required subjects to move to a different place around the board before recalling the position of the light. In the egocentric condition stimuli were presented in darkness, which eliminated allocentric cues. The KS patients were impaired at all delays of the short delay spatial memory tasks, suggesting poor encoding. On the allocentric and egocentric tasks the KS patients showed a comparable impairment in the two conditions, which worsened with delay. The patients with medial temporal lobe damage were unimpaired in the 'short delay' condition suggesting intact encoding and short-term memory for spatial information. However, the temporal lobe amnesics were impaired in the allocentric condition and showed accelerated loss of allocentric spatial information. In the egocentric condition, while the performance of one patient was impaired, the performance of the other was as good as controls. The authors suggested that allocentric spatial memory is sensitive to medial temporal lobe damage, but an intact medial temporal lobe may not be necessary for successful performance on an egocentric spatial memory task. They speculated that the egocentric deficits might have arisen due to impairments following injury in parietal or frontal cortices in the KS patients.

Neuropathology of Korsakoff's syndrome- Post-mortem neuropathology studies have provided variable evidence with regards to the structural basis of KS (Mair, Warrington, & Weiskrants, 1979; Mayes, Meudell, Mann, & Pickering, 1988; Victor, et al., 1989; Visser, Bischof, & Di Lollo, 1999). The first influential report on KS patients came from Victor, Adams & Collins (1971), which suggested that while mammillary body pathology is a consistent feature of KS, degenerative changes in the mediodorsal thalamic nucleus were better able to predict the degree of memory impairment. Later research highlighted that alcohol exposure itself particularly affected the size of the nuclei and the neural population within the anterior thalamus and mammillary bodies (Belzunegui, Insausti, Ibanez, & Gonzalo, 1995). Other subcortical areas that were noted to be affected by KS include pons, medulla, cerebellum and the hypothalamus (Harper, Dixon, Sheedy, & Garrick, 2003). Frontal cortices were also observed to be vulnerable to the influence of alcohol. Changes in parietal and frontal lobes have been observed on PET scans in alcoholics (Paller, et al., 1997) and frontal lobe dysfunction has also been correlated with the predominance of the executive-type symptoms demonstrated by KS patients (Adams, Gilman, Koeppe, & Kluin, 1995).

Crucial evidence for anterior thalamic involvement in Korsakoff's syndrome pathology came from a study by Harding and colleagues (2000). These researchers analyzed post-mortem the brain tissues of 8 documented KS patients and 5 non-amnesic alcoholics with Wernicke's encephalopathy (which were carefully identified according to diagnostic criteria as not having amnesia), 5 non-amnesic alcoholics without neuropathology, and 7 non-alcoholic controls. The percent of neurons remaining in the mammillary bodies, mediodorsal nucleus, and the ATN were calculated by using an optical dissector technique which permits to count nuclei in the sampling frames of coronal brain slices. Relative to controls, the Wernicke's encephalopathy patients on average had 53% of mammillary bodies cells and 52% of mediodorsal nucleus cells remaining, but showed little loss in the number of ATN cells with 86% of cells remaining. While the Korsakoff's syndrome patients lost more neural cells in the mammillary bodies (32% remaining) and the medial dorsal nucleus (36% remaining) the most pronounced reductions relative to the Wernicke's cases occurred in the ATN (47%

cell bodies remaining). The authors concluded that it was the neuronal loss in the ATN which was critical for the amnesic syndrome in KS, and not the degeneration of the mammillary bodies or the mediodorsal nucleus. A previous study by Harding and colleagues (1997) found no neuronal loss in the sub-regions of the hippocampus in the Korsakoff's patients, although there was a reduction in overall volume, which was attributed to the reduced white matter in alcoholics. The degeneration and volume loss in the cortical regions was comparable between alcoholics with and without KS. In conclusion, Harding and colleagues stated that it was the degeneration of the ATN that was critical for the presentation of amnesia in KS, while damage to other areas of the diencephalon and other structures, such as frontal and parietal cortices, may compound the amnestic syndrome and possibly contribute to individual variability.

In conclusion, cases of Korsakoff's Syndrome provide strong evidence of the contribution of diencephalic structures and in particular the ATN to memory functioning. As discussed, the memory impairments demonstrated by KS patients tend to be profound, affecting both recent and remote memories and are highly debilitating to KS patients. Unfortunately, no successful treatment strategy is yet available. However, some limited attempts have been made to improve cognitive functioning of the KS patients and these studies are reviewed below.

Recovery of Functioning in Korsakoff's Syndrome - Treatments for KS remain elusive. Victor and colleagues (1971) reported that 25% of their KS patients show unspecified recovery, 50% show improvements through time and 25% remain unchanged. Whilst it is unlikely that any established KS patient will show complete recovery, abstinence does result in improvements in cognitive functioning (Kopelman, 2002). In cases of Wernicke's encephalopathy the main line of treatment has been administration of thiamine, as deficiencies in proteins and electrolytes and vitamins, with the most characteristic deficiency being the lack of group B vitamins, have been noted to be the main risk factor for KS (Pitkin & Savage, 2004).

Cholinergic agents have recently been used to improve memory function in KS. Although there is some controversy, acetylcholinesterase inhibitors generally offer a partial benefit in Alzheimer's and other dementias characterized by memory deficits (Birks, Grimley Evans, Iakovidou, & Tsolaki, 2000; Birks, Melzer, & Beppu, 2000). Low levels of thiamine, which is a characteristic feature of KS, have been shown to affect acetylcholine production (Gibson, Jope, & Blass, 1975). Cell counting has shown that alcoholic dementia patients demonstrate a reduction in neurons in the basal forebrain, including the nucleus basalis of Meynert, the major source of cortical acetylcholine (Arendt, Bigl, Arendt & Tennstedt, 1983). It is natural to suppose therefore, that acetylcholinesterase inhibitors might similarly prove to be at least partially useful in KS. Kopelman (1995) and Lishman (1990) even proposed that biochemical manipulation of neurotransmitter systems may be a better prospect in KS than in Alzheimer's disease because the pathological process is progressive in the later disorder. Thus far, the number of studies where KS patients have been treated with acetylcholinesterase inhibitors is limited (Angunawela & Barker, 2001; Casadevall-Codina, et al., 2002; Cochrane, Cochrane, Jauhar, & Ashton 2005; Iga, Araki, Ishimoto, & Ohmori, 2001) with all studies being single-case designs involving 1 to 3 patients. Donepezil, an inhibitor of acetylcholinesterase, has been trialed with some moderate success. In a single-case study, Donepezil was reported to result in improvement in cognitive functioning in three cases of Korsakoff's syndrome examined. The improvements noted included memory abilities measures (as assessed on the Alzheimer's Disease Assessment Scale- cognitive subscale) (Cochrane, et al., 2005) as well as in general quality of life. However, the degree of success in each case has been hard to interpret because of a variety of factors including short treatment periods, lack of reporting of sequential cognitive testing, and confounding variables such as ongoing treatment with thiamine. It was also documented that the beneficial effects were not fully maintained once the treatment was discontinued (Cochrane, et al., 2005).

Another line of treatment has targeted the noradrenergic system. The reasoning behind this approach is based on observations that patients with KS demonstrate diminished central noradrenergic activity, which is associated with the lesions sustained

to the brainstem and diencephalic structures. Similarly, there is a body of evidence that experimental manipulations of central noradrenergic activity affect the ability of animals to learn and remember some conditioned behaviors (Mair & McEntee, 1983). Administration of clonidine (adrenergic agonist) has been reported to result in some modest improvements in cognitive status of the patients, resulting in the improved scores on measures of anterograde amnesia, but not retrograde amnesia or working memory (McEntee & Mair, 1980; McEntee, Mair & Langlais, 1981). However, a more recent study used a larger number (n = 18) of patients and failed to find any beneficial effects of clonidine (O'Carroll, et al., 1993).

Despite these trials of noradrenergic and cholinergic substances, there is still no fully successful therapeutic approach to KS. The treatment studies were mostly single-case or involving a small number of patients, and no double-blind placebo-controlled studies have yet been undertaken. The gains reported to have been made were mostly transient, with beneficial effects on cognitive functioning wearing off after treatment discontinuation.

2.4.2 Vascular syndromes of the thalamus

Vascular supply of the thalamus - Interruption of the blood supply to the medial thalamus, such as during stroke or infarct, can also lead to the development of amnesia. The deficits observed can vary between patients depending on the source of occlusion and the extent to which the territory is supplied by other sources. The precise detail of the blood supply to the thalamus also shows variability from one person to another (Schmahmann, 2003). These factors make the interpretation of the neurological correlates of impairments in vascular damage cases difficult and often controversial (Macchi & Jones, 1997).

The thalamus is supplied with the blood from four different sources (Schmahmann, 2003): 1. The tuberothalamic artery provides vascular supply to the reticular nucleus, ventral anterior nucleus (note: this is not an ATN), rostral part of the ventrolateral nucleus, ventral pole of the medial dorsal nucleus, **mammillothalamic tract,** ventral amygdalofugal pathway, ventral part of the internal medullary lamina and

anterior thalamic nuclei. 2. The paramedian artery provides vascular supply primarily to the dorsomedial nucleus, internal medullary lamina and intralaminar nuclei, the paraventricular nuclei, ventromedial part of the pulvinar, laterodorsal, the lateral posterior nuclei and ventral anterior nucleus. 3. The inferolateral peduncle artery gives vascular supply to the lateral thalamus, including the ventral posterior nucleus and the inferolateral part of the pulvinar. 4. The medial branches of the chorodial artery give vascular supply to subthalamic nucleus and midbrain, medial half of the medial geniculate nucleus and the posterior parts of the intralaminar nuclei and the pulvinar. The lateral branches of the chorodial artery supply the lateral geniculate nuclei, inferolateral region of the pulvinar, the lateral dorsal nucleus and the lateral posterior nucleus (Macchi, 1997; Schmahmann, 2003).

Given the above description of the blood supply to the thalamus, the pattern of deficits demonstrated after thalamic infarction is often complex and varies according to the thalamic hemisphere or the thalamic nuclei damaged. Left thalamic damage has been associated with verbal anterograde amnesia (Brown & Robbins, 1989; Parkin, Rees, Hunkin & Rose, 1994), intact short-term memory (Brown, et al., 1989), long-term anterograde and retrograde amnesia for verbal and visual information (Clarke, et al., 1994), dysphasia (Karussis, Leker & Abarmsky, 2000), loss of spontaneity (Kumar, Mashih & Pardo, 1996), ideomotor apraxia (Nadeau, Roeltgen, Sevush, Ballinger & Watson, 1994) and frontal lobe dysfunction (Pepin & Auray-Pepin, 1993). In contrast, damage to the right thalamus had been associated with visuo-spatial anterograde amnesia, global anterograde and retrograde amnesia (Sala, Spinnler, & Venneri, 1997), frontal disinhibition (Bogousslavsky, Regli & Uske, 1988), “dysexecutive syndrome” (Van der Werf, et al., 2000) and anosognosia (Karussis, et al., 2000).

The range of neuropsychological impairments demonstrated by thalamic infarct patients also varies depending on which specific thalamic nuclei appear to be affected by the infract. Infarcts in the medial thalamic nuclei have been associated with anterograde amnesia (Brown, et al., 1989; Parkin, et al., 1994) and both intact frontal lobe functions (Parkin, et al., 1994) and impaired frontal-lobe functions (Speedie & Heilman, 1982).

Infarcts involving the intralaminar nuclei have been noted to result in dysexecutive impairments (Van der Werf, Weerts, Jolles, Witter, Lindeboom & Scheltens, 1999; Van der Werf, et al., 2000). Some researchers also argued that the damage to the fibers that transverse the thalamus may be responsible for the amnesic deficits observed after thalamic infarction (Graff-Radford, Tranel, van Hoesen & Brandt, 1990; Van Der Werf, et al, 2000). Van der Werf and colleagues (2003) analyzed 22 cases of thalamic infarction, proven with MR imaging and who had established neuropsychological deficits, and found that damage to several distinct areas contribute to amnesia, although the nature of the memory deficits may be different. These areas included large areas in the anterior and medial parts of the thalamus, including the ATN, mediodorsal nuclei (MDn), midline and intralaminar structures. Posterior and lateral lesions on the contrary were not associated with memory problems. When memory problems consistent with an amnesic syndrome were examined, only one area proved to associate highly: a ventral area that overlaps the location of the mammillothalamic tract, although the lesions also encroached on the ventral MDn and internal medullary lamina. Van der Werf and colleagues argued that their data were in concord with the view put forward by Aggleton and Brown (1999), who state that a combined lesion to the mammillothalamic tract and adjacent internal medullary lamina white matter carrying fibers from the medial temporal lobe cortices to the MDn is associated with dense amnesia, characterized by both deficient recall and recognition.

As discussed below, however, not all the evidence from thalamic infarct cases is consistent with the conclusions of Van der Werf and colleagues (2003). The number of other cases where the infarct is confined to the anterior part of the thalamus is limited (Bogousslavsky, Regli, & Uske 1988; Clarke et al., 1994; Graff-Radford et al., 1984; 1985; Raymer, Moberg, Crosson, Nadeau & Rothi, 1997). Another particularly careful analysis of the lesion location was undertaken by Ghika-Schmid & Bogousslavsky (2000). They used systematic investigations, including neurological and neuropsychological examinations, computerized tomography and magnetic resonance imaging to analyze the brains of 12 patients (age range, 63+/-19 years) with an isolated anterior thalamic infarct. Magnetic resonance imaging revealed damage to the anterior

group of thalamic nuclei, the mammillothalamic tract, and the anterior part of the internal medullary lamina, with structural sparing of the dorsomedial and ventrolateral nuclei. All patients had anterograde memory impairment, with a delayed recall deficit, primarily verbal in the left-sided infarcts and visuospatial in the right-sided infarcts. The patients also demonstrated severe perseverative behavior, which was apparent in thinking, speech, and all memory and executive tasks, combined with increased sensitivity to interference. They also showed superimposition of mental activities normally processed sequentially (e.g. giving biographical information while working on a calculation test). In addition, all 12 patients (8 with a left-sided infarct, 4 with a right-sided infarct) had word-finding difficulties, 7 of 12 with impaired naming, 8 of 12 with dysarthia, and 5 of 12 with hypophonia. Comprehension, repetition, written abilities, and reasoning were consistently preserved in all patients, but apathy was noted. Dysexecutive features such as difficulty in programming motor sequences were also present. Visual neglect or topographic disorientation was found in 3 patients. Based on this data the authors concluded that memory as well as language and visuospatial problems can develop after thalamic damage. A case study by Rousseaux and colleagues (1991) which described a patient with unilateral anterior right thalamic infract who demonstrated global amnesia also pointed out that in addition to memory deficits the patient showed moderate impairments on attention and categorization tasks, suggesting frontal lobe involvement resulting in executive disability (Rousseaux, Kassiotis, Signoret, Cabaret & Petit, 1991).

A limited number of vascular studies have also tried to elucidate the contributions of other thalamic nuclei such as the mediodorsal and the intralaminar nuclei to memory functioning. Zoppelt and colleagues, (2003) compared the pattern of impairments following mediodorsal nuclei (MDn) and ventrolateral (VL) thalamic nuclei ischemic damage. The patients were assessed on the word list discrimination task and on the standard tests of memory and executive function and estimates of recollection and familiarity memory performance were derived. Comparisons with the control group revealed a significant impairment after MDn lesions on measures of working memory and free recall. By contrast, patients with ventrolateral lesions were impaired on verbal fluency. The authors argued that these findings offered support for the hypothesis of the

role of MDn in memory and the contribution of the ventrolateral nuclei to executive functions. Concerning recollection and familiarity, patients with MDn lesions were impaired only in recollection, but not in familiarity, whereas patients with ventrolateral lesions showed a combined deficit on both variables. Zoppelt and colleagues (Zoppelt, Koch, Schwarz, & Daum, 2003) argued that recollection deficits observed in patients with MDn lesions are based on the failure to accurately remember contextual information of the stimulus, pointing to a frontal-type memory dysfunction due to the connections of the MDn to the prefrontal cortex. However, there may be a differential pattern of memory impairment depending on the lesion site within the MDn. The patients with lesions predominantly in the lateral MDn showed a decrease only in recollection, while patients with lesions predominantly in the medial part of the MDn demonstrated deficits with respect to both recollection and familiarity.

In cases of vascular damage to the intralaminar nuclei, executive type problems have been most commonly reported (Bogousslavsky, et al., 1988; Daum & Ackerman, 1994; Van Der Werf, et al., 1999). Van der Werf and colleagues (1999) reported a case of a patient with an isolated lacunar infarction in the dorsal caudal intralaminar nuclei of the thalamus. The neuropsychological evaluation of the patient indicated problems with attention and concentration, executive disturbances, and memory deficits both in the visual and verbal domains, such as inability to recall words, recognize faces or reproduce a complex graphic design from memory. However, the patient was able to perform the visual association learning task at a normal level, with good retention after an interval of 30 minutes, which suggested that the automatic encoding was still functioning. The patient's recall of the complex figure did not deteriorate between immediate and delayed recall sessions and the recognition score on the verbal learning test was markedly better than would be expected from the delayed recall score. The researchers suggested that such performance on the psychometric tests indicates a normal rate of forgetting, unlike the anterograde amnesia, generally associated with the amnesic syndrome. However, explicit studies of forgetting suggest little differences in amnesics once initial encoding is established (Green & Kopelman, 2002). Van der Werf and colleagues (1999) argued that the memory deficits seen are secondary to a disruption in the use of mnemonic strategies

and planning abilities. This disruption is thought to result from a dysfunction of the prefrontal lobes, in turn induced by the loss of activating intralaminar thalamic input.

Early studies described amnesia due to thalamic damage as a disconnection syndrome, taken as a disconnection of the temporal cortex from the frontal cortex. Infracts in the thalamic region often disrupt the neural pathways which transverse the region of the infract, most often affecting the mammillothalamic tract (MMT) and the lateral internal medullary lamina (L-IML). A number of researchers suggested that diencephalic amnesia results from damage to either one or both of these pathways (Graff-Radford, et al., 1990; Markowitsch, 1988; Savage, Sweet, Castillo & Langlais, 1997; Van Der Werf, et al., 2000; Van Der Werf, et al., 2003). Anatomically the MMT is not simply a pathway from temporal to frontal regions, it connects the mammillary bodies with the ATN, which in turn projects to the prefrontal cortex, especially the cingulated cortex. The cingulate cortex projects to other prefrontal cortex regions and also to medial temporal lobe. Based on the evidence that the MMT contains fibers bound for the anterior nuclei Van der Werf and colleagues suggested that the infarctions affecting the anterior thalamic nuclei should produce the same deficits as damage to the MMT. In support of this notion Van der Werf and colleagues (2000) presented a meta-analysis of the available reports of thalamic amnesia following infracts (see Table 1). They noted that in all cases reviewed the amnesic syndrome was associated with lesions of the MTT, regardless of whether the infarction was in the anterior or middle thalamus, bar one patient. Conversely, nearly all the patients (11 vs 2) who did not have memory problems compatible with an amnesic syndrome had intact MMT. Apart from memory deficits most patients also demonstrated significant executive functioning problems (see Table 1), which may also be contributing to the presence of memory impairments, by affecting information organization abilities needed for subsequent successful retrieval.

Table 1: Amnesic and dysexecutive problems: relation to mammillothalamic tract, mediodorsal, internal medullary lamina and midline lesions across three different groups of thalamic infarction (adapted from Van Der Werf, et al., 2000)[a].

Group	**No. of patients with amnesia**	**No. of patients with amnesic syndrome**	**No. of patients with dysexectuive symptoms**	**No. of patients with damage to the mammillothalamic tract**	**No. of patients with damage to the mediodorsal nuclei**	**No. of patients with damage to the internal medullary lamina**	**No. of patients with damage to the midline nuclei**
Infarction located anteriorly (N=26)	25+, 1 no data	10+, 15 no data	15+, 1-, 10 no data	25 total, 10 of 10 amnesic syndrome patients	Of 15 dysexecutives: 9+, 3-; 3 not clear, 1 non-dysexecutive	Of 15 dysexecutives: 9+, 3-; 3 not clear, 1 non-dysexecutive	Of 15 dysexecutives: 6+, 9-; 1 non-dysexecutive
Infarction located in the middle portion of the thalamus (N=32)	23+, 8-, 1 no data	8+, 5-, 10 no data	12+, 3-, 17 no data	7 of 8 amnesic syndrome patients, 2 of 13 non-amnesic syndrome	Of 12 dysexecutive: 8+, 4- , of 3 non-dysexecutives 1+, 2-	Of 12 dysexceutives: 8+, 4-, of 3 non-dysexecutives 1+, 2-	Of 12 dysexecutives: 3+, 9-, of 3 non-dysexecutives 3-
Infarction in both regions (anterior and middle) (N=3)	3+	2+, 1-	3+	2 of 2 amnesic syndrome patients, 0 of 1 non-amnesic syndrome patient	3+	3+	3+

[a] = + indicates that amnesic or dysexecutive symptoms were present, - indicates they were not, amnesic syndrome = impairments in free recall and recognition, amnesia = recall deficits only; dysexecutive= as reflected by measures of executive functioning.

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Graff-Radford and colleagues (1990) suggested that the lateral internal medullary lamina pathway (L-IML), which also transverses the medial thalamus and has projections between many structures of the limbic system, may be contributing to thalamic amnesia. They described 2 patients who sustained damage to the ventral anterior nucleus and as a result developed amnesia characterized by deficits in anterograde verbal and visual learning, as well as retrograde deficits with the sparing of motor learning.

In conclusion, evidence from thalamic vascular cases provides further support for the involvement of anterior thalamus in diencephalic amnesia. Unfortunately the stroke syndromes are not specific to individual nuclei because even very small, focal ischemic lesions are seldom confined within the nuclear boundary. In cases of infarcts in the anterior thalamic region most typically damage to the mammillothalamic tract is also sustained. Indeed, damage to the mammillothalamic tract which carries projections from the mammillary bodies to the anterior thalamic nucleus may be the best predictor of memory deficits after thalamic strokes. However, uncertainty with the size and distribution of the lesion may explain the variable reports of executive function deficits in addition to amnesia in many cases.

Recovery of function after thalamic infarction. The incidence of cognitive impairments and changes in mood and personality after thalamic infarction as well as potentials for recovery are not known. The prognosis in terms of survival and general functioning after thalamic infarction correlates with the volume of haematoma, level of consciousness at onset, extent of motor weakness at presentation and presence of intraventricular extension and hydrocephalus (Chung, Caplan, Han, Pessin, Lee, & Kim, 1996). Prognosis after thalamic infarction is generally regarded as rather good, and at least better than after cerebral infarction or infractions in other subcortical structures (Buttner, Fuchs, Markert-Schwab, & Buckmaster, 1991). However, such prediction mostly emphasizes the low incidences of mortality and good recovery from motor deficits. Persistence of cognitive and psychiatric manifestations after tuberothalamic or paramedian stroke have been reported (Schmahmann, 2003), although no longitudinal studies have been performed.

2.4.3 Other cases of thalamic amnesia

Penetrating injuries - Penetrating injuries that affect the thalamus are rare but two cases have been reported. The first case is that of N.A. a male who at the age of 22 years sustained a penetrating brain injury with a miniature fencing foil (Squire, Amaral, Zola-Morgan, Kritchevsky, & Press, 1989). He developed severe amnesia primarily for verbal material and there was an absence of other detectable cognitive deficits. N.A. was extensively evaluated with a series of magnetic resonance imaging studies, which identified three major areas of damage. There was a prominent lesion in the left thalamus. The lesion involved the rostral group of intralaminar nuclei (central medial, paracentral, central lateral, rhomboid, and reuniens nuclei), the caudal group of intralaminar nuclei (centrum medianum and parafascicular nuclei), the ventral aspect of the mediodorsal nucleus, and the ventral lateral and ventral anterior nuclei. The patient's mammillary bodies were missing bilaterally. The trajectories of the mammillothalamic tract and postcommissural fornix were possibly also interrupted. The hippocampal formation was intact. The case of NA provided early evidence that amnesia can result when several diencephalic structures are damaged conjointly, including the internal medullary lamina, the intralaminar nuclei, the mediodorsal nucleus, and the mammillothalamic tract. The case of NA supports the findings noted in vascular infarct patients that damage to the mammillothalamic tract may be a predictor of memory deficits in cases of diencephalic damage.

Another case of diencephalic amnesia developed following a penetrating injury was reported in the patient B.J. This person sustained damage to the basal regions of the brain after the snooker cue entered his left nostril (Dusoir, Kapur, Byrnes, McKinstry & Hoare, 1990). He presented with a memory disorder which had clinical features of a dense amnesic syndrome, with both anterograde and retrograde amnesia. Formal memory testing was carried out 21 months after injury and demonstrated marked verbal memory impairment, as severe as that seen in patients with the amnesic syndrome, while performance on nonverbal memory tasks was relatively mildly impaired. He also had retrograde amnesia which affected the 6 month period before the injury. On other

cognitive tasks, the patient performed at an average or above average level, and there was no neuropsychological evidence of frontal lobe dysfunction. Neuroradiological investigations at various stages after his injury, however, failed to demonstrate a lesion in any of the thalamic nuclei, but lesions in the hypothalamus in the region of the mammillary bodies were noted. This case provided evidence that marked memory disorder after diencephalic injury can occur without direct pathology to the body of the thalamus and that structures in or adjacent to the hypothalamus, such as the mammillary bodies, may also play a role in human memory.

Thalamic cysts - Thalamic tumors and cysts account for about 1-1.5% of all brain tumors and are usually unilateral astrocytomas (Uchino, et al., 2002). Bilateral gliomas are very rare and only 16 cases have been documented. Main symptoms include personality change, confusion, memory loss, apathy, emotional liability and dementia, with sparing of motor and sensory functions. In bilateral gliomas cases the destruction of mediodorsal and intralaminar nuclei is commonly observed (Uchino, et al., 2002). Interesting recent evidence has emerged from studies of the 3rd ventricle (benign colloid) cysts. The tumors are removed surgically to control hydrocephalus, but as a consequence of tumors and also surgery atrophy in the fornix is often observed. Tsivilis, et al., (2008) reported on a cohort of 38 patients who underwent cyst removal 12 months prior to neuropsychological assessment. Memory deficits were widespread in this group, although only 3 patients had complete bilateral interruption of the fornix. Analysis of the post-surgery scans revealed that fornix atrophy was frequent, with 40% of both left and right fornix volumes being one SD below the mean of controls. Consistent damage was also found in mammillary bodies where 50% of the left and right volumes were abnormally small. The primary finding was that the mammillary body volume loss was correlated with poor performance of the patients on the memory tasks on the Wechsler Memory Scale-III. In contrast, performance on the recognition task (Doors and People Test) was not correlated with the volumes of either fornix or mamillary bodies. This pattern of impaired recall and relatively spared recognition following fornix-mammillary body atrophy lends further support to the Aggleton & Brown (1999) extended hippocampal system model. The model emphasizes that recollective recognition, which relies on

episodic memory, is impaired when the extended hippocampus system is damaged (e.g. following fornix damage). In contrast, familiarity-based recognition should not be affected by fornix damage as it relies on parahippocampal areas (e.g. perirhinal cortex). That is, they predicted the relative sparing of recognition following colloid cyst removal.

Prion diseases - A small number of rare nervous system disorders can be caused by mutant prions. A prion is a propagating infectious agent which is composed of protein. The prions propagate by transmitting a mis-folded protein state; the protein does not itself self-replicate and the process is dependent on the presence of the polypeptide in the host organism. Prions are hypothesized to infect and propagate by refolding abnormally into a structure which is able to convert normal molecules of the protein into the abnormally structured form. This altered structure is extremely stable and accumulates in infected tissue, causing tissue damage and cell death. All known prions induce the formation of an amyloid fold. The substantial number of mutant proteins is particularly present in the thalamus and associated with marked memory impairments.

Prion protein has been implicated in a number of diseases including a variant of Creutzfeldt-Jacok Disease (vCJD) in humans. The vCJD in humans in linked to bovine spongiform encephalopathy and manifests mainly as a psychological disorder with cognitive difficulties, such as impairments in language, vision, hearing and executive functioning (Kapur, et al., 2001, 2003). Kapur, et al., (2003) reported on 24 patients with vCJD examined retrospectively. While there was some variability in neuropsychological profiles, the overall pattern demonstrated was one of a combined cortical and subcortical dementia, with impaired performance being particularly prominent on tests of memory, executive function, speed of attention, and visuoperceptual reasoning. Across 16 cases where Wechsler Adult Intelligence Scale-Revised intelligence quotient (IQ) scores were available, the test profile was reflected by an invariably low performance IQ (<90 in all patients). All patients who received tests of verbal fluency, digit-symbol substitution and faces recognition memory showed deficits on these tests. Basic vocabulary, digit span and verbal reasoning skills were relatively preserved in most patients. In four cases who underwent more detailed cognitive testing, additional observations were made of

relatively intact long-term autobiographical memory and faces perception. Kapur and colleagues noted that cognitive impairment may be present as one of the earliest features of vCJD and it is possible that, at least in some cases, neuropsychological deficits precede the onset of psychiatric or neurological symptoms. Of relevance to the current discussion, neuropathological examinations revealed the presence of posterior thalamic/pulvinar high signal on MRI brain scans (Zeidler, et al., 2000). The high signal on the MRI is correlated with astrocytosis and neuronal loss, which mostly affects the anterior and medial thalamic nuclei. In another study Kapur, et al., (2001) described a single case of a patient who demonstrated an early onset cognitive deterioration. His neuropsychological profile included impaired ability to retain new episodic information, deficits on tests of retrieval from semantic memory, and impairments on tests of memory for public knowledge, such as famous personalities. Tests of executive function were also performed poorly. Picture recognition memory and autobiographical memory were relatively spared, as was performance on tests of face perception and complex copying ability. Post-mortem findings showed neuronal loss in the caudate, putamen, dorsal thalamus, cerebellum and occipital cortex and spongiform changes were also found in the entorhinal cortex and anterior thalamus.

Another rare condition associated with prion mutation is Fatal Familial Insomnia (FFI). Patients with this condition exhibit vigilance and sleep disturbances with inability to initiate and maintain sleep. Progressive cognitive changes are also noted and include changes in attention, working memory, temporal ordering of events, and frontal-lobe type functions such as planning of events. Although, patients exhibit memory problems, Montagna and colleagues (Montagna, Gambetti, Cortelli, & Lugaresi, 2003) suggested that the prominent vigilance abnormalities and the observation that general intelligence is preserved in FFI are incompatible with a definition of true dementia and more akin to a progressive confusional state. Montagna and colleagues (2003) also note that the most consistent neuropathological features observed in the FFI occur in the thalamus, with the most severe changes in the anterior ventral and mediodorsal nuclei, in which more than 50% of magnocellular and parvicellular neurons are lost (nearly 80% in some cases). This

loss is associated with reactive astrogliosis. Other thalamic nuclei, except for the pulvinar, are involved less consistently and less severely.

Degenerative disorders - Other disorders that affect the thalamus include multiple sclerosis, Parkinson's disease, and Alzheimer's dementia. In cases of multiple sclerosis the total reduction in volume in the thalamus (16.8% average volume loss) has been associated with a degree of cognitive decline demonstrated by the patients (Houtchens, et al., 2007). Autopsy examinations of Parkinson's patients demonstrated that the Lewy bodies (which are a hallmark of Parkinson's pathology) tend to consistently develop in thalamic nuclei (Rub, Del Tredici, Del Turco, & Braak, 2002). The involvement of the thalamus in Parkinson's is characterized by a hierarchical pattern: the nuclei belonging to the striatal loop (centromedian nucleus, ventral anterior nucleus), the cerebellar loop (ventral lateral nucleus), and sensory constituents (lateral geniculate body, ventral posterior medial nucleus, parvocellular part) exhibit only a few Lewy bodies and are lightly involved. The brunt of the Parkinson's-related pathology, however is seen in the central lateral nucleus, central medial nucleus, fasciculosus nucleus, limitans-suprageniculate-complex, paracentral nucleus, paraventricular nuclei, reuniens nucleus and subparafascicular nucleus. The rostral and caudal intralaminar are also among those extranigral regions that are particularly severely affected by the Parkinson's-related pathology. However, involvement of these thalamic nuclei is associated with the presence of extrapyramidal symptoms, not the degree of memory deficits demonstrated by patients (Henderson, Carpenter, Cartwright, & Halliday, 2000).

Alzheimer's dementia affects most regions in the limbic system, including the anterior thalamic nuclei, especially the anterodorsal sub-nucleus of the ATN. It has been suggested that these changes in the ATN play a part in the emergence of the severe memory problems (Braak & Braak 1991; Johnson, et al., 1998). Post-mortem examination revealed that both anterior thalamic and hippocampal changes occur relatively early in Alzheimer's pathology. Numerous neurofibrillary tangles and the neurophil threads are also noted in latero-dorsal nucleus, portions of the intralaminar

complex, the paraventricular and reuniens nucleus. Braak & Braak (1991) suggested that the neuropathological thalamic changes may be responsible for hampering the transport of information through limbic circuits, thus leading to the cognitive deficits observed in the sufferers.

Evidence from these additional neuropathological conditions further highlights the role of the thalamus in memory impairments, as well as aspects of cognition such as attention and self-regulation.

2.5 Summary

Neuropsychological studies of human cases of anterograde amnesia have struggled to provide definitive evidence concerning the neural basis amnesia. Although damage to the anterior thalamus is often observed in human conditions characterized by the presence of anterograde amnesia it is often accompanied by the damage in other proximal nuclei, as well as white matter tracts that transverse the area. Consequently, direct relationships between structure and function are difficult to ascertain, especially when the region of interest is relatively small. Hence, in order to be able to more clearly elucidate the functional role of the ATN one must turn to animal research where precise lesion placing allows for more direct observation of the relationship between function and structure. The following chapter addresses this issue.

Chapter 3

Animal models of diencephalic amnesia

3.1 Introduction

In this chapter a review of animal experimental findings pertaining to anterior thalamic involvement in memory functioning is presented. Different theories that aim to explain the various contributions of the diencephalic structures to information processing are discussed. Theoretical questions pertaining to the use of animal models in investigating memory functioning are also addressed.

3.2 Animal models of amnesia

As evident from the information discussed in Chapter 2 the functional contribution of anterior thalamus still remains somewhat unclear. In the human literature it is virtually impossible to observe a selective brain injury to an area which is very small, and the damage often overlaps more than one region. Animal models attempt to overcome such limitations by providing more precise placement of the lesion. Lesion studies do not examine the functions of the structure removed as such, but rather study the extent to which the brain can compensate in the absence of that structure. The traditional focus has been on describing overt structural damage (e.g. the degree of cell loss seen), with the assumption that other intact structures can perform normally apart from any disconnection of information caused by the lesion.

Animal models provide the advantage of detailed post-mortem examination, an ability to produce more precise lesions, as well as allow the direct comparison of the

lesioned animals to the controls in the pre and post surgery testing conditions and the effects of placebo and drug administration.

While animal research allows more direct observations between structure and function, the pattern of results may differ depending on the type of lesion technique used, size and location of lesions and the degree of damage to the surrounding structures, as well as the differences in methodology used to evaluate impairments. Hence careful examination and comparison of methodology is often required in order to be able to make comparisons between studies and draw overall conclusions.

Rats are the most common species used in the animal literature. Most of the basic anatomical connections between the subcortical and cortical structures of rats are similar to those in monkeys (Aggleton & Brown, 1999; Aggleton & Pearce, 2001; Gaffan, 1992, 1994; Kesner, Gilbert & Wallenstein, 2000; Morris, 2001).

3.3 Experimental Anterior Thalamic Lesions

In their influential review, Aggleton and Brown (1999) proposed that the anterior thalamic nuclei play a role in spatial memory that reflects their involvement in episodic-like processes. Table 2 provides an overview of the rat lesion studies completed in the last 20 years on the involvement of the anterior thalamic nuclei in memory. Permanent lesions to the ATN as well as lesions to the individual sub-nuclei produce impairments in spatial memory across many different memory tasks. These deficits remain despite the rats being extensively pre-trained before surgery, at least in T-maze and standard water maze tasks (Warburton, et al., 1999). Although one study (Sutherland & Rodriguez, 1989) found that pre-training can ameliorate the ATN induced deficits in the water maze, Warburton, et al., (1999) suggested that such discrepancies may be due to different demands of the tasks used, with the T-maze task being more difficult and therefore more sensitive to the lesion-induced impairments. The observation that the ATN are involved in spatial working-memory processing as well as the acquisition of spatial reference memory emphasizes the functional similarity between the ATN and the hippocampus.

Table 2. Summary of Rat and Mice Anterior Thalamic Lesion Studies with Assessment of Performance on an Array of Memory Tasks (partially adapted from Mitchell, 2004).

Year	Authors	Lesion site /Lesion method	Extra Damage	Behavioral Tasks	Training	Delays	Deficits
2009	Lopez, Wolff, Lecourtier, Cosquer, Bontempi, Dalrymple-Alford & Cassel	ATN, ILN/LT /NMDA	Minimal	1. Morris Water Maze acquisition and delayed re-testing	Post-op	5 days 25 days	ATN= impaired across conditions ILN/LT = impaired only at 25 days re-testing
2008	Wolff, Gibb, Cassel & Dalrymple-Alford	ATN, ILN/ NMDA	Minimal	1. Allocentric spatial reference memory in the Morris water maze 2. Left/right discrimination in a Y-maze	Post-op Post-op	-- --	ATN = impaired on 1 ILN = not impaired on 1 & 2
2007	Sziklas & Petrides	ATN/ Electrolytic	IAM; LD; MD	1. Visual-spatial conditional associative learning 2. 8 arm Radial Maze	Post-op Post-op	-- --	ATN = not impaired on 1 ATN = impaired on 2
2006	Gibb, Wolff & Dalrymple-Alford	ATN, LT, MT/ NMDA	ATN: LT and MT in one rat	1. Odor-place paired-associate task 2. Spatial and odor discrimination	Post-op Post-op	-- --	ATN= impaired on 1 LT = impaired on 1 MT = not impaired 1 ATN; LT; MT = not impaired on 2
2006	Mitchell & Dalrymple-Alford	ATN, LT/ NMDA	ATN: PT; IAM	1. Response working memory in a Plus maze 2. Spatial working memory in an 8-arm radial maze	Pre-op Post-op	-- 5s	LT = impaired on 1 ATN = impaired on 2
2006	Wolff, Gibb & Dalrymple-Alford	ATN/ NMDA	IAM; PT; LD	1. Memory for temporal order of a list of odors	Pre-op	3min	ATN = impaired on 1
2006	Frohardt, Bassett & Taube	AD, DT/NMDA	AD=Minimal damage to AV; AM; LD; PC	1. Food carrying task, with or without blindfold	Post-op	--	AD = mild impairment on 1 DT = severe impairment on 1
2005	Mitchell & Dalrymple-Alford	ATN, LT, MT/ NMDA	ATN= minor damage to	1. 12 arm radial maze 2. Memory for reward magnitude	Pre-op Post-op	5s 1-4s	ATN = impaired on 1 MT = impaired on 2 LT & MT = impaired on 3

			MT and moderate to LT region	3. Temporal order memory 4. Familiar vs novel object recognition	Post-op Post-op	1h 2h	ATN; LTN; MTN = not impaired on 4
2004	Henry, Petrides, St-Laurent & Sziklas	ATN unilateral x HPC/Electrolytic	?	1. Spatial conditional associative task 2. Delayed forced alternation	Post-op		ATNxHPC = impaired on 1 and 2
2004	Sziklas & Petrides	ATN/Electrolytic	PT; PVT	1. Egocentric conditional associative task in a T-maze	Post-op	--	ATN = not impaired on 1
2003	Corbit, Muir & Balleine	MD, ATN/ NMDA	minimal	1. Instrumental conditioning 2. Devaluation extinction tests	Post-op	-- --	ATN and MD = not impaired on 1 MD = impaired on 2
2003	Mair, Burk & Potter	ATN, PH, ATN-PH/ NMDA, RF	ATN:CL, LD, PC, PT, rostral MD	1. Varying choice DNM spatial Radial Maze	Pre-op	0-32s	All impaired initially, then in a delay-dependent fashion across further sessions
2003	Moran & Dalrymple-Alford	ATN; PRC/ NMDA	AT: PC, LD, CL	1. 12-arm radial maze 2. Spatial configuration 3. Spont Object rec	Post-op	5,14, 40 min	AT = impaired on 1 PRC = impaired on 2 ATN & PRC = not impaired on 3
2002	Mitchell Dalrymple-Alford & Christie	ATN Scopolamine Infusion 1,2.51,6.31,10,15 µg	Some fornix, LD	1. 12-arm radial maze-Inf after 6 forced visits. Inf prior to testing in standard version (doors)	Pre-op	10min 5sRI 5sRI	10 µg infusion increased errors during choice phase to both forced and free choice arms 10 µg = more working memory errors
2002	Van Groen Kadish & Wyss	AD/AV, AD/AV+, AD/AV/AM/ Ibotenic	Not indicated	1. Water maze	Post-op	--	All impaired on 1. AD/AV/AM showed no improvement across trials
2002 *	Ward-Robinson, Wilton, Muir, Honey, Vann & Aggleton	ATN/ NMDA	PVA, PT, PC, CL, Re	1. Non-spatial sensory preconditioning to fear 2. Conditioned taste aversion 3. T-maze spatial forced Alt	Post-op Post-op	-- -- --	ATN = not impaired on 1 & 2 ATN = impaired 3
2001	Alexinsky	ATN (ibotenic) MD (ibotenic) RSP, PPC (excision)	Not indicated	1. 3/8 baited radial maze (working and reference) 2. New Route –Pre-exp-Y/N 3. Contextual Light Change	Pre-op	-- -- --	MD = less correct visits on 1 ATN = more incorrect reference/working memory visits on 1 ATN; MD; PPC = impaired on 2 ATN = most repetitive errors on 3
2001	Chudasama,	PL, MD,	ATN:	1. Visual discriminations and	Pre-op and	--	MD = impaired on 1

	Bussey & Muir	ATN/NMDA	midline, slight dentate gyrus	reversal using touch screen VDU	post-op		ATN; PL= not impaired on 1
2001 *	Gaffan, Bannerman, Warburton & Aggleton	Expt 1: MB, ATN Expt 2: FX, RH/NMDA	ATN: CL, LD, PC, PT, PVA, Re	1. T-maze Spatial Forced Alt 2. Locomotor activity 3. Visual screen discriminations	Post-op	-- -- --	Expt 1: ATN; MB = impaired on 1 Expt 2: FX = higher activity on 2 FX, ATN, MB = impaired on 3 RH= not impaired on 3
2001 *	Warburton, Baird, Morgan, Muir & Aggleton	ATN-HPC Ipsi; ATN-HPC Contra/NMDA	Rostral CL LD, MD, PC, PT	1. T-maze Spatial Forced Alt 2. Water Maze and Probe trial 3. 8-arm Radial Maze	Post-op	-- -- --	ATN-HPC Contra = impaired on 1, 2, and 3
2001 *	Wilton, Baird, Muir, Honey & Aggleton	AD/LD NMDA	AV, AM	1. T-maze Spatial Forced Alt 2. Water Maze and Probe trial 3. Object-in-place 4. Spont Object recog	Post-op	-- -- -- 15 min	AD/LD = impaired on 1, 2 and 3 AD/LD = not impaired on 4
2000 *	Celerier, Ognard, Decorte & Beracochea	ATN, Alcohol induced/mice/Ibotenic acid for ATN	ATN: PVA, PC, PT, CL, LD Alc: severe MB, moderate ATN and CA1	1. T-maze Spatial Forced Alt and Sequential Alt 2. Non-spatial Temporal Alt 3. Auditory and Contextual Fear Conditioning	Post-op	30, 60s 15, 30,60s --	ATN= impaired on 1, 2 & 3 Alcohol = impaired on 1 sequential Alt only, and 3 contextual conditioning only
2000 *	Warburton, Baird, Morgan, Muir & Aggleton	ATN-FX Ipsi ATN-FX Contra ANT-FX Contra +HPC/ Cyotoxic	Rostral PC, CL, MD, LD	1. Spont Object Recog 2. Object location (in place) 3. T-maze Spatial Forced Alt 4. Water Maze 5. 8-arm Radial Maze 6. T-Maze Alt	Post-op	15min 5min 10s -- -- 10s	All impaired on 2, 3, 4, 5 & 6 No impairment on 1
1999	Sziklas & Petrides	ATN/ Electrolytic	IAM, partial LD, slight rostral MD, PC, PT, PVA	1. 8-arm Radial Maze 2. Spatial Visual Assoc 3. T-maze Visual Egocentric	Post-op	20s -- --	ATN = impaired on 1 &2 ATN = not impaired on 3
1999 *	Warburton & Aggleton	ATN, FX/ NMDA, RF	PVA, PT, Rostral PC, CL, CM,	1. Water Maze and Probe test 2. T-maze Spatial Forced Alt 3. Spont Object Recog	Post-op	-- 15s 15min	ATN and FX = impaired on 1 & 2; ATN worse on 1 ATN and FX = not impaired on 3

			MD, Re				
1999 *	Warburton, Morgan, Baird, Muir & Aggleton	ATN, FX/ NMDA, RF	PT, MD, Re, Rostral Cl, CM, PC, Rt, VA	1. Water maze 2. T-maze	Pre-op Pre-op		ATN = impaired on 1 but reacquired; ATN+damage = permanent deficit on 1 & 2 FX= impaired on 1 but reacquired
1997	Warburton, Baird & Aggleton	ATN, ATN+LD, FX/ NMDA, RF	Slight Re, rostral MD, PC, CL, CM	1. T-maze forced Alt 2. X-Maze Allocentric 3. X-Maze Egocentric Discrim	Post-op	0-30s -- 10s	ATN, ATN+LD, FX= impaired on 1 and delayed dependent & 2 ATN, ATN+LD, FX = not impaired on 3
1996 *	Aggleton, Hunt, Nagle & Neave	ATN, AM, AV/AD/ NMDA	ATN: PVA, PT, Re, Rt, rostral LD, MD, MB; shrunken AM: some PT; AV/AD: none	1. T-maze forced Alt Allocentric Alt 2. Egocentric discrim 3. 8-arm Radial Maze 90 degree rotation	Post-op	15s -- 60s	ATN, AM, AV/AD = all impaired on 1, ATN worse than others ATN, AM, AV/AD = not impaired on 2 ATN, AV/AD = impaired on 3 AM= not impaired on 3
1996	Byatt & Dalrymple-Alford	AV, AM/ RF	Some overlap between subnuclie, AD	1. 12-arm Radial Maze (Working and Reference)	Post-op	--	AV and AM = impaired on 1
1995 *	Aggleton, Neave, Nagle & Hunt	ATN, MB, FX,/ NMDA, RF	ATN: CL, LD, PT, PVA, Re	1. T-maze Spatial Forced Alt 2. Spont Object Recog	Post-op	10-40s 1, 15min	ATN = slower to acquire, delay dependent deficits on 1 ATN = not impaired on 2
1994 *	Beracochea & Jaffard	ATN; mice/ibotenic		1. T-maze 2. Delayed Alt 3. T-maze Seq Alt	Post-op	5min 6h 30s	ATN= impaired on 2 ATN= not impaired on 1 & 3
1991	Aggleton, Keith & Sahgal	FX, ATN, MB/NMDA RF	ATN: PT, PVA, PC, CL, Re	1. Operant DMTP	Pre-op	>0-32s	ATN & FX = impaired on 1
1991 *	Beracochea & Jaffard	ATN; mice, Ibotenic and Alcohol	?	1. T-maze Spont Alt	Post-op	30s	ATN = impaired on 1
1991	Peinado-Manzano & Pozo-Garcia	ATN, MD/Electrolytic	MD= 90%PV ATN=89%	1. Operant Delay Alt	Post-op	0-80s	ATN & MD = impaired on 1

			AM/AV also PT and rostral MD				
1989 *	Berachochea, Jaffard & Jarrard	ATN/mice/Ibotenic	?	1. T-maze 2. Temporal Alt 3. 8-arm Radial Maze 4. Spatial Reversal	Post-op	45s 15s -- --	ATN = impaired on 1 ATN = not impaired on 2, 3, & 4
1989	Sutherland & Rodriguez	ANT/mice/Electrolytic	?	1. Water maze working memory 2. Water maze reference memory	Post-op Pre-op	-- --	ATN = longer to reach platform on 1 ATN = not impaired on 2 for same position, but impaired on 2 for position change

Abbreviations: Alt = Alternation; ATN=anterior thalamic nuclei; AD= anterodorsal nucleus; AM = anteromedial nucleus; AV= anteroventral nucleus; CA1 = area CA1 of the hippocampus; CL = centrolateral; CM = central medial nuclei; Contra = Contra-lateral; DT= dorsal tegmental; FX = Fornix; HPC = hippocampus; IAM = interanteromedial nucleus; ILn = intralaminar nuclei; Ipsi = ipsilateral; IT= inteferotemporal; LD= laterodorsal nucleus; LT = lateral thalamus; MB = mammillary bodies; MD = mediodorsal nuclei; MT = medial thalamus; PC = paracentral nucleus; post-op = post-operative; PRC = perirhinal cortex; pre-op = pre-operative; PPC = posterior parietal cortex; PT = paratenial nucleus; PVA= anterior paraventricular nucleus; PV/PVP = paraventricular nucleus/posterior paraventricular nucleus; Re = reunions; RF = radiofrequency; Rh = rhomboid nucleus; RSP = retrpsplenial cortex; Seq = Sequential; Spont = Spontaneous; ? = no clear indication of lesion size; * = studies that utilized spatial working memory in a T-maze task to assess ATN induced impairments.

Another line of evidence that lends support to the notion that the ATN and the hippocampal formation form a functional system comes from studies that employ a cross-lesion method. In cross-lesion studies a unilateral lesion is placed in both structures of interest either in different cerebral hemispheres, or in the same hemisphere for both structures. The rationale behind such experiments is that unilateral lesions in different sites in opposite hemispheres will only have a severely disruptive effect if the two regions form a part of a functional system, whereas unilateral lesions made in the same hemisphere will have relatively little effect on behavior. Unilateral lesions to the ATN and contra-lateral unilateral lesions (different side) to the hippocampus or fornix produce impairments in spatial memory on the T-maze, water maze and 8-arm radial maze tasks. However, ipsi-lateral (same side) lesions produce only mild impairments which are significantly less than those following fornix lesions (Warburton, et al., 2000, 2001). Warburton and colleagues suggested that this evidence provides direct support for the theoretical notion that the ATN and hippocampus/fornix operate in conjunction as part of an integrated neural network during processing of spatial information.

Further supporting evidence for the interdependence between hippocampus and ATN comes from studies that also examined memory for temporal events. The separation of closely linked events across time may be crucial for memory of unique behavioral episodes, with the hippocampus being shown to support temporal order memory for both sequential spatial locations (Chiba, Kesner & Reynolds, 1994; Jackson, Kesner, Amann, 1998) and non-spatial items such as the position of items in a list of recently presented odors (Fortin, Agster, & Eichenbaum, 2002; Kesner, Gilbert, & Barua, 2002; Kesner & Rogers, 2004). Impairments in temporal order memory for the sequence of odor cues have also been observed after ATN lesions (Wolff, et al., 2006). Although a previous study on the temporal order memory after ATN lesions found no deficits (Mitchell & Dalrymple-Alford, 2005), procedural differences such as the length of time between sample and tests phases and the nature of stimuli presented probably contributed to the disparities observed.

With the exclusion of the temporal memory, there is little indication that ATN lesions impair memory for other classic non-spatial tasks, such as object recognition (Aggleton, Neave, Nagle, & Hunt, 1995), configural learning, or sensory preconditioning, (Moran & Dalrymple-Alford, 2003; Warburton & Aggleton, 1999; Ward-Robinson, Wilton, Muir, Honey, Vann & Aggleton, 2002). These tasks, however, are now acknowledged as generally insensitive to hippocampal lesions also (Warburton & Aggleton, 1999; Ward-Robinson, et al., 2002). ATN lesions may also impair go/no-go delayed alternation tasks by producing a delay-dependent deficit (Beracochea, Jaffard & Jarrad, 1989; Celerier, Ognard, Decorte & Beracochea, 2000; Peinado-Manzano & Pozo-Garcia, 1991), but the non-spatial characteristics of this task have been questioned (Ward-Robinson, et al., 2002). The ATN are not involved in egocentric type (motoric) learning, such as an ability to acquire a body turn rule (e.g. always go left) (Aggelton, et al., 1996; Warburton, et al., 1997; Wolff, et al., 2008).

While damage to the ATN and hippocampus results in a generally comparable pattern of impairment, particularly on tasks of working memory and spatial navigation, there is evidence that damage to these two brain regions may not always produce the same impairments, at least across different conditional associative learning tasks (Sziklas & Petrides, 1999; Sziklas & Petrides, 2007). A group of researchers led by Sziklas have demonstrated that the ability to form arbitrary associations, which is believed to test aspects of declarative memory, including episodic-like memory, can be differentially affected by ATN lesions depending on the type of task used. ATN lesions result in severe deficits on spatial-visual tasks which require learning an association between scenes and objects embedded in them (Sziklas & Petrides, 1999) or between different odors and their location (Gibb, et al., 2006). These tasks concern situations in which the selection of an object or odor is dependent on the spatial context and both ATN and hippocampus show comparable deficits on these tasks (Gibb, et al., 2006; Kesner, et al., 2002; Sziklas & Petrides, 1999; Sziklas, Lebel & Petrides, 1998; Sziklas, Petrides, & Leri, 1996). However, ATN animals do not demonstrate impairments on conditional tasks where they have to learn to make one of two motor responses (i.e. make a right or left turn; visual-motor) or an arm choice (visual-spatial) depending on which one of the two visual cues is

present (Sziklas & Petrides, 1999, 2004, 2007). In contrast, rats with dorsal hippocampal lesions show clear impairments on these two latter tasks (Sziklas, et al., 1996). Sziklas and Petrides (2007) stated that the visual-spatial task could not just be solved by the animals relying on egocentric strategy and hence the ATN rats must have been able to learn an association between two objects and two different locations. The authors suggested that the ATN may interact with the hippocampus in situations where spatial context instructs which of two objects embedded in the context to select (spatial-visual task), but the hippocampus does not require ATN input on tasks where arbitrary visual cues that are not embedded in a place instruct which place to chose (visual-spatial task). Additionally, Henry and colleagues (Henry, Petrides, St-Laurent & Sziklas, 2004) were able to demonstrate that disconnecting anterior thalamus and the hippocampus (a unilateral lesion in placed in the ATN and a contra-lateral lesion is placed in the hippocampus) significantly impairs the acquisition of the spatial–visual conditional learning task, although some learning was evident during training. Interestingly, the performance on the spatial-visual tasks does not seem to rely on the direct interaction between anterior thalamus and hippocampus via the fornix alone, because fornix transection does not affect performance on this task (Dumont, Pertides & Sziklas, 2007; Sziklas, et al., 1998; Sziklas & Petrides, 2002) or on the retrosplenial cortex alone (St-Laurent, Petrides & Sziklas, 2009). However disruption of both of these routes of communication (fornix and retrosplenial cortex) between the ATN and hippocampus produces severe deficits on the spatial conditional learning task (Dumont & Sziklas, 2007 cited in St-Laurent, et al., 2009). St-Laurent and colleagues (2009) proposed that this evidence supports the notion that the ATN and the hippocampus function interdependently on tasks that require the rat to choose an object based on its location, and that the interaction between these structures relies on both the fornix and the retrosplenial cortex but not either alone. However, ATN rats are able to use egocentric or place cues to solve a task, when they can select the correct location or motor response based on an associated visual cue. These different effects of ATN lesions across conditional spatial tasks implies that the plurality of hippocampal connections may compensate for at least some spatial memory functions associated with the ATN, but the reason why spatial-visual tasks differ from visual-spatial tasks is as yet unclear. It is

possible that directional cues are necessary in many spatial-dependent tasks, but not when other cues (objects) can be used (St. Laurent, et al., 2009).

3.4 Lesion effects following damage to the ATN sub-components

The evidence reviewed above shows that ATN lesions generally impair performance on tasks that are sensitive to hippocampal system function. However, questions have been raised whether damage to all or just some of the subcomponents is sufficient to produce the observed deficit. The ATN consist of three subdivisions anteroventral (AV), anteromedial (AM) and anterodorsal (AD) (see Fig 1). Generally the nuclei within the anterior thalamus tend to operate as an ensemble (Byatt & Dalrymple-Alford, 1996), although some subtle differences have been noted. Aggelton, et al., (1996) pointed out that the long-term persistent deficits on spatial memory tasks, such as T-maze alternation, are more likely to be obtained when damage to the whole complex is sustained, whereas AM only or AV plus AD lesions result in delayed task acquisition. The AV plus AD lesions were also more disruptive on the performance in the radial arm maze task, while the AM-only lesions had no effect. Lesions to the AV almost invariably cause damage to the AD because of their relative position in the rat brain, but lesions centered on the AD usually only cause dorsal AV damage. Van Groen, Kadish & Wyss (2002) demonstrated that while selective AD plus dorsal AV damage or AM damage produced impairments on the working and reference memory tasks in the water maze the animals were able to acquire the tasks over time. The total ATN damage produced severe impairment with no improvements evident across trails. The severity of the impairment was proportionate to the extent of the AM damage sustained in addition to the AV plus dorsal AD damage. Such evidence suggests that there might be subtle differences in the contribution of these regions to certain spatial tasks, which might be explained by the differences in anatomical properties. For example, the head direction cells are more prevalent in the AD (Taube, 1995). Taken together the evidence strongly suggests that lesions involving all three anterior thalamic nuclei are required to produce severe longer-lasting deficits on the tests of spatial memory processing. However, relatively minor damage to the ATN may potentially contribute to the severity of the amnesic syndrome when the insult is sustained elsewhere (Aggleton, et al., 1996; Byatt & Dalrymple-Alford, 1996).

3.5 Contribution of other nuclei in the medial thalamus to diencephalic amnesia

The review provided in Chapter 2 pointed out that the memory deficits observed in cases of diencephalic amnesia may vary as a function of the relative involvement of different thalamic regions. Initial investigations into the basis of diencephalic amnesia focused on the involvement of the mediodorsal nuclei (MDn) (Victor, et al., 1971). However, the lack of certainty over deficits observed after specific lesions to the MDn was partly responsible for a re-evaluation of the contribution of other thalamic nuclei, such as the ATN (summarized above) and also the Intralaminar nuclei (ILn). The ILn became of particular interest when their involvement was observed in pyrithiamine-induced thalamic deficiency (a rat model of chronic alcoholism) (Mair, 1994).

In contrast to the ATN, lesions to the MDn have not been consistently associated with spatial memory deficits, using either spatial working memory or reference memory tasks (Hunt & Aggleton, 1991, 1998; Kolb, Pittman, Sutherland & Whishaw, 1982; Neave, Sahgal, & Aggleton, 1993). Earlier studies that reported spatial deficits after MDn lesions found that these deficits were only evident when very large lesions were used that also encroached on the ATN (Stokes & Best, 1988; 1990). Later studies that examined spatial memory reported only very mild delay-dependent deficits after selective neurotoxic MDn lesions. For example, working memory for a lever or olfactory stimulus declined to about 80% correct at the longest, 13–20 s, delay after MDn lesions (Bailey & Mair, 2005; Burke & Mair, 1998). MDn has also been reported to be involved in recognition memory in the early studies, with deficits observed in both acquisition and performance of delayed-matching-to-sample and non-matching-to-sample object recognition tasks (Aggleton & Mishkin, 1983; Gaffan & Parker 2000; Hunt & Aggleton 1991; Mumby, Pinel & Dastur, 1993; Parker & Gaffan, 1997; Zola-Morgan & Squire 1985). However, the effects were not consistent and negative findings have also been reported (Hunt & Aggleton, 1998; Kornecook, Anzarut & Pinel, 1999; M'Harzi, Collery & Delacour, 1991).

One domain in which MDn lesions appear to play a more marked influence is memory for reward value and the anticipation of rewarding or reinforcing events (Corbit, Muir, & Balleine, 2003; Oyoshi, Nishijo, Asakura, Takamura, & Ono, 1996). These latter effects may have as their basis a ventro-lateral prefrontal, ventral basal ganglia, MDn neuroanatomical circuit (Mitchell & Dalrymple-Alford, 2005). In addition, there is some evidence that disruption to the MDn specifically influences flexible responding in delayed radial maze foraging and disrupts the ability to switch from a preferred response rule or strategy to a new strategy (Hunt & Aggleton, 1998) For example, rats with MDn lesions can distinguish which arm had been most recently visited (working memory), but the same rats are impaired at shifting from a preferred response rule. MDn has dense, reciprocal connections with prefrontal cortex, and damage to the prefrontal cortex can result in perseverative deficits (Kolb, 1984; Mishkin, 1964). Hunt & Aggleton (1998) argued that lesions to the MDn - prefrontal cortex pathways may disrupt prefrontal cortex function and in turn produce memory impairments.

Precise lesions to the ILn are often difficult to achieve, because of their small size and aggregate shape in the human brain. Hence, relatively limited evidence exists with regard to their involvement in memory. The interest in involvement of the ILn in memory first originated after the observation of their contribution to the pyrithiamine-induced thiamine deficiency (PTD) model (a model of chronic alcoholism). In the PTD model of brain damage lesions have been observed that are centered on the ILn (Mair, 1994) and internal medullary lamina pathway (IML) (Mumby, Cameli, & Glenn, 1999). These lesions, however, are not selective as damage to other structures such as the ATN (Langlais & Savage, 1995) and medial mammillary bodies (Mair, Knoth, Rabchenuk, & Langlais, 1991) have been noted particularly after longer exposure times to thiamine deficiency. Mair and others have conducted a number of behavioral studies that have implicated ILn in memory tasks, such working memory for an egocentric (body turn) response or for one of two retractable levers (Burke & Mair 1998; Mair, Burk & Porter, 1998; Savage, Castillo & Langlais, 1998; Young, Stevens, Converse & Mair., 1996). The memory deficits following ILn lesions are delay-independent (Bailey & Mair 2005; Burk & Mair 1998; Harrison & Mair 1996; Young, et al., 1996; Zhang, Burk, Glode, & Mair,

1998), which suggests that other factors that disrupt recall may be affected rather than memory. Van der Werf and colleagues (2000; Van der Werf, Witter & Groenewegen, 2002) proposed that ILn maintains a state of arousal for cortical regions that are involved in memory processing, with damage to the ILn affecting arousal, motivational and attention factors. In related work, Burk & Mair (2001) observed that ILn lesions impair response latency, which they felt was indicative of impaired motor initiation that affects one's ability to make voluntary movements. Based on this evidence the Mair's research group argued what ILn lesions are generally more comparable to that of dorsal prefrontal cortex and ventral striatum lesions than to the lesions of the hippocampal system. The recent perspective on the ILn function is that the substantial deficits associated with ILn lesions require damage that encroaches on other thalamic nuclei. The contribution of the rostral ILn is still uncertain. For example, a more recent study which utilized very precise lesion placings pointed to the importance of the rostral ILn in learning of odor-place paired-associative problems, which the researches attributed to disruptions of response or directional learning in the ILn lesioned rats (Gibb, et al., 2006) and another study has suggested that the rostral ILn lesions disrupt the consolidation of (remote) spatial memory (Lopez, et al., 2009).

3.5.1 Differential contributions of ATN, MDn and ILn to memory

Attempts have been made to compare the differential contributions of the ATN, MDn and ILn to memory. As expected, lesions to the ATN tend to consistently severely impair spatial memory performance, such as working and reference memory in the radial-arm maze and the Morris Water maze (Mitchell & Dalrymple-Alford, 2005; Warburton, Aggleton, & Muir, 1998). Selective ILn lesions with little or no involvement of the ATN and MDn were found to produce either no or only very mild and transient working memory deficits (Micthell & Dalrymple-Alford, 2005; Wolff, et al., 2008). In contrast, selective ILn lesions have been observed to negatively affect egocentric memory, which is not affected by ATN lesions (Mitchell & Dalrymple-Alford, 2006). However, these effects may be task-dependent, because egocentric deficits after ILn lesions have been reported on DNMS and a response-related working memory task in a cross-maze (Mair, et al., 1998; Mitchell & Dalrymple-Alford, 2005), but no egocentric impairment was

found after ILn lesions when the rats were trained on an egocentric reference memory task in a Y-maze (Wolff, et al., 2008). While the ILn lesions impaired retrieval of remote spatial memory in the water maze, only ATN lesions impaired initial acquisition of this task (Lopez, et al., 2009).

Dissociable effects between MDn and ATN have also been reported. For example, Chudasama, Bussey & Muir (2001) observed no impairments in attention in ATN rats on the five-choice serial reaction time tasks but the MDn rats demonstrated increased anticipatory responding during baseline performance and when the inter-trial interval was randomly varied during the serial reaction time task. In another study, medial thalamus lesions, which included the medial and central segments of the MDn and the intermediodorsal nucleus, resulted in impaired acquisition of the reward magnitude task, while having no effect on the spatial memory in the radial maze, opposite to the effects observed for ATN rats (Mitchell & Dalrymple-Alford, 2005). Both the lateral thalamus lesions, centered on the rostral ILn, and the ATN lesions were found to severely impair odor-place paired-associate learning, which was not affected by lesions to the medial regions of the MD (Gibb, et al., 2006).

The presence of dissociations between the anterior thalamic, medial and lateral limbic thalamic nuclei indicates that the manifestation of diencephalic amnesia may vary depending on the extent of thalamic injury and the specific memory task examined. It also explains in part the conflicting results often reported when large electrolytic lesions are performed in the region. Non-neurotoxic lesions disrupt fibers of passage, which may be a particularly relevant confound when anterior and lateral thalamic lesions are compared. Large electrolytic ATN lesions will disrupt fibers from the centrolateral nuclei and (especially) paracentral nuclei that course through the ATN region (Van der Werf, et al., 2002). As indicated earlier, it is also now acknowledged that previously reported findings of impairment on the hippocampal dependent maze-learning tasks after ILn (Mair, Burke, & Porter, 1998; Savage, Castillo, & Langlais, 1998) or MDn (Stokes & Best, 1988) lesions were primarily due to the damage that was sustained by the ATN

(Byatt & Dalrymple-Alford, 1996; Hunt & Aggleton, 1991). The contribution of thalamic aggregates to memory function can also explain the severe cases of amnesic syndrome observed in humans with relatively small structural damage to the thalamus (Marchetti, Carey, & Della Sala, 2005). Evidence that the thalamic aggregates may be responsible for different aspects of memory functioning underscores the importance of producing very selective ATN lesions and quantifying additional damage when attempting to elucidate the contribution of this particular region to memory.

In summary, the presence of impairments in allocentric memory acquisition after ATN lesions and absence of such impairments after MDn or ILn lesions adds support to the view that ATN damage is one of the key sources of episodic memory impairment in human cases of diencephalic amnesia (Aggleton & Brown, 1999). The relative contributions of ILn and MDn to diencephalic amnesia continue to be debated. Recent evidence that ILn lesions may impair recall of remote spatial memories, and by inference consolidation provides an additional perspective on the effects of limbic thalamus lesions and memory (Lopez, et al., 2009). Various theories have been postulated which attempt to explain the causes of the anterograde amnesia associated with damage to the diencephalic structures.

3.6 Theories of Medial Diencephalon Involvement in Memory

Aggleton and Brown (1999) highlighted the similarity of the syndrome produced by the damage to the medial temporal lobe structures and the diencephalic structures and revised Delay & Brion's idea (1969 cited in Aggleton and Brown, 1999) that both structures work in parallel. After reviewing neuropsychological evidence and the effects of lesions to different parts of the hippocampal complex, and the thalamic structures with which they are associated, Aggleton & Brown (1999) postulated a model of hippocampal-medial diencephalic interactions. In that model it was argued that an "extended hippocampal system" comprising the hippocampus, fornix, mammillary bodies and anterior thalamic nuclei, is vital for encoding and subsequent recall of episodic information. As a consequence damage to the component structures can result in

anterograde amnesia (Aggleton & Brown, 1999) (see Fig 2A). An important facet of this model was to suggest that recognition memory depends on two independent processes, only one of which is hippocampally dependent. One depends on the recollection of the event (remembering) and is hipocampally dependent; the other depends on a signal of stimulus familiarity ("knowing"), which does not require the hippocampus and is dependent on the perirhinal cortex in the temporal lobes (see Fig 2B). Damage to the extended hippocampal system causes deficits in spatial memory and in memory for relational information that typifies memory for autobiographical episodes, but spares recognition based only on familiarity (Aggleton, Vann, Oswald & Good, 2000; Holdstock, et al., 2002; Mayes, et al., 2004; Mayes, Isaac, Holdstock, Cariga, Gummer & Roberts, 2003; Moscovitch & McAndrews, 2002; Yonelinas, 2002; Yonelinas, et al., 2002). The other system, consisting of the perirhinal cortex and its connections to the dorsomedial nucleus of the thalamus (an extended perirhinal system), is necessary for item recognition based on familiarity which does not require access to the spatial-temporal context. Damage to this system will impair recognition of single items (Aggleton, et al., 2000).

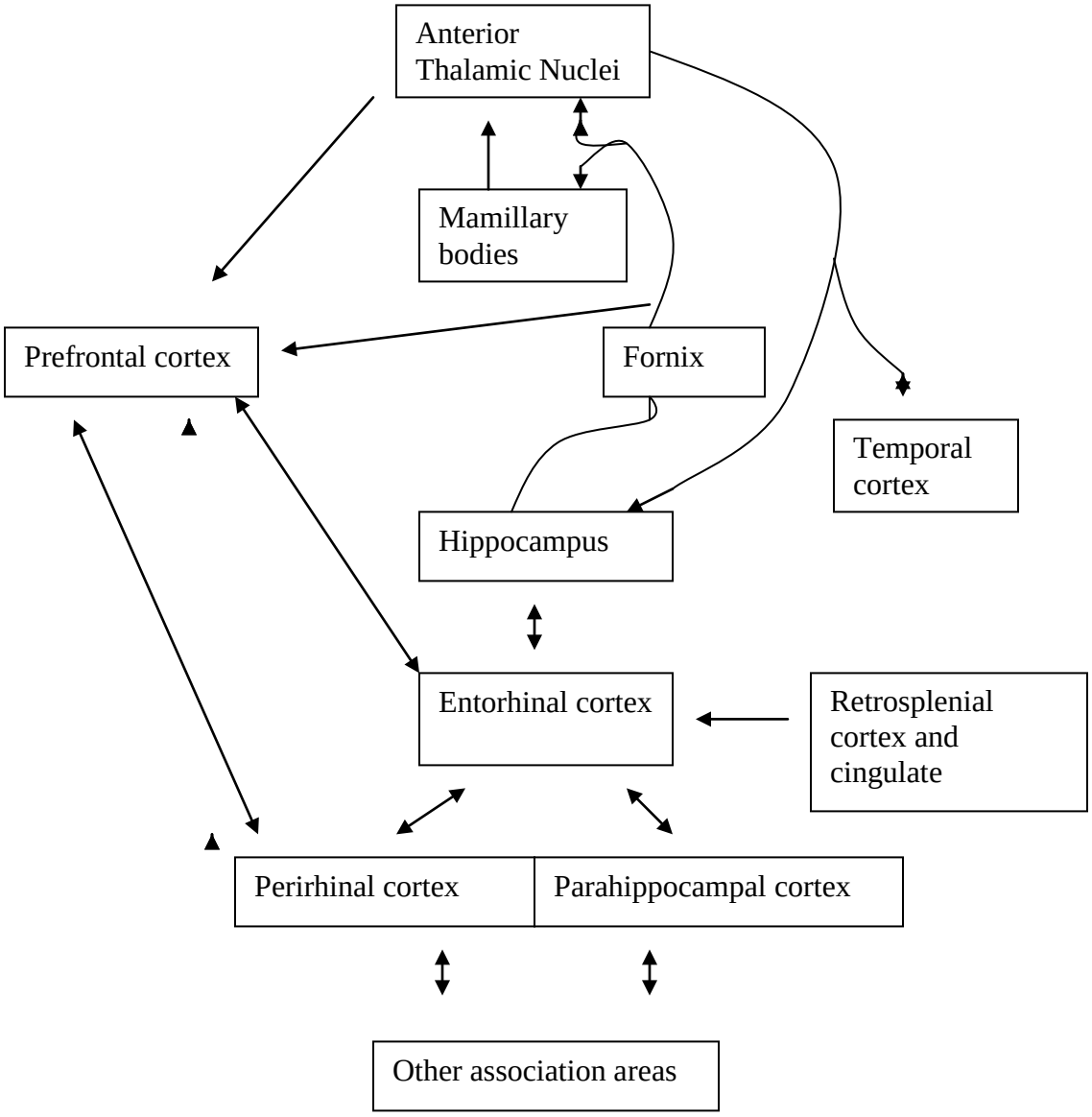

Fig 2A. Schematic diagram of the main pathways that allow encoding of episodic information and underlie recollective aspects of recognition. Adapted from Aggleton and Brown (1999).

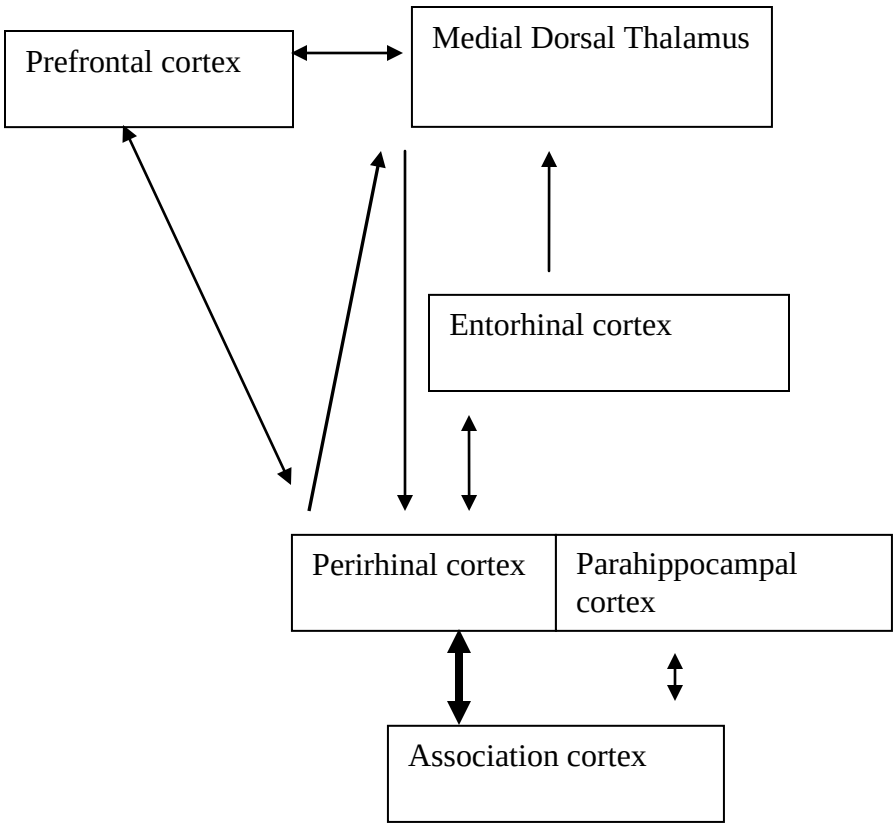

Fig 2B. Schematic diagram of the principal pathways underlying the detection of item familiarity. The relative thickness of the lines indicates the importance of the connections. Adapted from Aggleton & Brown (1999).

Aggelton and Brown (1999) further proposed that a reciprocal interaction exists between the hippocampus and the ATN. In support of their model, subsequent studies have demonstrated that ATN lesions can induce abnormalities in hippocampal activity as measured by the immediate-early gene activity (Jenkins, Dias, Amin & Aggleton, 2002a; Jenkins, et al., 2002b) and reduce the release of hippocampal acetylcholine (Savage, Chang & Gold, 2003). There is also evidence that navigation mechanisms that rely on head direction cells are dependent on the projections from the mammmillary bodies to the ATN and hence to the hippocampus (Basset, Zugaro, Muir, Golob, Muller & Taube, 2005). Moreover, as mentioned earlier, disconnection studies also demonstrated that ATN and hippocampus are interdependent for at least some aspects of spatial learning (Henry, et al., 2004; Warburton, et al., 2001).

The theory proposed by Aggleton and Brown caused substantial amount of debate. First, the specific function of the perirhinal-MDn axis has been questioned. While the role of perirhinal cortex in familiarity judgments is well documented, it is debated whether or not it also contributes to the spatial memory function. In several studies, Liu and Bilkey have found that lesions restricted to the perirhinal cortex cause modest performance deficits on tasks that require spatial memory, including the radial maze (Bilkey & Liu 2000; Liu & Bilkey, 1998; 1999; 2001). Other groups have not, however, found similar deficits (Bussey, Dias, Amin, Muir & Aggleton, 2001; Ennaceur & Aggleton, 1997; Moran & Dalrymple-Alford, 2003) and procedural variables such as maze construction and lesion techniques may explain these differences. The contribution of the MDn to recognition memory is also not straightforward, with some studies not finding MDn induced recognition impairments (Edelstyn, Ellis, Jenkinson & Sawyer, 2002; Markowitsch, 1999; Shuren, Jacobs & Heilman, 1997). Aggleton and Brown also explicitly avoided explaining the contribution of the ILn to amnesia. As indicated previously, Mair and colleagues (Burk & Mair, 2001; Mair, et al., 1998; Mair, Burk, Porter & Ley, 1999) suggested that in fact ILn plays a crucial role in thalamic amnesia. They cite the evidence from the pyrithiamine –deficiency model, where the extent of ILn damage was strongly correlated with the extent of behavioral impairment (Mair, et al., 1998) with rats performing poorly on the DNMS and DMS tasks. The Mair group claim that the pattern of functional impairment following ILn damage is markedly different from that associated with other thalamic damage, the hippocampus or the prefrontal cortex, with ILn rats demonstrating impairments on recognition type tasks (Burk & Mair, 1998; Mair, et al., 1998). Mair and colleagues suggested that the ILn therefore must have a separate influence in memory processing and does not just constitute an extension of either the hippocampus or the prefrontal cortex damage.

Another theoretical proposal has been put forward by Van der Werf and colleagues (Van der Werf, et al., 1999; 2000; 2002; 2003) who conducted extensive investigations of diencephalic amnesia following thalamic infraction in humans. They agree that the ATN play a role in memory (Van der Werf, et al., 2000; 2003) but argue that it is the contribution of the mammillothalamic tract that results in the amnesic syndrome demonstrated by humans. Furthermore, they suggest that the ILn maybe involved in frontal cognitive functions, as they are able to influence the function of the prefrontal cortex. The MDn are thought to contribute to the

dysexectuive problems observed in patients with thalamic lesions, although the authors note that it is not a one-to-one basis relationship as impairments in executive functions can be found in the absence of MDn lesions (Van der Werf, et al., 2000). Van der Werf and colleagues (2000) agree that the thalamic aggregates do not just relay information, but make specific contributions to the declarative memory processing. They suggested that ATN and MDn are involved in processing the content of stimuli for storage and recall, with ATN being responsible for what material is to be stored and remembered, while the MDn is involved in coordinating and selecting strategies that are used for retrieval. In their scheme, the ILn are involved in maintaining a state of arousal in the cortical regions which is needed for memory processing (Van der Werf, et al., 2003).

Another approach to understanding of diencephalic amnesia has been offered by Gabriel (1993). He conducted electrophysiological recordings of neurons in rabbits in the medial thalamus and cingulate cortices and was able to establish that the MDn and anterior cingulate are involved in the initial stages of discriminative avoidance learning task. On the other hand, electrophysiological changes in the ATN and posterior cingulate tend to occur at later stages of acquisition, suggesting that these structures are important for the maintenance of the learned information (Gabriel, 1993). The potential contribution of the ILn to memory is not addressed by the model.

Revisions of the Aggleton & Brown (1999) "extended hippocampus" model- More recently Aggleton (2008) refined that extended hippocampal system theory and proposed that the observed similarity between temporal lobe and diencephalic amnesia may be explained by the fact that both hippocampus and anterior thalamus are connected to the third region - retrosplenial cortex, which possibly functionally links the structures. In this revision, dysfunction in the retrosplenial cortex is seen to contribute to both temporal and diencephalic amnesia. Aggelton argued that the retrosplenial cortex represents an example of "covert pathology" in anterograde amnesia. Covert pathology is used to refer to an area that appears normal by the standard histological means and yet is functionally lesioned. In cases of temporal lobe amnesia it has been documented that ischemic damage to the hippocampus can produce more severe memory deficits than conventional lesions. For example, it has been observed in monkeys that the effects of ischemic hippocampal damage on tests of recognition memory are more disruptive than

conventional lesions even though the apparent extent of cell loss is comparable (Bachevalier & Meunier, 1996). Similarly, Mumby, Wood, Duva, Kornecook, Pinel & Phillips (1996) demonstrated that conventional hippocampal lesions produced no impairments on the recognition memory task, while severe deficits were observed following a vascular occlusion-induced hippocampal lesion, despite the extent of hippocampal damage being similar in both lesion methods. Aggleton (2008) proposed that these different functional outcomes are mediated by the presence of covert pathology in the retrosplenial cortex.

The retrosplenial cortex has extensive connections with the ATN (Shibata, 1998; Van Groen, Kadish & Wyss, 1999; Van Groen & Wyss, 1995). Figure 3 illustrates the location of the retrosplenial cortex in the rat brain and the relative positioning of the subregions of interest. The AV nuclei of the ATN have prominent connections with the retrosplenial cortex, while the AM projects more predominantly to the dorsal anterior cingulate than the retrosplenial cortices (Bentivoglio, Kultas-Ilinsky & Ilinsky, 1993; Van Groen et al., 1999). The rostral AM projects to layers I, V/VI of Rdg (retrosplenial dysgranular cortex) and layers I and V of the caudal part of Rga (retrosplenial granular a) and Rgb (retrosplenial rostral granular b). In contrast, the caudal AM projects to layers I and V of the rostral part of Rga and Rgb. Both AV and AD project to the full extent of the Rga and Rgb in a topographic manner. Neurons in caudal parts of AD and AV project to rostral retrosplenial cortex, while neurons in rostral AD and AV project to caudal retrosplenial cortex. The AV projects densely to layer I (Rgb) and lightly to layer IV (Rga) of the granular area and the AD projects with equal density to layers I, III and IV (Rga and Rgb) of the granular area (Shibata, 1993; Van Groen & Wyss, 1990; 1995).

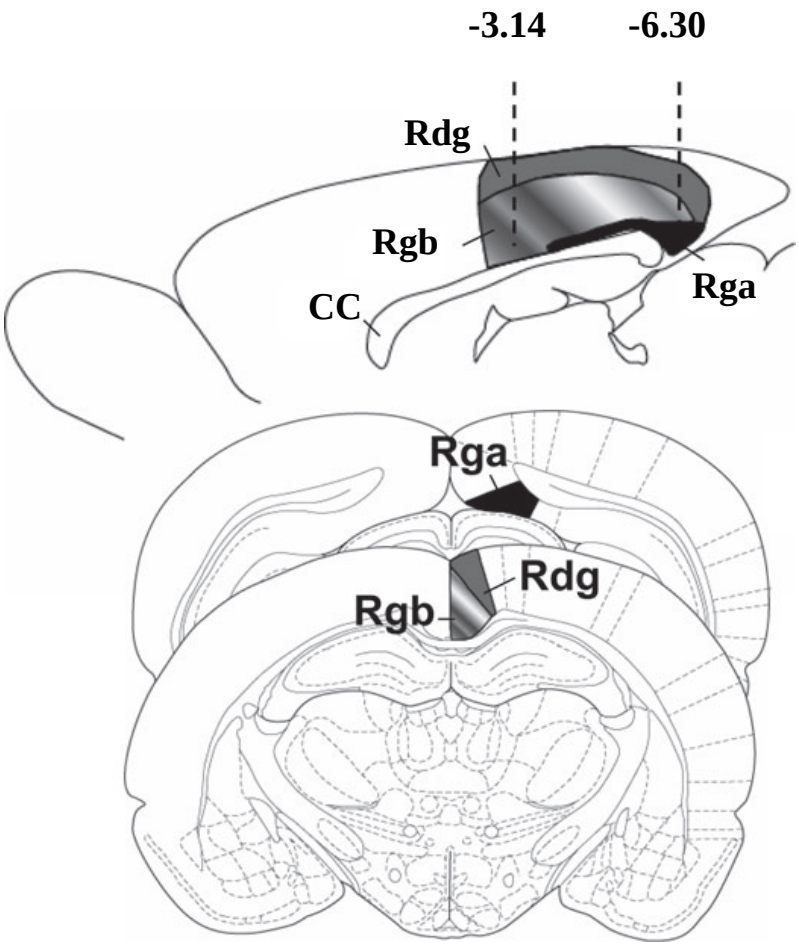

Fig 3. Retrosplenial cortex subregions. The sagittal (upper) and coronal (lower) sections show the location of the regions of interest. The numbers refer to the distance (mm) from bregma according to the atlas of Paxinos & Watson (1999). CC= corpus callosum, Rdg = retrosplenial dysgranular cortex; Rgb = retrosplenial granular rostral; Rga = retrosplenial granular caudal. Reproduced from Albasser, et al., (2007).

Neuropsychological studies have demonstrated that damage to the retrosplenial area can cause anterograde (Maguire, 2001; Rudge & Warrington, 1991; Valenstein, Bowers, Verfaellie, Heilman, Day, & Watson, 1987) and topographical amnesia (Maguire, 2001). In the animal literature, retrosplenial cortex lesions produce impairments in spatial memory (Aggleton & Vann, 2004; Cooper & Mizumori, 2001; Lukoyanov, Lukoyanova, Andrade & Paula-Barbosa, 2005; Vann & Aggleton, 2003; 2004) including learning the location of specific objects (Vann & Aggleton 2002). However, contrary results have also been reported. Extensive retrosplenial

cortex lesions have been noted to produce only a borderline (non-significant) effect on standard T-maze alternation (Harker & Wishaw 2002; Pothuizen, Aggleton, & Vann, 2008), and relatively mild allocentric deficits in the Morris water-maze (Harker & Whishaw, 2002; Sutherland, Vann & Aggleton, 2002; 2004; Van Groen, et al., 2004; Warburton, et al., 1998). To explain these discrepancies it has been suggested that the retrosplenial lesions may have a more selective effect on spatial working memory, disrupting only some of the strategies the animal can use to solve the maze. For example, retrosplenial lesions impaired T-maze alternation when it was heavily reliant on finding direction in the dark, or egocentric strategies (Pothuizen, et al., 2008). Burgess and co-workers have suggested that the retrosplenial cortex translates the allocentric representations into an egocentric framework (Bird & Burgess, 2008; Burgess, 2008). This function of the retrosplenial cortex could account for the impairments in direction and allocentric processing observed.

In order to establish whether retrosplenial cortex is a potential candidate for covert pathology in diencephalic amnesia it is necessary to look for some marker of neural function. The class of markers that were so far investigated are immediate early genes. Trans-synaptic activation can stimulate slow long-term changes in the post-synaptic neuron that involve the induction of gene expression. One of the categories of genes that are responsible for trans-synaptic activation are the immediate early genes. Their early activation combined with the fact that some immediate early genes (c-*fos*) are inducible transcription factors that can orchestrate the transcription of other down stream genes, places them as potential candidate makers for covert pathology. As immediate early genes are at the head of the cascade of changes it means that their abnormal activity may signal an array of other changes. Furthermore, some immediate early genes like c-*fos* have a role in synaptic processes thought to be central for memory and learning (Bozon, Davis & Laroche, 2002; Vann, et al., 2000a; 2000b).

Examination of early gene activity after anterior thalamic lesions revealed dramatic losses in early gene activation in the retrosplenial cortex (Jenkins, et al., 2004) (see Fig 4). Both bilateral and unilateral excitotoxic lesions of the rat anterior thalamus caused marked decreases in c-*fos* activity in the retrospleinal cortex (Jenkins, et al., 2002a; 2002b; 2004). Jenkins and colleagues have shown (as summarized by Aggleton, 2008) that 1) anterior thalamic lesions

cause 80% or more reductions in c-*fos* levels in the retrosplenial cortex as observed 4-6 weeks post-ATN lesion. The reductions in c-*fos* are most dramatic in the superficial layers of the Rga and Rgb; 2) anterior thalamic lesions cause c-*fos* depletions in rats 10 months post surgery and these depletions also become evident in the Rdg cortex and the deeper lamina of the Rga and Rgb; 3) anterior thalamic nuclei lesions do not affect just one immediate-early gene as matching patterns of *zif-268* depletions were also found; 4) the Fos changes are not specific to the lesion technique or rat strain; 5) this lesion-induced hypoactivty is not universal for all retrosplenial disconnections as lesions of the entorhinal cortex, a region that is also reciprocally connected to the retrosplenial cortex have little or no effect on Fos levels (Albasser, et al., 2007); 6) Anterior thalamic lesions do not alter the appearance of the retrosplenial cortex as determined by standard histological techniques and counts of Nissl stained cells (Jenkins, et al., 2004), although morphometric changes in the cells in the Rgd can be observed 4 weeks after ATN lesion.

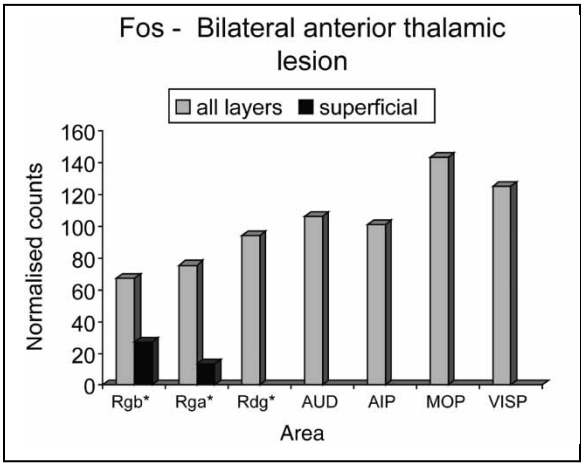

Fig 4. Anterior thalamic lesions and Fos activation (Jenkins, et al., 2002b data). Bar graphs showing c-Fos positive cell counts in retrosplenial cortex (Rga, Rgb, Rdg), as well as primary auditory cortex (AUD), insula cortex (AIP), motor cortex (MOP), and primary visual cortex (VISP) following excitotoxic lesions in the anterior thalamic nuclei. Counts in each region are compared with those from a control brain, and so a score of 100 reflects an identical count in the lesioned and intact hemispheres. By distinguishing the retrosplenial counts for the superficial layers (I–III) and for all layers it can be seen that anterior thalamic lesions have a much greater impact on superficial c-*fos* levels. From Aggleton (2008).

Jenkins and colleagues (2004) emphasized that this hypoactivity is not merely caused by global disconnection, as the anterior thalamic lesion leaves intact other inputs into the retrosplenial cortex (from the subiculum, postsubiculum, lateral dorsal thalamic nucleus, and enthorhinal cortex). The most extreme hypoactivity is observed in superficial cell layers of the retrosplenial cortex, while the deeper layers remain unaffected, even though the thalamic lesions leave intact a wide array of other excitatory inputs to the superficial retrosplenial cortex (Gonzalo-Ruiz, Morte & Lieberman, 1997). Given the evidence from human diencephalic infarcts (Van der Werf, et al., 2003) it is particularly interesting that the transection of the mammillothalamic tract was also sufficient to cause hypoactivity in the retrosplenial cortex similar to that observed after ATN lesions (Vann & Albasser, 2009). This lesion only indirectly disconnects the retrosplenial cortex (affecting the ATN inputs only) while all direct inputs remain intact. Electrophysiological studies have also demonstrated that following unilateral ATN lesions it was possible to induce long-term depression in the slice of the retrosplenial cortex in the unaffected hemisphere, but not in the slice ipsilateral to the lesion (Garden, et al., 2009).

Lesions to the hippocampus produce similar changes in the early-gene expression in the retrosplenial cortex as the anterior thalamic lesion (Albasser, et al., 2007). Clear reductions in both c-*fos* and *zif 268* were observed in both superficial and deep layers of the granular cortex, with there being no evidence of loss of neuronal numbers as seen on standard histology methods.

It was this pattern of results that led Aggleton group to suggest that both temporal lobe and diencephalic amnesia are characterized by hidden pathology in a common distal site, specifically the retrosplenial cortex. The authors point out that this hypothesis also has interesting implications in the human domain. For example, in Alzheimer's disease, hypoactivity in the posterior cingulate is often one of the first metabolic changes noted on the PET scan (Minoshima, Foster & Kuhl, 1994; Minoshima, Giordani, Berent, Frey, Foster & Kuhl, 1997) and it is also found in subjects with mild cognitive impairment which is often a prodromal stage of AD (Nestor, Fryer, Smielewski, & Hodges, 2003). In cases of Korsakoff's disease there is also evidence of posterior cingulate hypoactivity (Fazio, et al., 1992; Joyce, et al., 1994). This evidence suggests that retrosplenial dysfunction is a common feature of both temporal and diencephalic amnesias. A loss of retrosplenial cortex function may not only

exacerbate effects of the temporal and diencephalic lesions but may also help explain why the two types of amnesia share so many features.

The covert pathology theory of anterograde amnesia provides a basis for examining the possible recovery of functioning after anterior thalamic lesions. If no neuronal loss occurs in the retrosplenial cortex area, and yet there is evidence of hypoactivity which contributes to the amnesic syndrome, it is possible to speculate that any behavioral improvements observed following treatment administration after anterior thalamic lesions maybe a reflection of changes in the level of covert pathology elsewhere in the brain. Following ATN lesions the hippocampal system, prefrontal cortex system, and other thalamic inputs remain intact providing potential substrates for intervention which may also in turn effect the functioning of the retrosplenial cortex.

In summary, current theories of diencephalic amnesia which are based on animal and human research provide interesting insights into the neural basis of this condition. Accumulated evidence suggests that different medial thalamic nuclei are involved in different aspects of memory and each may have a specific and unique role to play. Novel suggestions that impaired ATN functioning can also negatively influence other ostensibly “healthy” brain regions offers exciting prospects particularly for the field of therapeutic intervention, where functional gains my be obtained via reactivation of these covert pathology regions. Clearly, the goals of therapeutic research are to eventually provide insights into the type of therapies or interventions which can be effectively used with humans suffering from diencephalic amnesia.

One of the many challenges researchers face in generalizing the animal results to the human domain is establishing whether the behavioral changes noted in animals actually reflect the changes in episodic memory abilities that one would ultimately want to enhance in amnesia sufferers. In the next section the approaches that are taken to test episodic memory in animals and the effectiveness of these approaches in assessing this construct are discussed.

3.7 Examining episodic memory in animals

While the animal models clearly have their advantages in terms of offering opportunity for high anatomical precision of lesion placement, and an ability to examine the physiological, pharmacological and molecular properties of the area of interest, the main caveat of this type of research is the generalizability of the findings across species. As key question in whether it is possible to assess episodic memory in animals.

Episodic memory in humans involves the conscious recollection of a unique past event that was personally experienced (Tulving, 1983). This is the type of memory which is impaired in human amnesia (Delay & Brion, 1969; Scoville & Milner 1957; Vargha-Khadem, Gadian, Watkins, Connelly, van Paesschen & Mishkin, 1997; Zola-Morgan & Squire, 1986). Studying episodic memory in animals is problematic due to the lack of experimental tasks for laboratory animals that model episodic memory. The difficulty in developing a task of episodic memory in animals arises due to the requirement for episodic memory to involve conscious recollection of the experience (Tulving 1983), which is extremely difficult to demonstrate without the use of language. Tulving (2002) even proposed that ‘mental time travel’ and ‘conscious recollection’ of personal experiences are such unique human-specific abilities that they have no counterpart in the animal kingdom. Other researchers (Clayton, Griffiths, Emery & Dickinson, 2001) argued instead that animals may possess and profit from an episodic memory system, particularly in terms of social interactions and foraging behavior. Animals that live in social groups and develop dominance hierarchies dependent on social interactions, and may need to remember an order in which to show appropriate social behavior, or recall specific social events (and its details in terms of what happened, where and when and who was involved) which led to a change in the hierarchy.

The definition of episodic memory provided by Tulving as memory that “receives and stores information about temporally dated episodes or events, and temporal-spatial relations between them” (Tulving, 1983) has been used to break down the episodic memory into its components (memory for “what”, “when” and “where”). One approach is to examine each individual component of episodic memory by itself and then to evaluate the situations which require two or more components together. That is, assessing memory for events by

demonstrating memory for an object (what), its location (where), and when it happened (when). Such "what-where-when" memory does not require evidence of either conscious recollection or awareness and has been demonstrated with some success in birds, and to a lesser degree of confidence in non-human primates and rats. Clayton and Dickinson (1998) allowed scrub jays to cache two types of food, one of which was preferred but more perishable over time. The food was cached in a number of spatial locations in the presence of a unique visual context. Following different delays the scrub jays remembered "when" food items were stored and searched preferentially for perishable food only soon after caching. They rapidly learned to avoid searching for perishable food after a longer interval during which the food decayed. Clayton and Dickinson (1998) argued that the spatial recovery preference of jays demonstrates memory of where and when the particular food items were cached, thereby fulfilling the behavioral criteria for episodic-like memory in non-human animals. However, the ability to cache for food relies on the natural behavior of the scrub jays, which makes it difficult to adapt this task to other species.

Attempts to replicate Clayton and Dickinson's (1998) findings in non-human mammals have yielded mixed results. While monkeys show reliable memory for the location of objects embedded in different visual background scenes presented on a computer monitor (Gaffan, 1994), and can remember after a single trial what kind of food was given to them and who gave it to them (Schwartz & Evans, 2001), or remember the order in which different types of food were presented (Schwartz, Hoffman & Evans, 2005), an integrated "what, where, and when" memory for unique experiences has not yet been shown in non-human primates. Bird and colleagues (Bird, Roberts, Abroms, Kit, & Crupi, 2003) asked whether rats, similar to scrub jays, are able to remember what kind of food they have cached in a modified radial-maze and where and when they did this. The rats, indeed, remembered the kind and location of two food items previously carried into single arms of the radial maze. However, despite the experience that a preferred kind of food cached at a specific location in a radial-maze was made unpalatable after one of two delays, the rats were unable to modify their foraging behavior to selectively search for a less-preferred but still edible food, which they had previously stored in a different location. Ergorul and Eichenbaum (2004) successfully trained rats to remember odor sequences presented in different locations in an open field. In the sample phase rats learned four unique sequences of odor stimuli presented in different places of the open field. In subsequent memory

tests, the rats were given the choice between an arbitrarily selected pair of odors in their original positions. The rats were rewarded for selecting the odor, which, during sampling, occurred earlier in the sequence. The rats were able to discriminate which odor had been presented first in the respective location, thus showing memory for “what, where, and when”. Ergorul and Eichenbaum (2004) also examined the contributions of odor and spatial cues to temporal order judgments in rats with hippocampal lesions. Their results suggest that intact rats, but not rats with hippocampal lesions, use a combination of spatial “where” and olfactory “what” cues to distinguish “when” the events occurred.

The difficulty researchers face in evaluating episodic memory in animals is the notion that animal's performance in delayed memory tasks might be guided by relative familiarity judgments rather than “conscious recollection” of a personal experience (Mayes & Roberts, 2001). The memory traces decay passively over time and hence an animal might judge the order of two caching episodes, (as in the scrub jay experiment) a sequence of odors, or objects presented sequentially by comparing the relative strengths of their memory traces.

Eacott and Norman (2004) suggested that in episodic memory, “when” serves only as an occasion setter to distinguish one experience from another similar experience and argued that the episodic-like memory in animals may be best defined as “what-where-which”. In monkeys and rats, scene memory has been considered as a measure of episodic like memory (Gaffan, 1994). In this task the animal learns about the location of a specific object within a unique background scene. However, such task again can be solved by familiarity of the objects seen. Eacott and colleagues (Eacott, Easton & Zinkivskay, 2005) also made attempts to develop tasks that assess familiarity and recollection in rats. The task relied on the rat’s innate preference to explore novel objects. The rats were trained in an E-maze where different contexts indicated different location of objects and hence the animal had to learn that the object location is stable within context but is different between contexts. Importantly, the objects were not visible to the rat in the test phase and the rat thus had to recollect where the novel object could be located depending on the context. While the animals demonstrated an ability to learn this task the response rate to the novel object was relatively low (65.2%). Although this task represents an attempt to test episodic

memory, difficulties with developing strong object preferences in animals mean that consistent and reliable results can be hard to obtain.

The various approaches described above provide an excellent start to the problems of evaluating episodic-type memory in rats, but for the most part researchers have to focus on simpler tasks that are highly sensitive to hippocampal system lesions (spatial memory tasks).

Over the years researchers have attempted to investigate what kind of information is selected in the environment by the animal and how this information is used to differentiate among locations. Some investigators suggested that animals preferentially learn about the location of the food in relation to all of the other cues in the environment (place learning) (Tolman, Ritchie, & Kalish, 1946) and others suggested that animals preferentially acquire a response tendency in which specific cues come to elicit a specific response – a response strategy (Blodgett & McCutchan, 1947). In a recent attempt to resolve this difference Skinner and colleagues (2003) conducted a number of ingenious experiments in the open field and T-maze to investigate what strategies the rats rely on to solve the T-maze. They observed that going to a specific place in the open field which is defined by specific distal cues is very difficult for the normal rat to learn, while the animals readily learn response and direction type solutions. Rats also found it difficult to learn a place strategy if they have to make competing responses, and go in an opposite direction (e.g. sometimes turn left and other times right, sometimes go east or west) to reach the food location that remained constant relative to the extra-maze cues. Direction and response are the two strategies that the animals will utilize more readily to solve the maze. The authors reported that rats are only able to utilize place strategy when they access the maze from different start points which allows for the presence of different (non-overlapping) extra-maze cues.

The ability of the animal to use and combine distal cues to guide their behavior in the environment independently of its relative position in space has been termed allocentric learning. The acquisition of allocentric spatial memory has been repeatedly demonstrated to be sensitive to hippocampal and ATN lesions (Aggelton & Pearce, 2001). One of the most frequently used tasks to assess the allocentric memory is a spatial working-memory task in a T or a cross maze. In this

task, as it is typically run, the subject is provided with a sample run in which it is permitted to enter only one arm of the T, the other arm being blocked. The subject is then returned to the base of the T and, after a delay, is permitted to run up the stem and choose either arm of the T. Reinforcement is only available in the arm that was not entered during the sample phase of the task. This task has repeatedly been utilized to show ATN induced memory impairment (Aggelton et al., 1995a; 1995b; 1996; Aggelton & Sahgal, 1993; Warburton & Aggleton, 1997; Warburton, Morgan, Baird, Muir, Aggleton, 1999). It also represented a key piece of evidence used by Aggleton & Brown (1999) in their review of diencephalic amnesia to support the notion of ATN involvement in spatial memory. The impairments demonstrated by ATN animals on the T-maze are severe and permanent and are not affected by pre-training (Warburton, et al., 1997; 1999).

The unique characteristic of the T-maze is that it cannot be solved by a fixed association between reward and location as the reward arms differ from trial to trial. Thus, the animal must remember what occurred on the sample phase of the trial to perform accurately in the choice phase, making it a working-memory type task. However, relatively few studies addressed the question as to what information the animal is actually using when trying to solve the T-maze. In a series of experiments Dudchenko (2001) attempted answer this question. Different delays were introduced between the sample and choice phase ranging from 10 seconds to 10 min and rats were tested either in the presence or absence of landmarks (spatial cues). Both cued and non-cued groups were able to solve the maze successfully and remember which arm they have selected for at least 5 min, although the performance declined at the 10min delay. When the spatial cues were scrambled, the spatial-cued rats demonstrated a decline in performance, suggesting that they relied on the extra maze cues to guide their performance to a great extent. The non-cued rats on the other hand were able to solve the maze by relying on egocentric or internal /response strategies, such as being able to remember the body turn made. When the egocentric cues were disrupted by getting the rats to run through an alternation box during the delay, the non-cued animals were still able to solve the maze at above chance levels. In other words, normal rats appear to use the spatial relationship between the maze arms and salient extra-maze cues when such landmarks are available, but yet also perform above chance levels when such spatial relationships are disrupted. Dudchenko (2001) suggested that under normal circumstances rats use a combination of strategies such as extra-maze landmarks (when

available) and a representation of the last turn made to solve the maze, which is in line with Skinner's and colleagues (2003) suggestion that the rats rely on multiple strategies to solve the maze.

The Aggelton laboratory (Aggleton, et al., 1995a; 1996; Warburton, et al., 1997) attempted to examine what maze strategies get disrupted when animals sustain lesions to the ATN. The researchers tested whether the ATN rats used an allocentric (spatially-based) or egocentric response strategy on the T-maze task by using probe trials. On the probe trials the rat started the choice phase on in an arm located 180 degrees from the start arm used in the sample phase. An allocentric strategy would predict that the rats would successfully choose the alternate location (although they would be making the same body turn); while a response strategy would suggest that the rats would incorrectly return to the same locations (by alternating a body turn). The sham rats were able to successfully solve the 180 degree problem trials demonstrating 77%-80% across studies mean correct responses on the first session, improving to 90% correct by the end of training (16-20 sessions). The scores of the ATN group for the 180 degree condition were at chance level (50-60% across studies), while these rats were able to perform well above chance when permitted to solve the task in the standard procedure (65-77% across studies). This suggests that the ATN lesion disrupted the rats' ability to use the allocentric cues, with the animals needing to rely on other strategies such as egocentric cues in order to solve the standard alternation task. Aggelton and colleagues (1996) tested this hypothesis further by examining whether the rats are able to learn to always make the same body turn and then reverse the turn. They trained the rats to always make a left or a right body turn irrespective of which start arm in the cross-maze was used. The ATN animals did not demonstrate any impairment, performing at the level of controls on this task, and being able to use egocentric or motoric cues to aid their performance. Subsequent replications of the allocentric spatial alternation in the T-maze with ATN animals have further confirmed that the deficits observed result from the rats' inability to use the extra-maze cues to guide responses (see Table 2 for summary of studies).

3.8 Summary

While assessing episodic-memory as we observe it in humans, remains to be a challenge in a laboratory setting, some very promising advances have been made in the area, especailly to the extent that the spatial memory tasks used assess the ability of the animal to form a “mental snapshot” of the environment. A T-maze or a modified T-maze (cross-maze task) is one of the spatial working memory tasks that has been associated with robust and permanent impairments in performance after ATN lesions. The performance of the ATN animals on this particular task has also been used as a primary evidence to support the notion that ATN constitute a part of the extended hippocampal system involved in spatial memory processing. A T-maze task on which reliable and seemingly permanent behavioural impairments can be obtained following an ATN lesion was therefore selected in the current thesis as an important first stage in the attempt to examine recovery of functioning after ATN lesions.

Chapter 4

Environmental enrichment as a therapeutic tool

4.1 Introduction

The concept of environmental enrichment (EE) is reviewed in this chapter. The contribution of EE to brain plasticity is discussed and examples of how EE is implemented are provided. The review focuses mainly on the behavioral effects of EE as observed in animal studies which try to model some kind of brain damage seen in humans. The impact of EE on the central nervous system is also considered with an emphasis on the neurobiological effects which may underlie behavioral changes.

4.2 Defining enriched environment

In 1949 Donald Hebb postulated a use-induced hypothesis of plasticity of the CNS, arguing for an increased effectiveness of environmental conditions that provide enhanced opportunities for physical and social stimulation and/or interaction in improving brain functions. However, it was not until the sixties and seventies when a major shift occurred in beliefs about brain plasticity, and more specifically about the effects of environment on brain development and adaptation. In the late seventies research began to emerge that the environment may have a role in treating brain-damaged subjects, this leading to the substantial body of new research being generated that examined the environment as a therapeutic tool (Greenough, et al., 1976; Jones & Smith, 1980).

A group of researchers headed by Rosenzweig had previously conducted a series of systematic experiments that explored the effects of EE on cognition. These

experiments provided a model of housing conditions commonly used today in enrichment experiments. In the standard housing condition, rats were housed in groups of 3-6 per standard cage without objects. In the EE conditions, the rats were housed in much larger groups of 8-12 animals per large cage, which provided numerous objects and an increased opportunity for social interaction. Furthermore, the variety of objects (boxes, ladders, cups, tubes) were changed daily and provided an opportunity for sensory and motor stimulation. In many early experiments, another condition was employed - an impoverished environment condition, in which a rat was housed in isolation in a small cage, with no objects. Housing rats in isolation was common place and remains a typical feature even today when rats (or mice) have received an experimental brain injury.

Although some studies have investigated the impact of pre-operative enrichment on recovery from brain damage or from childhood, adult or age-related experiences, the main focus on this chapter is on the effects of post-operative experiences on recovery. This approach is adopted because modifying the environment post-operatively as a therapeutic procedure if of more relevance for clinical purposes than a pre-operative manipulation.

Across the last three decades Will and colleagues have systematically reviewed the field on environmental enrichment (Will, 1981; Will & Kelche, 1992; Will, et al., 2004). The reviewed evidence clearly points to the fact that enrichment often, but not in every case, has a positive effect on recovery from brain damage. A few studies have documented adverse effects, although the results were mixed with both negative and either neutral or positive effects observed depending on the task used (see Will & Kelche, 1992 for review). The prevalence of studies that demonstrated at least no deleterious effect led Will and colleagues (2004) to conclude that enrichment is a low risk therapeutic tool, but the effectiveness of enrichment in reversing the behavioral effects of brain damage is still debated.

4.3 The generality of enrichment effects

Over the last 30 years the generality of enrichment effects has been broadened. Evidence is now available that EE can induce behavioral effects after various kinds of brain damage, after various periods of differential exposure, in various behavioral testing situations, at different ages and in both genders, and in various rat strains and even other species.

While the early studies mostly used rats, mice and occasionally rabbits, subsequent studies have also used other species such as non-human primates, cats, gerbils, deer-mice and chickadees (Cornwell & Overman, 1981; Feeney, Gonzalez & Law, 1982; Hovda & Feeney, 1984; Lovely, Gregor, Roy & Edgerton, 1986; Mohammed, et al., 2002). Post-operative enrichment has been noted to facilitate learning in the Hebb-Williams maze in both normal kittens and kittens with neonatal lesions of the marginal and posterolateral gyri (Cornwell & Overman, 1981). In both cats and monkeys enrichment prevented the development of unilateral vestibular neurectomy (Lacour, 1984, cited in Will & Kelche, 1992). Enrichment was also shown to be more important in promoting recovery in squirrel monkeys than simple motor training (Plautz, Milliken, & Nudo, 2000). Exercise is generally not as effective as enrichment especially in terms of promoting spatial learning (Black, Isaacs, Anderson, Alcantara, & Greenough, 1990; Gomez-Pinilla, So, & Kesslak, 1998; Kleim, Lussnig, Schwarz, Comery, & Greenough, 1996; Kleim, Vij, Ballard, & Greenough, 1997), so the social component afforded by the EE is seen as critical. Only a few studies have examined the impact of the social component on its own (Einon, Morgan, & Will, 1980; Finger & Stein, 1982). Einon and colleagues (1980) were able to demonstrate that both social housing and environmental enrichment improved the performance of rats with hippocampal lesions on a radial maze task, but this improvement was greater when social grouping and physical complexity were combined. Will and colleagues (Will, Toniolo, Kelche, Pallage, Deluzarche, & Misslin, 1986) found that introducing different objects into the cages of isolated rats was sufficient to produce behavioral changes in hippocampal lesioned rats on tasks of reaction to a novel environment and to novel objects. Neuronal morphology studies identified that the combination of social and physical complexity resulted in increased thickness in the

rats' cortex in comparison to the effects of each condition on its own (Diamond, 1988). The combination of social condition and exposure to new stimulus objects seem necessary for animals to gain a full effect of the enrichment.

In the early experiments designed to explore the impact of an enriched environment on the brain, post-weaned intact rats were maintained in environment from 25 to 105 days of age as there was no available data on how long it would take to create chemical and structural changes in the brain. Over time, evidence emerged that the differences in cortical thickness with 80 day exposure to the EE was not as great as with 30 day exposure (Bennett, Diamond, Krech, & Rosenzweig, 1964; Diamond, Krech, & Rosenzweig, 1964). Consequently, the length of exposure period was reduced from 80 days to 30 days, then 15 days, 7 and even 4 days. At each of the intervals animals from EE showed increases in cerebral thickness in some areas, but not others. For example, 80 days exposure did not induce changes in somatosensory cortices while the 30 day exposure did. The occipital cortex showed changes after 30 and 80 days exposure, but the gains were larger at 30 days (Bennett, et al., 1964). Bennett and colleagues (1964) suggested that the lengthy exposure may possibly become monotonous with its effects decreasing over time. One early study also suggested that a few hours of exposure to EE per day results in similar alterations in neurotransmitters and brain weight as continuous 24hr/day exposure (Rosenzweig, Love & Bennett, 1968), and later experiments indicate that as little as 4 days of environmental complexity can be sufficient to induce alterations in dendiritic morphology (Wallace, Kilman, Withers & Greenough, 1992). In studies that specifically focus on post-operative enrichment 30 days continuous enrichment is the most commonly used paradigm, although exposure times of 60 and 90 days, as well as continuous exposure for a 24-hour period, or for just several hours a day, have been used with comparable success (Bindu, Rekha, & Kutty, 2005; Diamond, 1988; Diamond, et al., 1964, Kolb & Gibb, 1991; Turner & Greenough, 1985; Rosenzweig, et al., 1968; Zolman & Morimoto, 1962). Positive effects of EE on behavior were documented even when the introduction to the EE was delayed 275 days after basal forebrain lesion (Paban, et al., 2005).

The initial EE research has focused mainly on studying its beneficial effects on recovery from surgical lesions (see Table 3). Behavioral recovery has been observed after visual cortex (Cornwell & Overman, 1981; Delay, 1988); sensory motor cortex (Christie & Dalrymple-Alford, 1994); cortical (Rose, Attree, Brooks & Johnson, 1998; Schwartz, 1964); basal forebrain (De Bartolo, et al., 2008; Fréchette, Rennie & Pappas, 2009); hippocampal (Einon, et al., 1980; Galani, et al., 1997; Kelche & Will 1982; Pacteau, Einon & Sinden, 1989); hypothalamic (Wolgin & Teitelbaum, 1978); reticular thalamus, (Sauro, et al, 2001); subiculum (Bindu, et al., 2005; Dhanushkodi, Bindu, Raju & Kutty, 2007); medial prefrontal cortex (Waddell, Pistell, Heldt, & Falls, 2000), and fimbria-fornix (Van Rijzingen, Gispen, & Spruijt, 1997) lesions. More recently, the generality of enrichment effects has been broadened to include other kinds of brain injury. The latest studies have often used enrichment to promote recovery in animal models of degenerative disorders. EE has been found to delay the onset of motor symptoms in Huntington's disease transgenic R6/1 model and in the more severe R6/2 model, exposure to EE was associated with the rescued levels of BDNF (brain-derived neurotrophic factor) in the striatum and hippocampus (Ferrer, Goutan, Marín, Rey & Ribalta, 2000; Spires, et al., 2004; Van Dellen, et al., 2000; Zuccato, et al., 2001). Similarly, long-term exposure (6 months and more) to enrichment was found to result in global improvements in cognitive function as assessed on tasks of working memory, reference learning, and recognition/identification and in some cases also resulted in decreased amyloid deposition in the mouse model of Alzheimer's disease (Arendash, Garcia, Costa, Cracchiolo, Wefes & Potter, 2004; Lazarov, et al., 2005). In animal models of developmental type disorders, such as in fragile X knockout mice (Grossman et al., 2001) or in Ts65Dn partially trisomic mice (Down Syndrome model) (Baamonde, Martinez-Cue, Lumbreras, Paz, Dierssen & Florez, 2001; Martinez-Cue, et al., 2002) exposure to EE also enhanced working memory functioning. Models of hereditary conditions such as dwarfism benefited from EE exposure resulting in improved learning on Hebb-Williams and T-maze tasks (Bouchon & Will, 1982). Recovery following from more extensive trauma to the brain such as in cases of traumatic brain injury (Hoffman et al., 2008; Kline et al., 2007) or stroke (Briones, et al., 2004; Puurunen, Jolkkonen, Sirvio, Haapalinna, & Sivenius, 2001) was also promoted by exposure to enrichment.

Improved performance on the Morris Water maze has consistently been observed in animals exposed to EE following a middle cerebral artery occlusion (Ronnback, et al., 2005).

Table 3. Summary of studies that examine the effects of enrichment-type conditions on recovery from focal surgical lesions.

Year	Authors	Lesion/ Species	Enrichment protocol	Behavioral tasks	Outcome
2009	Fréchette, Rennie & Pappas	Forebrain cholinergic lesion/7-day-old rats	EE exposure from weaning for 42 days	Working-memory spatial navigation in MWM	EE= improved
2008	De Bartolo Leggio, Mandolesi, Foti, Gelfo, Ferlazzo & Petrosini	Basal forebrain/rats	Reared in EE from day 21 to 90	Serial learning task	EE= improved
2007	Dhanushkodi, Bindu, Raju & Kutty	Ventral subiculum/rats	Post-op EE exposure for 10 days	8-arm radial maze	EE=improved
2005	Bindu, Alladi, Mansooralikhan, Srikumar, Raju & Kutty	Ventral subiculum/rats	Post-op EE exposure for 6h/day for 10 days	8-arm radial maze, MWM	EE= improved on the radial maze but not MWM
2005	Paban, Jaffard, Chambon, Malafosse & Alescio-Lautier	Medial septum and nucleus basalis magnocelluralis/rats	Post-op EE exposure from day 275-500	Non-matching to position in the T-maze, object-recognition, object-location, open field	EE= improved performance on matching to position, object recognition but not object location or open field
2001*	Sauro, Sweeney & Saari	Reticular thalamus/rats			EE=improved
2000*	Waddell, Deni, Garrett & Zrull	Medial prefrontal cortex/rats			EE=improved
1998	Galani, Coutureau, & Kelche,	Hippocampus, subiculum, ethorhinal/rats	Post-op EE exposure for 30 days	8-arm radial maze	EE= improvement only in subiculum rats
1997	Galani,, Jarrard, Will, & Kelche,	Hippocampus, subiculum, ethorhinal/rats	Post-op EE exposure for 30 days	Reaction to novelty, MWM, Hebb-Williams maze	EE= hippocampus rats improved on Hebb-Williams but not on MWM and novelty task; no effect in other lesion groups
1997	Van Rijzingen, Gispen & Spruijit	Bilateral or unilateral transection of fimbria-fornix bundles/rats	Post-op EE exposure for 6 weeks	MWM	EE= less severe deficit in bilaterally lesioned rats in time to swim to platform

					and time spent in the edge zone
1994	Christie & Dalrymple-Alford	Unilateral sensori-motor cortex/rats	Post-op EE exposure for 30 days	Beam walking	EE= improved
1991	Kolb & Gibb	Cortical/rats	Post-op EE exposure for 90 days	Measures of body weight, claw cutting, food hoarding, tongue extension, spatial navigation in the MWM, running wheel activity	EE= improved on all parameters but not food hoarding and grooming
1989	Pacteau, Einon & Sinden	Dorsal hippocampus/ weanling rats	Post-op EE exposure for 30 days vs. social and isolated	Radial arm maze, plus maze, spontaneous alternation in a T-maze	EE= improved performance on the T maze but not radial arm maze
1988	Dalrymple-Alford, Kelche, Eclancher & Will	Septum/rats	Pre-op rearing in EE for 57 days	Open field, 12-arm radial maze	EE= no improvement on both tasks, bar increased ambulation in an open field.
1988	Rose, Dell, Love & Davey	Unilateral cortical/rats	Post-op EE exposure for 30 days	Go/No-Go reversal task	EE= no improvement
1987	Dalrymple-Alford & Kelche	Septum/ weaning rats	EE exposure at weaning till 57 days	Open field and 12 arm radial maze	EE= lowered ambulation in the open field but no effect on the radial maze
1987	Gentile, Beheshti & Held	Sensorimotor cortex /rats	Pre-op: EE exposure for 2h/day for 25 days; or access to activity wheel, or impoverished. Post-op all impoverished.	Locomotor testing in a runway	EE= enhanced locomotion; running wheel rats recovered more slowly
1987	Kelche, Dalrymple-Alford & Will	Fimbria-fornix transection/rats	Post-op EE exposure versus isolated housing	8-arm radial maze	EE = improved only the sham rats but no improvement in lesioned rats
1987	Kolb & Elliott	Frontal decortication/1-5 day old rats	Post-op EE from 1-5 days to adulthood	Tongue extension	EE= improved in 1 and 5 day operated rats
1987	Rose, Davey, Love & Dell	Unilateral neocortical lesion/rats	Post-op EE for 30 days	Bracelet test	EE= no recovery
1986	Will, Toniolo, Kelche, Pallage, Deluzarche & Misslin	Dorsal hippocampus/ 30-day old rats	Isolated with or without objects plus 5 min daily exposure to new cage	Reaction to novel object and novel environment, 8-arm radial maze	Exposure to objects= more exploration of novel objects, improved on radial maze, no differences in reaction to novel environment
1985	Albert, Walsh & Longley	Septal, medial accumbens and medial hypothalamus/weanling	Post-op group or isolated rearing for 6-13 days	Defensiveness to experimenter	Social rearing= reduced defensiveness after septal and medial accumbens

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		rats			lesions but not after hypothalamus lesions
1985	Dalrymple-Alford & Kelche	Dorsal hippocampus/rats	Post-op EE exposure for 30 days	Place learning	EE=improved
1985	Held, Gordon & Gentile	Sensorimotor cortex/rats	EE exposure for 2h/day for 30 days during immediate pre and/or post-op period versus impoverished	Locomotion in a runway	Pre-op EE= no deficits in locomotion; Post-op EE= reduced deficit.
1984	Dalrymple-Alford & Benton	Dorsal hippocampus/ weaning rats	Isolation rearingversus social raring	Open field, runway task, shuttle box task	Social rearing = improved on all tasks
1984	Whishaw, Zaborowski & Kolb	Hemidicortication in rats at birth or adulthood	Post-op EE exposure for 90 days	MWM	EE= improved MWM in adults, but no further improvement in neonates
1983	Will, Deluzarche & Kelche	Dorsal hippocampus/rats	EE exposure for 7, 15 or 23 days post-op, social or isolated	Spontaneous alternation	EE= improved across all durations
1982	Engellenner, Goodlett, Burright & Donovick	Septal/mice	Reared in EE or restricted conditions from weaning to 60 days and returned post-surgery	Handling reactivity; open field stress reactivity; MWM spatial discrimination	EE= improved on all tests
1982	Goodlett, Engellenner, Burright & Donovick	Septal/mice	5 weeks of post-weaning reared in continuous EE, or restricted or switched from EE to restriction 24h post surgery	Reactivity to handling	EE= reduced reactivity to handling; switch to restriction= increase in reactivity
1981	Cornwell & Overman	Marginal and posterolateral gyri/kittens	Post-operative rearing in EE	Maze learning and form and grating differentiation	EE= improvement on maze learning
1981	Will, Kelche & Deluzarche	Entorhinal/rats	Post-op EE exposure 35 or 60 days vs impoverished	Spontaneous alternation; Hebb-Williams maze	EE= no improvement on alternation; unclear effects on Hebb-Williams
1980	Einon, Morgan & Will	Dorsal hippocampus/rats	Post-op EE-exposure for 30 days vs. social or impoverished	Radial maze and motor transfer	EE and social = improved on radial maze and motor transfer
1979	Will & Kelche	Dorsal hippocampus/rats	Post-op EE exposure for 30 or 60 days	Two-way active avoidance response acquisition and extinction in shuttle-box	EE= improved in both
1978	Kelche & Will	Dorsal Hippocampus/rats	EE exposure for 60 days post-op vs impoverished	Hebb-Williams maze	EE= improved
1978	Wolgin & Teittelbaum,	Hypothalamus/30-day old	Sensory stimulation	Feeding	Sensory stimulation

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		cats			=improved
1977	Will, Rosenzweig, Bennett, Herbert & Morimoto	Occipital cortex/rats	Post-op EE exposure for 60 days for 24h/day or for 2h/day vs impoverished	Hebb-Williams maze	EE= improved for both
1976	Will & Rosenzweig	Occipital cortex/rats	Post-op EE exposure for 60 days vs impoverished	Hebb-Williams maze	EE= improved
1973	Donovick, Burright & Swilder	Septum/ rats	Rearing in EE	Exploration, fluid consumption	EE=improved
1964	Schwartz	Posterior cortex/ neonatal rats	Rearing in EE for 44 days	Hebb-Williams maze	EE=improved
1959	Smith	Anterior temporal cortex/rats	Post-op EE exposure for 72 days	Hebb-Williams maze	EE=improved

Abbreviations: EE = enriched environment; MWM = Morris Water Maze; *= the article is not fully accessible through public domain literature searchers.

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Environmental treatments have also been used successfully with intact animals in order to delay or offset the impact of normal aging or exposure to adverse conditions. Exposure to enrichment has been correlated with a better performance in older animals on different tasks of learning such as Morris Water Maze and Hebb-Williams maze (Bennett, McRae, Levy & Frick, 2006; Kempermann, Gast & Gage, 2002; Kobayashi, Ohashi & Ando, 2002; Milgram, et al., 2005; Mohammed, et al., 2002) as well as neural plasticity such as changes in dendrite branching, (Kolb, Gibb, & Gorny, 2003), spine density (Kolb, et al., 2003) and neurogenesis (Kempermann, et al, 2002). At other end of the age spectrum, the adverse effects of early life experiences such as neonatal hypoxia (Pereira, et al., 2007; Pereira, Strapasson, Nabinger, Achaval, & Netto, 2008); maternal exposure to alcohol (Hannigan, O'Leary-Moore & Berman, 2007), inadequate life conditions such as low maternal care (Bredy, Humpartzoomian, Cain, & Meaney, 2001), social isolation (Hellemans, Benge, & Olmstead, 2001) and exposure to toxins (Lee, Anderson, Zuck, Lidsky, & Schneider, 2000) can also in some cases be substantially eliminated by rearing in enriched conditions.

Overall, numerous reports are now available which indicate that improvements after brain damage can be obtained if an animal is exposed to enriched environment. However, the specificity of enrichment effects remains uncertain, with lesion studies in particular reporting "mixed" results, even with hippocampal system lesions.

4.4 Specificity of enrichment effects

The specificity of EE effects has been most readily observed in studies which employ focal brain lesions. As noted above, exposure to EE has been found to enhance recovery after many brain lesions, including lesions to the limbic system (Dalrymple-Alford & Benton, 1984; Dalrymple-Alford, Kelche, Eclancher & Will, 1988; Donovick, Burright, & Swidler, 1973; Einon, et al., 1980; Galani, et al., 1997). The hippocampal lesioned rats were shown to demonstrate improved performance on the Hebb-Williams maze (Kelche & Will, 1978); T-maze (Pacteau, et al., 1989; Will, Deluzarche & Kelche, 1983); radial maze (Einon, et al., 1980); place learning (Dalrymple-Alford & Kelche, 1985) and avoidance response (Will & Kelche, 1979) tasks following enrichment. In contrast to the

improved performance usually found following exposure to enrichment after hippocampal lesions, enrichment does not seem to affect recovery on the Hebb-Williams maze when damage to the septum, (Dalrymple-Alford & Kelche, 1987) or the entorhinal cortex (Will, Kelche & Deluzarche, 1981) is sustained or on the radial arm maze following damage to the fimbria-fornix pathways (Kelche, Dalrymple-Alford & Will, 1987). Mixed enrichment effects were also reported in a study by Galani and colleagues (1997) who examined the effects of EE on lesions to subcomponents of the hippocampal system, including the hippocampus, entorhinal cortex and the subiculum. Rats with hippocampal damage showed improved performance on working and reference memory tasks which are ordinarily difficult for the hippocampal rats to solve, demonstrating a decreased response latency to find a platform in the water maze, increased time spent in the previously correct quadrant during the probe trial and better learning on the Hebb-Williams task. The enriched hippocampal rats also demonstrated reduced locomotion. While lesions to the entorhinal cortex produced similar behavioral impairments as hippocampal lesions, enrichment did not promote recovery in the entorhinal rats on the Hebb-Williams task. The authors argued that these differences in therapeutic effects were due to the different type of errors committed by the animals on the tasks. The greatest effect of housing in the hippocampal rats was found in the number of repetitive or perseverative errors committed by rats on the Hebb-Williams task, while the entorhinal rats made less perseverative errors. Given that perseveration, as well as locomotion, are affected by enriched housing, the authors suggested that the main effect of recovering from brain damage in the enriched environment was due to decrease in activity with a resulting improvement in attention. Lesions to the subiculum induced only a mild impairment in the probe-trial on the water-maze task and the impairment was not improved by enrichment exposure. More recent studies (Bindu et al., 2005; Dhanushkodi, Bindu, Raju, & Kutty, 2007) reported that lesions to the subiculum resulted in severe spatial deficits in both radial maze and water maze. However, post-operative enrichment after subiculum lesions has brought recovery in the 8-arm radial maze but not the water maze. To explain the differences observed the authors tried to examine the different spatial demands of each task. They have noted that on the water maze task the EE rats continued to swim around the edge, hence never encountering an opportunity to get to the

platform and demonstrating a comparable number of errors on this task to the non-enriched rats. In concert with the argument made by Galani and colleagues (1997), Bindu and colleagues (2005) suggested that enrichment probably promotes the use of non-hippocampal mediated escape strategies rather than re-instates the spatial strategy per se.

Bindu and colleagues (2005) have also questioned whether the different motivational aspects of each task can contribute to task specificity observed. The rats trained in the radial maze task are food deprived and it is possible that food deprivation may alter sensory, motor, and motivational aspects of behavior. These aspects are lacking in rats exposed to the water maze. Previously, Dalrymple-Alford and colleagues (1985) found that 10 days of post-operative differential housing in rats with dorsal hippocampal lesions at the age of 30 days produced opposite effects in two behavioral tasks, the Morris water maze and a dry version of the same task. In the latter task, a small food cup filled with dry food pellets was hidden under the sawdust in a circular arena. The sawdust maze and the water maze were located in the same room, thus equating the spatial memory requirements. However, exposure to EE had no beneficial effects on performance in the water maze, while the same animals demonstrated improvements on the dry version. In attempt to explain such effects Will & Kelche (1992) suggested that enriched housing condition may influence the level of stress, sensory, motor, temporal and motivational aspects of the tasks and differential housing conditions possibly alter these aspects differentially and according to the performance.

Rose and colleagues (Rose, Dell, Love, & Davey, 1988) have concluded that task specificity of enrichment effects points to the importance of carefully defining recovery. They paid particular attention to differentiating between compensation and recovery by using unilateral neocortical lesions, hence avoiding total loss of sensory modality, and then examining behavioral deficits not thought to be primarily due to sensory loss. They suggested that their results were consistent with the view that enrichment facilitates compensation and not recovery (Rose, Davey, Love, & Dell, 1987). Similarly, Held and colleagues (1985) reported that both pre-operative and postoperative enriched housing attenuated beam walking after sensorimotor cortex lesions but normal responses were

only observed in the pre-operatively enriched rats while the post-operatively enriched rats still demonstrated aberrant pathology (Held, Gordon, & Gentile, 1985). Compensation might also underlie the neuromorphological data reported by Kolb and Gibb (1991), who noted that EE produced similar effects in intact and lesioned animals without modifying the lesion-induced plastic processes. This evidence has led Stein and colleagues (Stein, Finger, & Hart, 1983; Stein & Hoffman, 2003) to suggest that CNS insults may lead the animals to alter their behavioral strategies such that a problem can be solved in a new or different way, with enrichment effects thus reflecting the ability of the system to compensate for the damage sustained, rather than induce restitution of premorbid behavior.

The absence of EE effects after damage to the afferent-efferent systems of the hippocampus is more difficult to explain. Attempting to explain why EE can be an effective therapeutic tool after hippocampus damage, but not after lesions to the fimbria-fornix Will and colleagues (2004) suggested that as the fimbria-fornix lesions result in serotonergic deinnvervation of the hippocampus and/or possibly prevent the cell proliferation in the dentate gyrus. Neurogenesis in the dentate gyrus is known to be one of the effects of EE (Kempermann, Kuhn & Gage, 1998). However, no experimental evidence exists to support neurogenesis as a mechanism of recovery. It is clear that careful analysis of lesion effects is important for understanding of the putative EE benefits and could be best achieved in a single experiment with different lesions but same housing, behavioral and neurological assessments.

As the above summary highlights, enrichment is generally regarded as a treatment strategy that seems to have a beneficial effect on behavior following a wide-range of brain insults. It is safe and relatively easy to administer. However, the treatment effects are not always achievable across all testing conditions and lesion types. The specificity that exists can potentially be explained by the underlying neuronal plasticity that may help promote behavioral recovery.

4.5 Neurobiological effects of enrichment

Despite the vast data accumulated on the positive behavioral effects of enrichment, the mediators of improved performance following enrichment remain largely unclear. Morphological and neurochemical changes associated with enrichment have been identified, which are presumed to contribute to memory enhancement.

Early enrichment experiments showed that it promotes morphological changes, such as increases in cortical weight and thickness (Bennett, et al., 1964). Work by Greenough (1976) suggested that cortical changes are caused by either enhanced dendritic branching or synaptogenesis. Later experiments demonstrated that increases in dendritic branching, synaptic contact areas and the number of synapses per neuron can occur in the occipital cortex of rats after exposure to EE (Churchill, Galvez, Colcombe, Swain, Kramer, & Greenough, 2002). In intact animals, reach training (i.e. reaching with a forelimb to access a reward) has been shown to selectively alter dendiritic branching in layers II and III of pyramidal neurons in rat motor-somatosensory forelimb cortex (Whithers & Greenough, 1989). Housing in EE also significantly increases the number of dendrite spines both in cortical layers II-III and V-VI, indicating that activity in the EE leads to general stimulation of dendritic spines (Johansson & Belichenko, 2002). As neurons in layers II-III have extensive connections with other cortical areas Hess and colleagues (1996) suggested that synaptic plasticity in cortico-cortical connections promote cortical map re-organization (Hess, Aizenman & Donoghue, 1996). Morphological changes also occur in the hippocampus in response to enriched environment, including increased thickness (Walsh, Budtz-Olsen, Penny & Cummins, 1969) increased dendritic spine density in the CA1 area (Moser, Trommald, & Andersen, 1994), increased synaptic transmission (Green & Greenough, 1986) and increased neurogenesis in the dentate gyrus (Kempermann, Kuhn, & Gage, 1997). The latter effect has been implicated, but never proven in the context of EE induced enhancement of performance in hippocampal-dependent tasks (Bruel-Jungerman, Laroche, & Rampon, 2005; Nilsson, et al., 1999). However a more recent study by Meshi and colleagues (2006) demonstrated that EE had a beneficial effect on spatial learning and anxiety-like behavior despite the hippocampal neurogenesis being blocked.

At the neurochemical level, neurotrophins and neurotransmitters have been suggested to participate in the plasticity induced by EE (Mohammed, et al., 2002; van Praag, Schinder, Christie, Toni, Palmer & Gage, 2002). Among the neurotransmitters, norepinephrine and serotonin may be of special interest with regard to the effects of EE because they both play an important role in the modulation of brain plasticity (Gu, 2002). Nevertheless, a role for norepinephrine in the behavioral effects of EE has not been consolidated, as most studies show that norepinephrine depletion has no impact on behavioral expression in EE exposed rats (Benloucif, Bennet & Rosenzweig, 1995; Murtha, Pappas, & Raman, 1990; but see Mohammed, Jonsson, & Archer, 1986). Concerning serotonin, recent study by Galani and colleagues (2007) demonstrated that exposure to EE resulted in a significant behavioral improvements in serotonin depleted rats. Exposure to enrichment reduced the serotonin levels in the ventral hippocampus (particularly sham rats), increased serotonin turnover in the entire hippocampus (particularly in lesioned rats) and augmented the norepinephrine levels in the dorsal hippocampus (in both lesioned and sham rats); but no such alterations were found in the frontoparietal cortex. The authors suggested that enrichment can still exert its beneficial behavioral effects even when the serotonergic system is depleted. Changes in acetylcholine and dopamine levels were also noted in the prefrontal cortex of rats exposed to EE and have been linked to reduced levels of stress demonstrated by aged and young animals in the open field tasks (Del Arco, et al., 2007; Segovia, Del Arco, de Blas, Garrido, & Mora, 2008; Segovia et al., 2009).

In contrast to the substantial amount of research on EE induced neuro-morphological and neuroanatomical changes in intact animals, the data concerning brain modification after brain damage is limited and somewhat controversial. Early studies demonstrated the EE induced changes on gross neuroanatomical measures following cortical lesions and malnutrition (Katz, et al., 1980; Rosenzweig & Bennett, 1976), and increases in the weight of residual cortex after various bilateral cortical lesions (Rosenzweig, 1984). Kolb & Elliot (1987) showed that EE experience could attenuate the thinning of cerebral cortex induced by neonatal frontal lesions, but the effect was age

dependent, being most pronounced in rats who underwent surgery at 5 days and less pronounced in animals who sustained lesions at 1 day of age. Kelche & Will (1982) carried out an experiment that demonstrated that hippocampal lesions significantly decreased the branching and number of spines of basilar dendrites in layer V pyramidal cells of area 17 in both post-operatively enriched and impoverished rats. The cytological measures were only affected by postoperative rearing conditions in sham-operated rats. Ip and colleagues (Ip, Giza, Griesbach, & Hovda, 2002) subjected postnatal rat pups to a fluid percussion injury and then housed them in EE (17 days) or standard conditions. The dendritic density and dendritic branching was analysed in frontal, parietal, and occipital cortices. Rearing in EE induced an increase in dendritic density, primarily within the occipital cortex. Fluid percussion injury induced an increase in dendritic density, primarily in regions remote from the injury site, namely contralateral parietal cortex and ipsilateral and contralateral occipital cortex. In injured animals subsequently housed in EE, the injury appeared to inhibit the experience-dependent dendritic density effects of EE. However, an unexpected enhancement of dendritic density was seen in the ipsilateral occipital cortex, indicating the region-specific sensitivity to experience-dependent plasticity (Ip, et al., 2002). Johansson & Belichenko (2002) occluded the middle cerebral artery in rats and then housed them in a standard or in an enriched environment for 3 weeks. They subsequently observed that in intact rats, the number of dendritic spines was significantly higher in the enriched group than in the standard group in all layers of the pyramidal neurons in the somatosensory cortex. Contralateral to the infarct, pyramidal neurons in layers II/III, which have extensive intracortical connections that may play a role in cortical plasticity, had significantly more spines in the enriched group than in the standard group. They conclude that housing rats in an enriched environment significantly increases spine density in superficial cortical layers in intact and lesioned brain and this effect may mediate compensatory processes which underlie behavioral changes demonstrated after ischemic rats' exposure to EE (Biernakie & Corbett, 2001; Grabowski, Sorensen, Mattsson, Zimmer, & Johansson, 1995; Johansson & Ohlsson, 1996).

In attempt to explain the morphological changes that occur in response to enrichment, Will (1981) originally speculated that trophic factors such as NGF might be involved in the mediation of differential housing effects. To sustain synaptic plasticity neurons utilize several neurotrophic factors (nerve growth NGF and brain derived neurotrophic factor BDNF). The role of NGF in regulating neuronal survival and maintaining functional plasticity during adulthood is well established (Barde, 1989; 1994). Many studies have suggested that NGF may be essential for maintenance of the cholinergic neuron phenotype in the nuclei of the basal forebrain, and low level of NGF has been linked to the effects of cognitive decline associated with aging (Carswell, 1993; Henriksson, Soderstrom, Gower, Ebendal, Winblad & Mohammed, 1992). NGF has also been shown to influence the functional correlates of the limbic system and infusion of NGF has been demonstrated to enhance spatial memory (Backman, et al., 1996; Chen & Gage, 1995). BDNF has been found to affect neuroplasticity in terms of long-term potentiation and depression (Korte, Kang, Bonhoeffer, & Schuman, 1998).

A number of studies generated by a group of researches in Sweden have now linked changes in NGF to the effects of EE exposure. For example, Pham and colleagues (1999) demonstrated that exposure to EE resulted in increase of NGF in the hippocampus, particularly in the brains of non-handled rats. The NGF changes were also accompanied by improved performance of the enriched non-handled rats on spatial learning tasks (Pham, Soderstrom, Winblad, & Mohammed, 1999). Furthermore, studies have also confirmed that EE enhances the expression of mRNA's that encode for trophic factors (NGF, BDNF, NT-3, GDNF) increasing the amount of NGF protein and the density of NGF receptors (Falkenberg, Mohammed, Henriksson, Persson, Winblad & Lindefors, 1992; Mohammed, Winblad, Ebendal, & Larkfors, 1990, Mohammed, et al., 2002; Pham, Winblad, Granholm, & Mohammed, 2002; Young, Lawlor, Leone, Dragunow, & During, 1999; Zhu, Yee, Nyffeler, Winblad, Feldon, & Mohammed, 2006). These mechanisms may prevent spontaneous apoptosis and provide a mechanism of long-term neuroprotection. Following brain insults EE also increases the levels of BDNF in ischemic rats (Puurunen, et al., 2001; Zhao, Lein, He, Smith, Aston, & Gage, 2001), as well as increases synapse density in the CA1 region in CA1 NMDAR-1 knockout mice. Although gene expression changes have been most intensively studied in the

hippocampus, cortical changes have also been observed. Rats with sensorimotor cortex infarcts demonstrated gene-up-regulation in the contralateral to the infarct cortex area following exposure to EE (Keyvani, Sachser, Witte, & Paulus, 2004).

It is well accepted now that neurons are continually born from endogenous stem cells and added to the hippocampal dentate gyrus (as well as other brain areas such as olfactory bulbs) throughout life, a process which is termed adult neurogenesis. However, adult hippocampal neurogenesis declines precipitously with age. The functional importance of neurogenesis in the adult hippocampus is still uncertain (Eriksson, 2003). Nevertheless, improved performance in learning and memory tasks under conditions that stimulate neurogenesis such as enrichment and running (Kempermann, et al., 1997; van Praag, Kempermann & Gage, 1999) as well as stimulation of neurogenesis in learning situations (Gould, Tanapat, Hastings, & Shors, 1999; Shors, Miesegaes, Beylin, Zhao, Rydel, & Gould, 2001) suggest that newly formed granular cells of the adult dentate gyrus become an integral part of hippocampal functioning. In fact, it was shown that the newly formed neurons in the dentate gyrus which were generated under the running condition were integrated into the hippocampal circuitry, determined by intracellular recording of individually labeled neurons (van Praag, et al., 2000). EE has repeatedly been demonstrated to promote hippocampal neurogenesis. Levels of BrdU which mark newly generated neurons have been shown to increase in the hippocampus dentate gyrus of intact rats after EE exposure (Tashiro, Makino, & Gage, 2007). Kempermann, Kuhn, Gage (1997) demonstrated that significantly more new neurons existed in the dentate gyrus of the hippocampus of mice exposed to an enriched environment compared with littermates housed in standard cages. The enriched mice had a larger hippocampal granular cell layer and 15% more granular cell neurons in the dentate gyrus. A further study by Kempermann and colleagues (2002) demonstrated that after intact 10-months old rats were exposed to 10 months of continuous enrichment hippocampal neurogenesis was still observed. This finding indicates that the stimulatory effect of enrichment does not wear off on continued exposure. Kempermann and colleagues (2002) suggested that presence of increased adult hippocampal neurogenesis was not a reflection of an acute response to a novel stimulus, as environmental complexity did not change besides regular

re-arrangements, but rather indicated a persistently elevated enrichment-induced baseline level of neurogenesis. Using the expression of immediate early gene products, c-*fos* and *zif268*, as indicators of recently activated neurons, Tashiro and colleagues (2007) showed that previous exposure to an enriched environment increased the total number of new neurons and the number of new neurons responding to re-exposure to the same environment. The increase in the density of activated new neurons occurred specifically in response to exposure to the same environment but not to a different experience. Furthermore, these experience-specific modifications were affected exclusively by previous exposure around the second week after neuronal birth but not later than 3 weeks. Thus, the animal's experience within a critical period during an immature stage of new neurons determines the survival and population response of the new neurons and may affect later neural representation of the experience in the dentate gyrus. The researchers argued that the experience-specific functional modification through adult neurogenesis could possibly be a mechanism by which new neurons exert a long-term influence on the function of the dentate gyrus related to learning and memory.

Increasing adult neurogenesis by environmental enrichment is presumed to be associated with improvement in learning tasks, but adult neurogenesis cannot be made responsible for all functional changes associated with environmental enrichment. Abolishing adult neurogenesis by irradiation did not lead to the disappearance of functional benefits from environmental enrichment – at least within the scope of the tests applied, such as spatial working memory, long-term conditional rule retention and spatial reversal (Hernández-Rabaza, et al., 2009). Although the study does not disprove that enrichment might exert functional effects by increasing adult neurogenesis, it demonstrates that correlation is not causality.

The expression of immediate early genes (IEG's) has been postulated as one of the molecular mechanisms that control plastic changes in the CNS (Wallace, Withers, Weiler, George, Clayton, & Greenough, 1995). Wallace and colleagues demonstrated that the zinc-finger IEG nerve growth factor induced gene-A (NGFI-A) is up-regulated in the brains of rats exposed to EE. Expression of this IEG was lower in animals that were simply manipulated or left undisturbed. A recent study showed that EE caused a complex

profile of gene expression in the striatum of adolescent mice, which consisted of the up-regulation of 48 genes that are implicated in various functions, such as cell proliferation and differentiation, intracellular signaling, transcription and translation, as well as structural changes and cell metabolism (Thiriet, et al., 2008). Increased levels of Arc (which is the IEG that labels dendrites) have been demonstrated in intact animals in the cortex as well as CA1, CA2, CA3 and to a lesser extent in the dentate gyrus after exposure to EE (Pinaud, Penner, Robertson, & Currie, 2001).

Changes in the IEG can also occur in EE-exposed brain-injured animals. Up-regulation in Fos levels have been noted in the granular cell layer in the dentate gyrus in ischemic rats who underwent enrichment and water-maze training (Puurunen, et al., 2001). It is interesting however, that enrichment by itself without testing did not produce changes in the Fos levels, suggesting that activation by explicit training is needed to reveal the effects of EE housing on the function of the dentate gyrus.

Thus, there are now sufficient data available that point to the fact that enrichment is associated with changes at the cellular level, including the promotion and survival of new adult neurons in the dentate gyrus. Although the functional significance of neurogenesis is not well understood it has been suggested that the new born pathways may contribute to the establishment of long-term potentiation, which in turn contributes to the behavioral improvements on the tasks of memory (Garthe, Behr & Kempermann, 2009).

4.6 Interaction between environment and drugs

Studies that have addressed the issue of interaction between environmental manipulation and drug administration are limited. Most of the research has focused on investigating the potential impact of enrichment on reversal of deleterious effects induced by pre and post-natal exposure to various drugs and neurotoxic substances such as beta-blockers (Ryan & Pappas, 1990), alcohol (Hannigan, O'Leary-Moore, & Berman, 2007; Rema & Ebner, 1999; Wainwright, Levesque, Krempulec, Bulman-Fleming, & McCutcheon, 1993), toxins (Lee, et al., 2000), cocaine (Neugebauer, Cunningham, Zhu, Bryant, Middleton & Dwoskin, 2004; Solinas, Thiriet, El Rawas, Lardeux, & Jaber, 2009) and diazepam

(unpublished research cited in Johansson & Belichenko, 2002). However, little is known about possible augmentation of enrichment effect by drug administration. Selegiline, has been shown to reduce behavior and cognitive deficits following focal brain ischemia both by itself and in combination with enriched environment exposure (Puurunen, et al., 2001). Conversely, amphetamine had no additional effects on ischemic rats housed in enriched environment (Johansson, Mattsson, & Ohlsson, 1997), although exposure to EE reduces amphetamine-induced impulsivity (Perry, Stairs, & Bardo, 2008).

Overall, the interaction between drugs and environment and the question as to whether the effects of EE exposure can be further potentiated by drug administration requires further experimental attention.

4.7 Application of experimental data on enrichment to human populations

Enrichment findings in studies with rodents cannot be easily applied to the human condition. What is called “enriched” under laboratory conditions is arguably substantially impoverished against a rodent habitat in the wild (for a more detailed discussion, see Smith & Corrow, 2005; van Praag, et al, 2002). However, the key point with EE paradigm is not to gain a precise knowledge of individual stimuli sufficient to elicit the effect of interest, as these will certainly greatly differ between rodents and humans, but to show a more fundamental process, the brain’s potential for plasticity, and the influence of experience on the degree of this plasticity. In the field of traumatic brain injury and stroke rehabilitation it has long been known that intensive rehabilitative input provided by trained medical staff, and a coordinated multidisciplinary team input including education of patients and families, has vastly beneficial effects on the recovery of the patient (Beaulieu, Rivard, Hudon, Beaudoin, Saucier, & Remondin, 2002; Cole, Paulos, Cole, & Tankard, 2009; Johansson, 2000). No study has yet demonstrated whether such beneficial effects are due to the rehabilitation input and time spent in different therapies or to the non-specific effect of a more stimulating environment or to other factors such as patients’ expectations or placebo effect.

A substantial amount of literature has considered the importance of cognitive enrichment earlier in life on cognitive ability at a later time. Anstey & Christensen (2000) identified 32 studies that examined various predictors of age-associated decline, with education being one of the major predictors. These studies show that education has a general protective effect with respect to developing dementia. However, a detailed review of age studies by Kramer, Behrer, Colcombe, Dong, & Greenough (2004) highlighted the notion that education effects are process specific, and mainly affect crystallised abilities later in life, having little impact on the fluid-type intelligence, such as working memory or processing speed capacity. A longitudinal Nun Study conducted by Riley and colleagues (2005) found that the high levels of linguistic ability in early life were associated later in life with lower incidence of cognitive impairment, and with a lower incidence of neuropathological measures associated with Alzheimer's disease (AD) (Riley, Snowdon, Desrosiers, & Markesbery, 2005). These findings suggest that the factors responsible for poor performance earlier in life contribute to both levels of education and to the subsequent development of impairments. Winnock, Letenneur, Jacqmin-Gadda, Dallongeville, Amouyel, & Dartigues (2002) reported a higher incidence of APOE4 allele, associated with a higher incidence of dementia, in low educated subjects, suggesting the possibility that APOE phenotype has an influence on cognition throughout life.

By definition, cognitive enrichment is different from education, and there are reports that enrichment later in life, related to the complexity of the work environment has a positive effect on intellectual functioning. Engaging in cognitively stimulating activities such as reading magazines and books and playing chess at an older age was associated with reduced risk of AD four years later (Wilson, Bennett, Bienias, & Aggarwal, 2002). Cognitive training of subjects who have already demonstrated mild to moderate AD seems to have more limited effects. Moore and colleagues (2001) trained mild to moderate AD patients on different tasks of attention and memory and while the subjects were able to demonstrate improvements that were maintained at one month follow-up, the improvements were specific to the cognitive tests used (Moore, Sandman, McGrady, & Kesslak, 2001).

A cognitive reserve hypothesis has been widely cited as one of the possible mechanisms of positive effects of education on cognitive function in older individuals. The hypothesis suggests that high levels of education are able to delay the clinical expression of dementia, because of the brain's ability to utilise available neural structures as a back-up or reserve. The hypothesis relies on the assumptions that some kind of neuroplasticity linked to cognitive experiences provides more neural reserve to support normal cognitive functioning despite the presence of brain pathology. The cognitive reserve hypothesis is supported by the evidence that rate of cognitive decline in AD is directly related to the educational background (Andel, Vigen, Mack, Clark & Gatz, 2006).

Overall, human literature points to the relationship between cognitive experiences early in life and cognitive decline associated with aging. Cognitive enrichment, as assessed by education and job complexity, has protective effects against the development of dementia. The effects may be limited however, with crystallised abilities showing more protection than fluid skills. Enrichment that occurs following brain insults also tends to be associated with beneficial outcomes, however it becomes more difficult to ascertain which components of the stimulating environment are associated with positive behavioural changes. The human studies, provide evidence of plasticity of the human brain in response to the environment, and consequently also support the use of EE in animal models.

4.8 Summary

Environmental enrichment in the laboratory setting provides an easily administered, accessible, low risk and yet potentially powerful therapeutic tool for brain damage. The effects of enrichment may not always be uniform, and lesion-type as well as task specificity exists. Despite mixed findings, there is sufficient evidence of positive EE effects on memory function, particularly after hippocampal system damage, to suggest it could be a viable candidate for therapeutic intervention following ATN lesions.

Chapter 5

Cerebrolysin as a Therapeutic Tool

5.1 Introduction

This chapter reviews the pharmacodynamics of the drug Cerebrolysin. The neurobiological properties of the drug and modes of action are discussed. The literature pertaining to the effectiveness of this compound in improving learning and memory in both the human and animal domain is presented.

5.2 Pharmacological properties of Cerebrolysin

Cerebrolysin (Cere) is a commercially available drug (EBEWE Pharmaceuticals Ltd., Austria). It is a systemically injected brain-derived peptide preparation, produced by standardized enzymatic breakdown of lipid free porcine brain proteins (Paier, Windisch, & Eggenreich, 1992). Cerebrolysin consists of approximately 25% low-molecular-weight peptides (<10kDa), based on the total nitrogen content, stabilized with amino acids. The manufacturers of the drug suggest that Cerebrolysin has a multimodal action and expresses itself in several ways: 1) via a neurotrophic effect by inducing neuronal differentiation and hence producing protection against different ischaemic and neurotoxic lesions; 2) by regulation and improvement of neuronal metabolism which prevents lactacidosis in hypoxic or ischemic episodes, 2) modulation of synaptic plasticity which corresponds to improved behavior and learning.

The mechanism of the putative neuroprotective effect of Cere is still unknown. The assumption is that this brain derived peptide may have neurotrophic effects. Cere was found to show clear neuroprotective properties after different types of lesions in vitro

and in vivo, resembling the pharmacological activities of naturally occurring nerve growth factors such as NGF or BDNF (Windisch, Gschanes, & Hutter–Paier, 1988). Satou and colleagues (2000) reported that Cerebrolysin exerts a neurotrophic activity similar to NGF on chicken dorsal root ganglia. Akai, Hiruma, Sato, & Ivamoton (1992) investigated the regeneration of the cholinergic cells in the medial septal nucleus after axonal transaction by cutting the fimbria-fornix and found that Cerebrolysin was able to prevent the degeneration of medial septal cholinergic neurons, causing on average 49.9% of neurons to survive and the Cere protection was achieved after a peripheral injection, indicating that the small molecules were able to penetrate the blood-brain barrier in pharmacodynamically significant amounts. Comparison of intracerebroventricullar versus intraperitoneal injections of Cerebrolysin supported this finding (Gschanes, Valoušková, & Windisch**,** 1997). Gschanes and colleagues continuously infused high (0.57 mg/day) and low doses of Cerebrolysin (0.0057 mg/day) into the lateral ventricle as well as administered Cerebrolysin daily via intraperitoneal injections (100 mg/day) to ischemic animals. The animals' swimming speed and spatial memory was then assessed on the Morris Water maze. The low dose iv. and ip. rats demonstrated comparable improvements in performance, recovering functioning up to the levels of intact controls. The high iv. dose resulted in disturbance in motor speed and spatial memory. The ability of Cerebrolysin to cross the blood-brain barrier which makes invasive application strategies like intracerebroventricular infusion unnecessary, contributes to its uniqueness as an NGF-like compound for potential neurotherapy.

Due to its neurothrophic like characteristics Cerebrolysin was also found to be in treatment of neurodegenerative disorders such as Multiple Sclerosis, Alzheimer's dementia, Parkinson's and Huntington's diseases by inducing protection against cell death or apoptosis. In a study that examined cultured chick embryonic neurons (in vitro) the addition of Cerebrolysin resulted in significant percentage reduction in cells showing apoptosis (Hartbauer, Hutter-Paier, Skofitsch, & Windisch, 2001). Reduction of apoptosis levels following Cerebrolysin administration was also demonstrated in a mouse model of Alzheimer's disease (APP-tg) (Rockenstein, et al., 2006; Rockenstein, Adame, Mante, Moessler, Windisch, & Masliah, 2003) as well as in-vitro models of brain ischemia

(Gutmann, Hutter-Paier, Skofitsch, Windisch, & Gmeinbauer, 2002; Schauer, et al., 2005). The mechanism of action leading to an antiapoptotic effect of Cerebrolysin still needs to be determined. A possible explanation of the apoptosis preventing effect of Cerebrolysin has been provided by Wronski and colleagues (Wronksi, Tompa, Hutter-Paier, Crailsheim, Friedrich, & Windisch, 2000) who examined the effects of Cerebrolysin on calcium-dependent cysteine protease calpain. Over-activated calpain has been suggested to be a key factor in a destruction of cytoskeletal proteins involved in the pathophysiology of ischemia and AD and activation of calpain has been implicated in excitotoxic and in some types of apoptotic cell death (Siman & Noszek, 1988; Squier, Miller, Malkinson, & Cohen, 1994). Wronski and colleagues were able to observe that administration of Cerebrolysin in-vitro resulted in the inhibition of the calcium-dependent protease calpain, supporting the notion that the drug can prevent cell death.

Cerebrolysin has also been shown to enhance the growth of neurons in tissue cultures. Some of the earlier original research on Cerebrolysin reported stimulated nerve growth in vitro (Lindner, Grosse, Matthies, & Kirsche, 1975). The density of cells or number of cells per volume unit, rate of mitosis and phases of mitotic non-neuronal cells were changed by Cerebrolysin. Cerebrolysin was also shown to increase synapse density (Mallory, Honer, Hsu, Johnson, Rockenstein, & Masliah, 1999; Reinprecht, Gschanes, Windisch, & Fachbach, 1999; Rockenstein, et al., 2002). In both young (6-weeks) and old (24 month) rats, a 7 day or a 19 day treatment with Cerebrolysin resulted in marked increase of synaptophysin-immunoreactive presynaptic terminals in entorhinal cortex, the dentate gyrus, and hippocampal subfields CA1, CA2, and CA3 (Reinprecht, et al., 1999; Windholz, Gschanes, Windisch, & Fachbach, 2000), suggesting that the Cerebrolysin induced increase in synapse density may possibly underlie the improved learning and memory functions observed in treated rats.

Increases in glutamate receptors have also been postulated as another mechanism of action of Cerebrolysin. Glutamate has been implicated in the learning processes and marked reductions in density of glutamate receptors have been noted in the hippocampus of the AD patients. Old rats that have been treated with Cerebrolysin not only

demonstrated improved performance on the Morris water maze but also were noted to have increased number of glutamate receptors in the hippocampus (Eder, Reinprecht, Schreiner, Skofitsch & Windisch, 2001; Gschanes, et al., 2000). In attempt to further elucidate the therapeutic action of Cerebrolysin a more recent study examined its effects on kynurenic acid (KYNA) (Baran & Kepplinger, 2008). KYNA is an endogenous metabolite of the kynerenine pathway of trytophan degradation and is an antagonist of the ionotropic excitatory amino acid, glutamate, and nicotinic cholinergic receptors. Increased KYNA levels have been observed in AD and elderly subjects (Baran, Jellinger, & Deecke, 1999) as well as patients with Down syndrome (Baran, Cairns & Lubec, 1996) and with schizophrenia (Schwarcz, Rassoulpour, Wu, Medoff, & Tamminga, 2001). In vitro Cerebrolysin administration to human and rat brain homogenates has been found to lower KYNA formation, leading to the speculation that glutamenergic and cholinergic neurotransmission is modulated via that mechanism, thus contributing to therapeutic effects of Cerebrolysin (Baran & Kepplinger, 2008).

Cerebrolysin has also been found to promote adult neurogenesis. Presently, study of the events that lead to increased neurogenesis is being actively investigated. One of these events is activation of neurogenesis via the release of neurotrophins (Magavi, Leavitt, & Macklis, 2000). As was outlined in the previous chapter (Chapter 4) environmental enrichment is one of the most reliable therapeutic tools that can lead to neurogenesis. Rats held under a combination of both complex inanimate and social stimulation show enhanced dentate gyrus neurogenesis as well as dendritic arborization (Gould, et al., 1999; Nilsson, et al., 1999; Young, et al., 1999). Enhancement of neurogenesis by an enriched environment appears to be mediated mainly by the inhibition of spontaneous apoptosis and prolonged survival of the progenitors (Kepmermann, et al., 1997). Administration of Cerebrolysin was also shown to promote neurogenesis in the dentate gyrus, potentially by inhibition of the spontaneous apoptosis of progenitors, and increases in the number of BrdU-positive newborn cells (Tatebayashi, Lee, Li, Iqbal, & Grundke-Iqbal, 2003). Tatebayashi and colleagues (2003) argued that Cerebrolysin probably rescued one of the daughter cells of a parent progenitor, which otherwise would have died by apoptosis. Furthermore, increased neurogenesis was also associated with

improved escape latencies observed in Cerebrolysin treated 8-12 months old rats on the Morris water maze (Tatebayashi, et al., 2003).

Other than its neurotrophic-like properties Cerebrolysin has also be implicated in glucose metabolism. Boado and Pardridge (1999) demonstrated that Cerebrolysin improves the transport of glucose from blood to the brain. Cerebrolysin increased the expression of the BBB GLUT1 glucose transporter gene in cultures of brain endothelial cells. The activity of this protein, which plays a pivotal role in the transport of glucose from blood to the brain, is modified under pathological conditions. By increasing the expression of BBB GLUT1 Cerebrolysin may accelerate repair of the BBB in the regions compromised by hypoxia and thus contribute to their functional recovery, as manifested by the absence of hemorrhagic infarctions in Cerebrolysin treated rats (Boado & Pardridge, 1999).

The neuroprotective properties of Cerebrolysin have also been investigated in various animal models of brain disorders, such as Alzheimer's disease and stroke. Although the precise mechanism leading to the neurodegeneration in AD is not fully understood, studies suggest that alterations in the processing of amyloid precursor protein (APP) result in the accumulation of some A-beta and APP C-terminal products might play a key role in the pathogenesis of AD (Kamenetz, et al., 2003). Cerebrolysin has been shown to ameliorate the neurodegeneration and reduce amyloid burden in an animal model of AD (Rockenstein, et al., 2002). It also reduced amyloid pathology by decreasing APP production and protolysis (Rockenstein, et al., 2006). Moreover in the APPtg mice administration of Cerebrolysin ameliorated changes in neurogenesis in the dentate gyrus subgranular zone, and the changes were correlated with reduced deposition of amyloid aggregates (Rockenstein, et al., 2007).

In animal models of stroke intravenous Cerebrolysin reduced mortality by about 50% after bilateral carotid artery occlusion in rats (Schwab, Lutum, & Seufert, 1997) and reduced infarct size by 60% as well as the loss of MAP-2 immunoreactivity (Schwab, et al., 1998). Enhancement of the forelimb and hindlimb placement and better performance

on the body swing tests was also observed in Cerebrolysin treated rats that underwent MCA occlusion (Ren, et al., 2007). At the cellular level Cerebrolysin has been shown to ameliorate the effects of oxidative cell stress (Gonzalez, Francis, & Castellano, 1998; Sugita, Kondo, Kanazawa, Itou, & Mizuno, 1993).

While the key mechanisms of Cerebrolysin action are still not well understood, the accumulated data indicate that it most likely includes its promotion of neurogenesis by acting as a neurotrophic agent. The trophic properties make Cerebrolysin particularly valuable as a possible therapeutic agent in conditions which are not reversible, and where its trophic properties may assist in slowing the progression of the disease (such as in dementia) or in reducing negative outcomes (such as in strokes). Human data have now accumulated that further supports the use of Cerebrolysin as a neuroprotective drug.

5.3 Application of Cerebrolysin in humans

For the last 30 years Cerebrolysin has been prescribed as an anti-ageing substance (Ukraintseva, Arbeev, Michalsky, & Yashin, 2004) being most extensively used with Alzheimer's disease (AD) patients (see Table 4). The first therapeutic attempts for AD have focused on the deficits of central cholinergic neurotransmission (Patel, 1995). However, direct cholinergic replacement strategies turned out to be ineffective but the use of cholinesterase inhibitors (AChEI's) produced improvements in mild to moderate AD (Farlow & Evans, 1998; Taylor, 1998). The first substance used was Tacrine which produced modest but reproducible improvements in cognitive performance as assessed on the Alzheimer's Disease Assessment Scale - cognitive subscale (ADAS-cog) and treatment also resulted in improvements in Global Rating Scales (Davis, et al., 1992). The use of Tacrine though was limited by its substantial side-effects and hepatoxicity (Knapp, et al., 1994). Another substance that became available was Donepezil which was better tolerated and could be administered orally (Shintani & Uchida, 1997; Rogers, Doody, Mohs, Friedhoff, 1998; Rogers, Farlow, Doody, Mohs & Friedhoff, 1998). The dual-acting AChEI Rivastigmine, showed more cholinergic side-effects, but of mild to moderate severity (Anand, Gharabawi, & Enz, 1996). Both compounds, Donepezil and Rivastigmine produced improvements in cognitive functioning, global rating, and

activities of daily living. Other available drugs, Metrifonate (Williams, 1999) and Galanthamine (Birks, 2006) also produced similar effects to Donepezil and Rivastigmine. Interestingly, despite the considerable differences between compounds in the strength of enzyme inhibition all these agents are associated with comparable levels of improvement. The main limitation of AChEI's is that drug withdrawal leads to rapid loss of the gains made to the level of placebo-treated controls (Kaduszkiewicz, & Hoffman, 2008; Lledo, Alonso & Grubb, 2006; Rogers, 1998a, b). Another line of treatment has focused on the NMDA antagonist Memantine, which seems to have a similar efficacy to that of cholinergic drugs, but with best results being obtained in moderate to severe forms of AD (Reisberg, Doody, Stöffler, Schmitt, Ferris, & Möbius, 2003; Tariot, Farlow, Grossberg, Graham, McDonald, & Gergel, 2004). Stabilization strategies aiming at delaying disease progression rather than symptom improvement have become the focus of current drug development. Several avenues of research are being explored including anti-oxidants, anti-inflammatory drugs, statins, drugs that counteract amyloid deposition or toxicity (Lledo, et al., 2006). However, recent reviews of pharmacological treatments of AD have emphasized the need to develop drugs that exert neuroprotective effects (Becker & Greig, 2008). Clinical trials with intracerebroventricular application of NGF in AD proved to be disappointing due to side effects such as hyperalgesia and weight loss, and no improvements in cognition could also be shown (Olson, et al., 1992; Seiger, Nordberg & von Holst, 1993). However, there were improvements in cerebral blood-flow (Nordberg, et al., 1997). Scientific attempts to improve neurotrophic therapy for neurodegenerative disorders are continuing, which aim to overcome problems with blood-brain barrier penetration and also look at creating substances that act on different growth factor receptors.

As Cerebrolysin's small molecules are able to penetrate the blood-brain barrier in pharmacodynamically significant amounts, thus allowing it to be administered peripherally (intravenously), it presents a viable treatment option in AD cases. Furthermore its neurotrophic properties and ability to reduce amyloid formation (Rockenstein, et al., 2002) and improve glucose transporter expression (Boado & Pardridge, 1999) demonstrated in the laboratory settings support the trialing of

Cerebrolysin as a possible agent for the treatment of AD (Frick, Price, Koliatsos & Markowska, 1997).

Studies with Cerebrolysin in patients suffering from AD have demonstrated some clinical benefits (Kofler, 1990). In the last 7-8 years a number of major double-blind placebo-controlled studies were conducted which evaluated the efficacy of Cerebrolysin in AD (see Table 4 for citations). The sample sizes in the studies varied between 53-178 patients and included mild to moderate cases of AD as assessed on the Mini-Mental State Examination. In majority of studies Cerebrolysin was administered intravenously at a doze of 30 ml mixed in 100 ml saline for 5 days a week for between 4-12 weeks. The primary outcome measures included the ADAS-cog (Alzheimer's Disease Assessment Scale- cognitive subscale), which is a psychometric scale consisting of 11 items that evaluate selective aspects of memory, orientation, reasoning and carrying out instructions; the clinical impression measures: Clinical Interater Based Impression of Change (CIBIC) or Clinical Global Impression/Change (CGI/C) which are similar instruments that use information obtained during independent clinical interview to assess disease severity and progression; and measures of Activities of Daily Living (ADL). All of the double-blind placebo controlled studies were able to detect statistically significant improvements in global impression rating (Alvarez, et al., 2006; Bae, et al., 2000; Panisset, et al., 2002 Ruether, et al., 1994; 2001; Ruther, Ritter, Apecechea, Freytag, Gmeinbauer & Windisch, 2000; Xiao, Yan & Yao, 2000) and some also detected trends towards improvements in ADL rating (Alvarez, et al., 2006; Ruther, et al., 2001; Panisset, et al., 2002). Positive cognitive changes were less consistently observed with improvements on ADAS-cog, MMSE and the Trail Making Test detected in some (Bae, et al., 2000; Ruether, et al., 1994; 2000; 2001; Xiao, et al., 2000) but not other studies (Alvarez, et al., 2006; Panisset, et al., 2002). Although the patients in the Panisset and colleagues study (2002) had a low baseline cognitive impairment which led to the ceiling effects. Alvarez and colleagues (2006) argued that the variability in the impact of Cerebrolysin on cognitive functioning may be attributable to the dose-related profile of activity of Cerebrolysin on cognition and behavior in AD. The authors documented that improvements on ADAS-cog could be

observed at a lower 10 ml dose, while the standard 30 ml (used in other studies) resulted in some positive cognitive changes, the magnitude of difference failed to reach significance. The high 60 ml dose had a positive effect on global rating and on the neuropsychiatric symptoms displayed. Alvarez and colleagues (2006) suggested that Cerebrolysin might improve negative symptoms at cognition-enhancing doses and exert tranquilizing and/ or antidepressant-like effects at higher doses in AD. Importantly, in all three dose levels no significant adverse effects have been documented.

Table 4: Summary of randomized, double-blind, placebo-controlled studies conducted on the effectiveness of Cerebrolysin in human populations.

Year	Authors	Patient population	Outcome variables	Treatment regime
2006	Alvarez, Cacabelos. Laredo, Couceiro, Sampedro, Varela, Corzo, Fernandez-Novoa, Vargas, Aleixandre, Linares, Granizo, Muresanu, Moessler	**AD**: Age: ≥50 (N=279); MMSE 14-25; Modified Ischemic Score ≤4	Significant difference to placebo group 10 ml = improved on ADAS-cog (-4.099 points) and CIBI/C = 65% 30 ml = improved on CIBI/C = 60% 60 ml = improved on CIBI/C = 58.8% and NPI = (-5.4 points) No difference to placebo group for all doses on MMSE DAD	10, 30, 60 ml/day for 5days/week, for 4 weeks, then 2days/week for 8wks
2005	Ladurner, Kalvach, Moessler	**Stroke**: (N= 146) 24h post stroke onset	Significant difference to placebo group on SST ($p < 0.05$) CNS score for days 1,3,7,14, and 21 but not at 90 days On two measures of the Barthel Index - feeding and incontinence No difference to placebo group on MMSE apart from the right-sided stroke subgroup.	50ml/day for 21days
2002	Panisset, Gauthier, Moessler, Windisch	**AD**: Age ≥60; (N =192) MMSE 10-26; Hachinski Ischemic Score ≤4	Significant difference to placebo group on CIBI/C = (-0.44) points at 12 weeks No significant difference to placebo on ADAS-cog MMSE Slight improvement on DAD ($p = 0.093$).	30ml/day, 5days/week, for 12 weeks; assessed at week 4, 12, and 24
2002	Ruether, Alvarez, Rainer, Moessler, (subgroup of Ruether, et al. 2001)	**Moderate to Severe AD**: Age=50-80; (N=109); MMSE <20	Significant difference to placebo group on CGI/C ($p = 0.004$) ADAS-cog = 4.1 points Maintained at 28 weeks follow-up.	Same as Ruether et al. (2001)
2001	Ruether, Alvarez, Rainer, Moessler	**AD:** Age = 50-80; (N=149); MMSE 14-24; Hachinski Ischemic Score ≤4	Significant difference to placebo group on CGI (p =0.004) ADAS-cog = 3.2 points	30ml/day, 5 days/week, for 4 weeks repeated after a 2-months treatment free interval

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2000	Bae, Cho, Cho, Hoon, Choi, Lee, Jung, Kim, Lee, Choi, Cho, Lee	**AD**: Age ≥50; (N = 53) MMSE 10-24; Hachinski Ischemic Score ≤4	Significant difference to placebo group on ADAS-Cog (p = .02) CGIS/C (p = .01) MMSE (p = .04) No significant difference to placebo on GDS ADL	30ml/day, 5days/week, for 4 weeks
2000	Ruether, Rither, Apecechea, Freytag, Gmeinbauer, Windisch	same sample at Ruether et al., (1994) after 28 weeks follow-up	Significant difference to placebo group on SCAG = (- 11.1) points No difference to placebo on ZVT CGI NAI Bf-s	Same as for Ruether et al., 1994
2000	Xiao, Yan, Yao	**AD**: Age =55-85; (N=154); MMSE 14-25; Reisberg score: 3-4; Hachinski Ischemic Score ≤4	Significant difference to placebo group on MMSE = 2.5 points CGI = 72% improvement SCAG = (-6.2) points NAI = (-3.61) ZVT-1 = (-43.9) No difference to placebo group on ADL	30ml/day, 5days/week, for 4 weeks
1994	Ruether, Rither, Apecechea, Freytag, Windisch	**AD**: Age = 55-85; (N = 120); MMSE 15-25; Reisberg score: 3-4; Hachinski Ischemic Score ≤4	Significant difference to placebo group on ZVT-G = (-24.2) points SCAG = (-17.4) points CGI = 61.2% improvement NAI = (-11) points Bf-s = (-14.7) points	30ml/day, 5days/week, for 4 weeks

Abbreviations: AD = Alzheimer's Disease, ADAS-cog/noncog =Alzheimer's Disease Assessment Scale-Cognitive and Non-cognitive Subscales, ADL = Activities of Daily Living; Bf-s = Brief function scale; CNS = Canadian Neurological Scale, CGI = Clinical Global Impressions, CGI/C = Clinical Global Impressions /Change, CIBI/C = Clinical Interview Based Impression of Change, DAD = Disability Assessment in Dementia; GDS = Geriatric Depression Scale; MMSE = Mini-Mental State Examination; NAI = Nuremberg Activities Inventory; SCAG = Geriatric Clinical Assessment-Sandoz; SST = Syndrome Short Test; ZVT -G/ ZVT-1 = Trail Making Test.

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Unfortunately, no head-to-head studies comparing the effectiveness of Cerebrolysin versus that of AChEI's have been conducted. Comparisons of outcomes of Cerebrolysin administration and AChEI's suggest that both agents have similar effectiveness. Administration of AChEI's was reported to result in improvements in cognitive functioning on the ADAS-cog of 1.6 to 3.9 points (Anand, et al., 1996; Kaduszkiewicz & Hoffman, 2008; Raskind, Peskind, Wessel, & Yuan, 2000). Cerebrolysin administration was documented to produce improvements of 3.2 to 4.1 points. Considering that estimated rates of cognitive decline on the ADAS-cog in one year can be about 9 points for untreated AD patients, stabilization of decline is of clinical relevance. Another factor that supports the use of Cerebrolysin is its low toxicity. Unfortunately, AChEI's are associated with the presence of adverse effects such as vomiting, diarrhea, and weight loss with these being significantly more prevalent in the treatment groups in comparison to controls (Kaduszkiewicz & Hoffman, 2008). While Cerebrolysin can be associated with some mild adverse effects including urinary tract infections, depressive symptoms, dizziness and fever the frequency of these adverse effects (2-17%) is not significantly different from that observed in the control groups (Alvarez, et al., 2006; Panisset, et al., 2002; Ruether, et al., 2001). Furthermore no changes in blood parameters or vital signs occur with Cerebrolysin administration even at higher doses (Alvarez, et al., 2006). The treatment effects of cholinergic drugs tend to diminish sharply after drug withdrawal. For example, Rogers and colleagues (1998) reported that the effect of Donepezil was lost within the 6 weeks after cessation. In contrast, Ruether and colleagues (2002) demonstrated a persisting efficacy of Cerebrolysin over a period of 6 months after cessation of active treatment. Panisset and colleagues (2002) detected a significant treatment effect for Cerebrolysin 2 months after the treatment was stopped and a study by Ruether and colleagues (2002) again identified a persisting effect after 3 months following drug withdrawal. Alvarez and colleagues (2006) were able to demonstrate that the 10 ml Cerebrolysin dose was superior over placebo in producing changes on the cognitive and global impression measures that were maintained at 24-weeks post-treatment. The 10 ml group demonstrated a more than threefold increased probability of global function improvements at 24-weeks as well as 2 point improvement on the ADAS-cog from baseline.

In attempt to further examine the effectiveness of Cerebrolysin recent meta-analytical study evaluated 6 clinical trails comparing Cerebrolysin with placebo in mild to moderate AD (Wei, He, Wang, Su, & Chen, 2007). The results confirmed that Cerebrolysin could significantly improve the clinical global impression of AD patients with a log(OR) of 1.1799 (95% confidence interval, 0.7463 to 1.6135, p <0.05). However, no statistically significant effect was detected for ADAS-cog scale, while a slight and significant effect size: 0.78 (95% confidence interval 1.39 to 0.17) was recognized for MMSE. The reason the authors cited for the lack of effect on cognitive measures is the limited number of studies included (four used ADAS-cog and three used MMSE), additionally the meta-analysis combined the data from 3 dose groups in the Alvarez, et al., (2006) study, which masked the therapeutic effect of the 10ml dose on cognition. The results of the meta-analytic study point to the fact that large-scale multi-centre studies are needed to provide further evidence on the efficacy of Cerebrolysin on cognitive functioning in Alzheimer's dementia.

Another line of therapy where Cerebrolysin has been suggested to be beneficial is stroke rehabilitation. In the recent years, considerable efforts have been made to develop therapeutic strategies for stroke. Thrombolytic therapy which needs to be administered 3h after the onset of stroke has been shown to limit neurological damage (Zhang, Chopp, Jia, Cui, Lu, & Zhang, 2009). Aspirin has also be tried but seems to be more effective as a preventative in reducing stroke fatalities rather than in improving functional outcomes in stroke survivors (Rao, 2009). Efforts have also been made to develop neuroprotective therapies that can intervene at various stages of ischemic cascade including calcium channel blockers, NMDA antagonists, glutamate release inhibitors, sodium channel blockers, free oxygen radical scavenges and potassium channel agonists among others, but all have produced inconsistent results and some also showed safety problems (See Enlimomab Acute Stroke Trial Investigators, 2001 for review). While BDNF and insulin-like growth factor (IGF) have been used in animal models of stroke (Schäbitz, Schwab, Spranger, & Hacke, 1997) the safety of such therapy in humans has been questioned (Clark, Albers, Madden, & Hamilton, 2000). Considering these issues in stroke therapy

Cerebrolysin was deemed as a possible viable therapeutic tool. Preliminary clinical studies have indicated that Cerebrolysin has a potential therapeutic effect in stroke rehabilitation in terms of improved motor and cognitive function (Barolin, Koppi & Kapeller, 1996; Gusev, et al., 1994). So far only one randomized double-blind, placebo-controlled study was conducted which administered Cerebrolysin to stroke patients (Ladurner, et al., 2005), although another study is now under way (Hong, Moessler, Bornstein, Brainin, & Heiss, 2009). One hundred and forty six patients with mild to moderate stroke severity participated in this study and Cerebrolysin was administered within 24 hours of onset of stroke via intravenous injection at 50ml for 21 days. The patients were followed up at 1, 3, 7, 14, 21 and 90 days. Cerebrolysin treated patients were able to demonstrate significant improvements in their level of cognitive function on the Syndrome Short Test with this improvement being evident at 90 days post-stroke follow-up. The Syndrome Short Test findings were complemented by similar although non-significant improvement on the MMSE with patients demonstrating a nearly one point gain especially at days 1, and 3. No significant changes however were observed on the neurological measures (Canadian Neurological Scale) with the Cerebrolysin group only demonstrating some improvement in Motor Weakness by the end of treatment, but not at 90 day follow-up. The authors reasoned that the mild to moderate baseline patterns of impairment of patients in the study possibly prevented the observation of any neurological changes due to ceiling effects. Despite the limited efficacy of Cerebrolysin observed in this study, due to a relatively small sample size and a mild average severity of impairment of patients, the results still appear to be promising, especially if one considers the limited beneficial effects reported for other therapeutic approaches for ischemic stroke. In addition the excellent safety record of Cerebrolysin (i.e. absence of significant adverse effects) also warrants further investigations of Cerebrolysin in ischemic stroke.

Additionally, some exploratory studies have been conducted that have demonstrated the effectiveness of Cerebrolysin in other conditions. In post-acute cases of moderately-severe TBI, administration of 30ml/day for 4 weeks of Cerebrolysin resulted in decrease in slow EEG and increase in fast EEG activity (Alvarez, et al., 2008). The

authors argued that improvements in EEG profile may underlie positive changes in cognitive measures of attention and working memory that were observed in treated patients. Administration of Cerebrolysin to children with ADHD, as well as to children with compromised immune status has resulted in reduction of illness rates and improvements on immunological parameters (Sotnikova, Gromova, Novikova & Burtsev, 2000; Sotnikova, Gromova & Novicova, 2002).

Overall, in the human domain the treatment effects of Cerebrolysin have most extensively been tested in Alzheimer's disease patients and limited amount evidence exists that support the use of Cerebrolysin in stroke and TBI patients. In cases of Alzheimer's disease in particular, Cerebrolysin has been shown to improve the measures of clinical global impression, however the impact of Cerebrolysin on patients' cognitive functioning and activities of daily living is less well established. So far, only a small number of large-scale double-blind placebo-controlled studies which could provide more convincing evidence on the efficacy of Cerebrolysin have been conducted. It is important to note that other available treatment of AD and stroke at present also have limited effectiveness and in comparison to these treatment options Cerebrolysin has a major advantage of being much better tolerated, and being superior in its is safety profile. More evidence on Cerebrolysin's clinical effectiveness is required, in that regard animal models can be useful in guiding research and identifying potential treatment targets.

5.4 Lesion models and Cerebrolysin

The number of studies which focused primarily on the behavioral effects of Cerebrolysin and its postulated effectiveness in reducing memory impairment and enhancing learning in animal models has been limited. The only available evidence so far comes from a group led by Francis-Turner and colleagues who examined the Cerebrolysin's therapeutic effects after focal lesions to the fimbria-fornix and sensorimotor cortex.

Considering that fornix constitutes part of the extended hippocampal system proposed by Aggleton & Brown (1999) to be important for encoding and recall of episodic information, the evidence concerning the possibility of recovery of function after

fimbria-fornix lesions is of particular relevance to the current thesis. Francis-Turner and Valoušková conducted two studies involving Cerebrolysin administration after fimbria-fornix lesions. The first study employed aspiration lesions to the fimbria-fornix (Frances-Turner & Valoušková, 1996). Young adult naïve rats were first pre-trained on the Morris Water Maze for 3 consecutive days, eight trials per day before undergoing surgery and 14 day treatment with Cerebrolysin. The Cerebrolysin was administered intraperitoneally at a does of 2.5mg/kg. After treatment the rats were assessed on retrograde memory of the pre-operatively trained spatial task (ability to recall the location of the platform) for 1 day/four trials and on an anterograde memory test in which a platform was moved to new location for 3 days, 8 trials/day. The lesion only group demonstrated a strong anterograde amnesia as well as retrograde amnesia, showing longer escape latencies in both acquisition and retention tests in comparison to controls. The Cerebrolysin group however did not demonstrate impairments on either anterograde or retrograde tasks and were not significantly different in their performance to the intact controls, suggesting that Cerebrolysin contributed to substantial recovery of functioning in that group. The authors also compared Cerebrolysin effectiveness to other naturally occurring trophic factors such as nerve growth factor (NGF) and basic fibroblast growth factor (b-FGF). The NGF administration resulted in reduced anterograde but not retrograde amnesia demonstrated by the fimbria-fornix animals, while in contrast, Cerebrolysin eliminated both types of amnesia. The b-FGF administration did not have any beneficial effects on performance. The authors concluded that Cerebrolysin can promote recovery of spatial memory following fimbria-fornix lesions and the action of Cerebrolysin is more widespread than the effects of NGF or b-FGF.

In their second study, Francis-Turner, Valoušková & Mokry (1996) administered Cerebrolysin, NGF or b-FGF treatment to rats with unilateral fimbria-fornix lesions either for 2 or 4 weeks and then examined the long-term effects of the compound by re-testing the animals on the Morris Water Maze 6 months post-surgery for their ability to retrieve the old position of the platform. The 2 week Cerebrolysin, NGF or b-FGF administration did not result in significant behavioral changes, with treatment groups not differing statistically from controls at 6 months post-surgery re-testing. However, the 4-week

treatment improved performance of both Cerebrolysin and b-FGF groups to a level of intact controls, with both groups demonstrating similar trajectory lengths to controls. Based on these findings the authors argued that at least a 4-week administration of Cerebrolysin is needed to produce permanent behavioral changes.

Another study (Koroleva, Korolev, Mares, Pastalkova, & Bures, 1999) which examined the effects of Cerebrolysin on recovery after hippocampal system damage exposed the rats to CO poisoning and then administered Cerebrolysin for 2 weeks (2.5mg/kg) intraperoitoneally. CO exposure produces hippocampal damage and in this study was associated with marked reductions in cell density in the CA1 region. Ten days after CO exposure (early test) the rats were tested on the spatial reference memory task in the Morris Water Maze for 3 days. The Cerebrolysin treated rats demonstrated shorter escape latencies in comparison to the CO exposed no-treatment group on the second and third day of training in the water maze. Both groups were impaired on the first day of the early test. The animals were then again re-tested on the Morris Water Maze for 3 days 20 days after CO exposure (late test) and this time the Cerebrolysin rats also demonstrated shorter escape latencies on day one of training as well as on day three (differences in escape latencies Cere group = 7s vs. no-Cere group = 14 s). The researchers suggested that the Cerebrolysin promoted better spatial learning in the early test, which then led to better performance of the animals on the first day of the late test. The researchers also conducted electrophysiological recordings in the hippocampus and the cortex and suggested that the behavioral recovery was associated with hippocampal recovery manifested by increased amplitude of spreading depression waves.

Valoušková and Gschanes (1999) evaluated whether Cerebrolysin administration can also improve spatial memory performance following aspiration lesions to the sensori-motor cortex. The lesioned rats' ability to navigate to a place in a Morris Water Maze was assessed immediately after 2-weeks of Cerebrolysin treatment and 7 to 8 months later. Cerebrolysin was also administered either intraperitoneally or infused into the cortical wound. The effects of Cerebrolysin were compared to those of NGF and b-FGF. The lesioned rats that did not receive treatment demonstrated spatial memory

impairments on the water maze, reflected in longer escape latencies and trajectory length and these impairments were permanent, lasting 8 months. Only rats treated with Cerebrolysin demonstrated comparable escape latencies to sham controls at 2 weeks after the lesion test. The behavioral effect was similar in magnitude whether Cerebrolysin was administered intraperitoneally or into the cortical wound. No short-term effect of NGF or b-FGF was found. At 8 months re-test the Cerebrolysin effect weakened, with Cerebrolysin rats demonstrating similar escape latencies to the lesioned controls. Only administration of b-FGF was associated with improved performance on the delayed test. The authors argued that longer Cerebrolysin treatment times of at least 4 weeks are required to produce lasting behavioral changes as was demonstrated in the Francis-Turner, et al., (1996) study.

Valoušková & Francis-Turner (1998) further extended their findings by evaluating whether Cerebrolysin can produce behavioral changes if administered 4 months after cortical ablation surgery. Spatial learning and memory was evaluated in the Morris Water maze and assessed at three different intervals: 1) post lesion; 2) 4 weeks post lesion and post 4-week Cerebrolysin treatment and 3) 4 months after Cerebrolysin treatment completion. Spatial learning was markedly impaired in all lesioned groups prior to Cerebrolysin administration. Following 4 weeks of Cerebrolysin treatment the treated rats demonstrated marked improvements in trajectory length although did not reach the level of intact controls. Four months later the Cerebrolysin group continued to demonstrate shorter escape trajectories in comparison to the lesion only group. Thus, Cerebrolysin was able to produce beneficial effects on memory functioning even when administered substantial time after the initial brain insult although the magnitude of improvement was smaller then when Cerebrolysin was administered immediately following a lesion. The authors also compared different Cerebrolysin doses (2.5mg/kg vs 1.25mg/kg) in this study. The standard 2.5mg/kg dose was more effective in promoting behavioral recovery immediately post-administration. While a half dose of 1.25mg/kg was slightly more effective in promoting recovery at 4 months after treatment completion. The authors argued that different doses may need to be used depending on whether the treatment is administered at an acute or chronic stage.

The data from focal lesion studies that investigated the effects of Cerebrolysin so far indicates that Cerebrolysin can act to improve spatial memory after various types of brain lesions, including lesions to the hippocampal system. It can exert beneficial effects either when administered soon after the insult or when the administration is delayed some months post-surgery. Longer-term treatment with Cerebrolysin (up to a month) is more effective in producing lasting behavioral changes than short-term administrations. Cerebrolysin also seems to be more effective in producing behavioral changes than the naturally occurring neurotrophins such as NGF or b-FGF, at least in an acute stage.

5.5 Summary

Cerebrolysin represents a unique neurotrophic compound which seems to have neurotrophic activity similar to that of naturally occurring growth factors, with the added benefit of being low in toxicity and effective when administered peripherally. In humans, Cerebrolysin administration was noted to improve everyday functioning of Alzheimer's disease and stroke patients. Although the neurobiological properties of Cerebrolysin are still not well understood it has been noted to promote neurogenesis and synaptogenesis especially in the hippocampal system and prevent apoptotic cell death. Animal studies shown that Cerebrolysin administration can induce behavioral changes when administered following ischemia, in animal models of degenerative disorders (for e.g. APP-tg mice) and following focal brain lesions, including lesions to the hippocampal system, such the fimbria-fornix and CO induced hippocampal damage. The ability of Cerebrolysin to improve spatial memory after fimbria-fornix and hippocampal damage, as well as the observation that Cerebrolysin may delay cognitive decline in Alzheimer's dementia patients who are known to demonstrate anterior thalamic pathology (see Chapter 2) make it a viable candidate for therapy after anterior thalamic lesions.

Chapter 6

Effects of immediate post-surgery exposure to enriched environments on recovery of function after anterior thalamic lesions

This chapter documents the first of the four experiments (see Chapters 7, 8, 9) that evaluated the possibility of recovery of function after anterior thalamic lesions. In the current experiment rats received lesions to the anterior thalamus and immediately after recovery from surgery were exposed to enriched environments for a period of 30 days prior to being tested on spatial and non-spatial memory tasks.

6.1 Introduction

As discussed in Chapter 2 considerable clinical evidence has accumulated that the integrity of the thalamus is crucial for normal memory (Schmahmann, 2003; Van der Werf, et al., 2003). Clinical observations supported by experimental data (discussed in Chapter 3) indicate that severe deficits in spatial and temporal order memory exist after ATN lesions, even when subtotal lesions are used that minimise confounds from damage to adjacent thalamic structures (see Chapter 3). This lesion evidence, its similarity to many effects of hippocampal lesions, and the ATN's various direct and indirect neural connections with the hippocampal system support an influential proposal that the ATN constitute a critical nodal point in an extended brain system responsible for at least some aspects of normal episodic memory (Aggleton & Brown, 1999; Vann & Aggleton, 2004).

The current study provides a first examination of whether memory deficits produced by ATN lesions are amenable to experimental intervention. Recovery of

function in robust animal models would encourage the possibility for therapeutic intervention in clinical cases of diencephalic amnesia. This study employed post-operative enriched housing as a therapeutic tool because enrichment enhances brain plasticity with beneficial effects after many types of brain injury, including hippocampal system lesions (Dalrymple-Alford & Kelche, 1985; Kolb & Whishaw, 1998; Will, et al., 2004). A 30-day continuous period of enrichment was used as it represents a standard length of exposure used in experimental lesion studies (Galani, et al., 1997, 1998).

The primary interest was spatial working memory using a preoperatively trained T-maze non-matching-to-place task. As discussed in Chapter 3 this alternation task has revealed severe and apparently robust memory deficits after ATN lesions (Aggleton, et al., 1995a; Aggleton & Brown, 1999; Warburton & Aggleton, 1999; Warburton, et al., 1999; Ward-Robinson, et al., 2002), especially when a cross-maze procedure is used that emphasises the use of spatial cues and minimises the rats' ability to benefit from egocentric (response-based) strategy (Aggleton, et al., 1996; Warburton, et al., 1997). Although traditionally the enrichment literature has employed other tasks such as the Morris Water maze to examine treatment effects (see Will, et al., 2004), the cross-maze was chosen here with an aim to examine the possible impact of enrichment on the seemingly permanent and well-established ATN induced deficit. Additionally two other behavioural tasks were employed. One task examined the ability to discriminate between fixed locations in a radial-arm maze, using problems with different spatial pattern separations, which provides an evaluation of reference memory. The differentiation or separation between cues might be required for the animal to successful solve a spatial memory task when there is an overlap between cues. This ability has been termed pattern separation and describes a mechanism for separating partially overlapping patterns of activation, so that one pattern may be retrieved as separate from other patterns. Gilbert and colleagues (Gilbert, Kesner & DeCoteau, 1998) demonstrated that performance of the dentate gyrus lesioned rats on the spatial pattern separation task increased as a function of increased spatial separation between the correct object and the foil on the choice phases. Fornix lesions were shown to spare the acquisition of easy spatial separations/discriminations (widely separated arms), but impair acquisition of difficult

discriminations (close/adjacent arms; McDonald & White, 1995). While there is evidence available to suggest that pattern separation is subsumed under hippocampal function, little is known about whether ATN plays a similar role. The final task was a non-spatial memory task which examined the memory for reward values, based on the anticipatory contrast paradigm developed by Flaherty and colleagues (Flaherty, Turovsky & Krauss, 1994). ATN has previously been demonstrated to not be involved in working memory for reward value (Mitchell & Dalrymple-Alford, 2003) and rats with hippocampal lesions also do not demonstrate impairments in their ability to adjust their behaviour in anticipation of a higher reward value (Gilbert & Kesner, 2002). This particular task represented an alternative to a spatial task of separation. It was not expected that ATN induced impairment be observed on the anticipatory contrast task. This non-spatial task was regarded as a behavioural control for any non-specific ATN lesion effects, such as change in motivation.

6.2 Materials and Methods

6.2.1 Animals

Forty-six female PVGc hooded rats were used (6-7 months old, between 148-171 g at surgery). Testing occurred during the dark phase of their reversed 12-hr light cycle. Body weights were restricted to 85-90% of free-feeding weight during testing, with free food access for the surgery and recovery period and during subsequent differential housing in enriched versus standard cages. All protocols in this study conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Ethics Committee University of Canterbury (see Appendix A).

6.2.2 General Surgery Procedure

Rats were anesthetized with sodium pentobarbitone (75 mg/kg i.p.) 20 min after atropine (0.18 mg/kg i.p.) and supplemented by subcutaneous mepivacaine (3.0 mg/kg) and ketofen (0.50 mg/kg). They were placed in a stereotaxic frame with atraumatic ear bars (Kopf, Tujunga, CA), and the incisor bar was set at −7.5 mm below the interaural line to

minimize damage to the fornix. Bilateral lesion sites were used to maximize ATN damage, directed at the anteroventral nucleus (AV) and the anteromedial nucleus (AM). To improve target accuracy, one of five anterior–posterior coordinates was used based on an individual rat's bregma to lambda (B–L) distance (in millimetres). For the AV lesion, the AP coordinates from bregma were: −2.2 for B-L = 6.0 and 6.1; −2.3 for B-L = 6.2 and 6.3; −2.4 for B-L = 6.4, 6.5 and 6.6; −2.5 for B-L = 6.7 and 6.8; −2.6 for B-L = 6.9 and 7.0; and −2.7 for B-L = 7.1 and 7.2. The AV lesion was ±1.65 mm lateral from the midline and −5.50 mm ventral from dura. The AM lesions followed an identical scheme except that lesions were placed ±0.90 mm lateral from the midline, −5.80 mm ventral from dura and AP was 0.1 mm more anterior than for the AV lesion. Either 0.12 μl (AV lesion) or 0.09 μl (AM lesion) of 0.12 M N-methyl-d-aspartic acid (NMDA; Sigma, Castle Hills, NSW, Australia) in phosphate buffer (pH 7.2) was infused over 3 min at each site, using an automated Stoelting microinfusion pump and a 1-μl Hamilton syringe (Reno, NV). The needle was left in situ for further 3 minutes for diffusion at each site before slow retraction. ATN sham controls received the same surgical procedure, but the needle was lowered to 1.50 mm above the lesion coordinates and no material was infused to avoid accidental damage to surrounding structures.

6.2.3 General Housing Procedure

Prior to surgery all rats were housed in standard (small group) housing conditions of 3 or 4 rats per opaque plastic cage (50 cm long by 30 cm wide by 23 cm high). Following surgery all rats were housed individually for a recovery period of 5 days and then, based on matched preoperative spatial working memory performance, pairs of rats were randomly assigned to either an enriched environment condition (EE) for 30 days or the standard housing conditions (SC). No behavioural testing occurred during the 30 days of enrichment. The rats in the EE groups were housed post-operatively with 11 or 12 rats in enrichment cages, made of wire mesh (85 cm long by 60 cm wide by 30 cm high) with a solid metal floor covered with sawdust (see Fig. 5 for an example of EE housing). An array of objects, such as Perspex tunnels, PVC tubing, an occasional running wheel, metal chains, ladders, boxes, glass cups and plates, and plastic toys were placed in these cages and were changed on a daily basis, plus the position of food and water and the

placement of the enrichment cages in the colony room were varied every 2 to 4 days. The rats in the EE group were then re-housed in standard conditions (i.e., 3 or 4 rats per cage) with their cage mates from the same enriched environment cage. All rats (from standard and enriched cages) were food deprived prior to retesting for spatial working memory which started at 40 days post-surgery. After the end of continuous enrichment and thereafter the rats from enriched cages were returned to the enriched environment for a period of 1.5 to 2 hours at the end of each day, followed by their daily food ration on return to the standard cages. After confirmation of accurate lesions (see Lesion Evaluation), the final group numbers were: SC-SHAM, *n* = 11; SC-ATN, *n* = 8; EE-SHAM, *n* = 12; and EE-ATN, *n* = 7 (4 SC-ATN rats and 4 EE-ATN rats were excluded solely on the basis of histology).

Fig 5. A photograph depicting an example of an enrichment cage arrangement. A wire-mesh cage with objects and a group of 12 rats.

6.2.4 Apparatus and behavioural testing

Spatial working memory in the cross-maze. Spatial working memory was tested using a T-maze configuration (Fig 6), embedded in a cross-maze raised 75 cm above the floor. The wooden runways were 10.5 cm wide and painted gray, with 2.5 cm high galvanised

steel edges. The two stems were 1 m long with a guillotine door located 28 cm from each end to create a North (N) and a South (S) start area. The two goal arms were 40 cm long, the end of which included a raised wooden food well (2.5 cm diameter, 1 cm deep). Wooden blocks (10.5 cm wide by 30 cm high by 10 cm deep) were used to restrict access to any stem or arm. The maze was located diagonally in a windowless room (334 cm by 322 cm) which contained a number of distal cues (to the side of the goal arms, computer, poster, desk and chair on one side, curtain on the other; beyond the start areas, closed door at one end, hub to a radial arm maze at the other). The horizontal distance between an end of the maze and the nearest surface varied from 25 cm to 70 cm. Diffuse lighting was provided by overhead fluorescent lights.

Fig 6. A photograph depicting the arrangement of the cross-maze apparatus and the experimental room set-up.

All rats were trained to criterion on the spatial working memory task prior to surgery and then retested for 10 sessions at 45 days post-surgery on the same task. Six trials were conducted per session and sessions were conducted on alternate days for pre-surgery training with half the rats tested per day for 7 days a week, and on consecutive days for 7 days a week at post-surgery/post-enrichment re-testing. Pre-surgery, rats were familiarized individually to the maze over 3 days with chocolate pieces (1 g)

freely available on the arms and then the food wells. The rats were initially trained for 6 or 7 sessions (to 85% accuracy on 2 consecutive sessions) using the start area in the N or the S stem for half the six daily trials and with the same start area used for both the "sample" and "test" (choice) run for any given trial. Correct performance on the test run required the rat to choose the alternate arm from that previously visited during the sample run of that trial (reinforced spatial alternation). To ensure that the rats were not simply using an egocentric strategy (alternating body turn) from sample to test runs, a pseudorandom half of the trials on each of the subsequent 10-14 preoperative sessions (again to criterion of 85% accuracy for two sessions) used the opposite start area for the test run (e.g., S for the sample run and N for the test run for a given trial). The other half of the daily trials used the same start area, such as only S or only N, for both sample and test runs. On the sample run, the rat was placed in the start area, the door raised, and the rat allowed to enter one open arm, due to the placement of wooden blocks on the alternate arm and stem area, where it was confined for between 5 and 10 seconds while it ate a two 1 gram chocolate food rewards. It was then picked up and returned to the appropriate start area for a delay of between 5 and 10 seconds while the arm barriers at the choice point were removed or repositioned as required. When the door was raised for the test run the rat was thus allowed a free choice between the two maze arms (hind foot down that arm; no retracing). If the rat chose the previously blocked arm (non-matching alternation; hind foot down that arm) it was rewarded with three (1 g) food rewards, confined in that arm for between 5 and 10 seconds while it ate the reward and returned to the home cage. If the rat returned to the arm previously visited on the sample run it was confined to that arm for 10 seconds and returned to the home cage (no correction trials). The rats were tested in groups of 3 or 4 with each rat having one trial in turn, so that the inter-trial interval was approximately 3-4 min. Each rat experienced a pseudorandom sequence of correct arm choices (left or right), N or S start position for the sample run, and same vs. opposite start position for the test run, which varied across rats and sessions (adapted from Fellows, 1967).

Pattern Separation for Reward Magnitude: Pattern Separation for Reward Magnitude was conducted only post-surgery, starting 3 days after cross-maze re-testing. The testing

was conducted in a different room to that of the cross-maze testing. Testing was conducted in standard opaque test cages, (similar to those used for standard housing) which were positioned on cage trolleys and covered by a weighed down Perspex lid; sawdust covered the cage floor. The same animals were always run in the same cages. The lickometer apparatus was suspended inside the testing cage (see Fig 7). It consisted of a 300 ml glass bottle with a metal spout which was inserted into a metal holding chamber, suspended 3 cm above the cage floor, in such a way that the tip of the spout was positioned just inside the small (1 cm diameter) opening in the chamber. Licks were recorded via contact relay circuits connected to the metal drinking spout and were counted using a specifically written computer program. Sucrose solutions were mixed on weight basis from tap water and commercial sugar approximately one hour before the start of each experimental session. The rats continued on the food deprivation regime, (weights restricted to 85% of ad libitum). Water was always available in the home cages, but removed for 15 minutes prior to testing. Rats were first put into lickometer cages for 25 min on Day One for familiarization. Condensed milk was applied on the spout to encourage the rats to drink, but the water contained no sucrose. For the next two days of pre-training the rats received a 2% sugar solution in the bottle for 25 min which ensured that all rats were drinking from the bottle. After completion of the pre-training stage, three test phases began which were conducted in a similar manner as described in Gilbert and Kesner (2002). The successive phases were of 10 day duration each, with testing occurring 5 days a week, with all rats tested each day.

Fig 7. A photograph depicting the lickometer apparatus.

For Phase One the rats were exposed to 2% sucrose solution, followed by no bottle and then a 32% sucrose solution. The rat was placed into the lickometer cage for 25 min with no water (bottle upright), which was followed by *one* trial per day, which consisted of:

Sample 1: 2% sucrose solution for 180 seconds

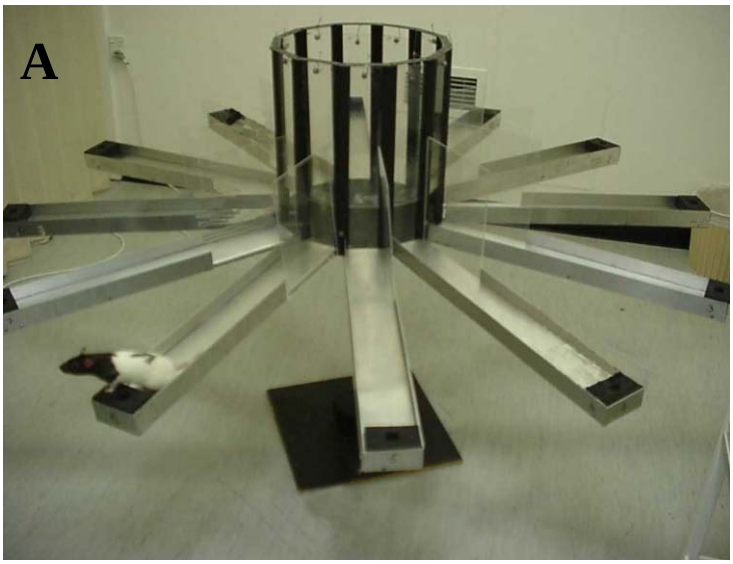
Sample 2: no bottle for 15 seconds

Sample 3: 32% sucrose solution for 180 seconds

Phases Two and Three followed the same procedure except that in Phase Two the concentration of the sucrose solution was 16% for Sample 3 and in Phase Three it was 8% for Sample 3. Three day breaks were inserted between each testing phase. Following testing for each day the standard animals were returned to their standard home cages and the enriched animals were put into the enrichment cages for 1.5 to 2 hours.

Spatial pattern separation problems. (Behavioural testing was conducted by Mark Ottley, (a 4th year project student), under the guidance of the author and primary supervisor). A12-arm radial maze was used (85 cm above floor, 1.65 m diameter), which comprised a 35-cm-wide wooden hub (painted black) and 12 aluminium arms (65 cm long by 8.5 cm wide, 3 cm high borders) (Fig 8A). Single clear Perspex barriers (20 cm long by 25 cm high) extended along each arm from the central hub to discourage the rats from jumping across arms. A black wooden block (5 cm long by 8.5 cm wide by 3 cm high) containing a food well (2 cm diameter, 1 cm deep) was located at the end of each arm and housed inaccessible food (one gram chocolate pieces) to provide constant olfactory cues. Clear Perspex guillotine doors, each with a white horizontal label (4.5 cm wide by 2.5 cm high) centred 3 cm above the door's base, controlled access to the arms and could be raised singly or together by an overhead pulley system. The radial maze was located in the same room where the previous cross-maze testing was conducted. Acquisition of three successive spatial problems (fixed rewarded locations in the maze) began at 110 days post-surgery. For each of the 12 daily (massed) trials, access was allowed only to a designated rewarded arm and one of two non-rewarded arms, with the left / right position of the non-reward arm determined using a pseudorandom sequence (Fellows, 1967). The problems used two non-reward arms that were: i) 5 or 6 arms distant from the reward arm in the first problem (wide separation; for example, arm 12 was rewarded and arm 6 and arm 7 were the non-rewarded arms); ii) 4 arms distant in the second problem (intermediate separation; for example, arm 9 was rewarded and arm 1 and arm 5 were the non-rewarded arms); and iii) 1 arm distant in the third problem (close separation; for example, arm 3 was rewarded and arm 2 and arm 4 were the non-rewarded arms) (Fig 8B). For any given rat no arm that was used as a reward or non-reward arm in one problem was used for that rat in any subsequent problem. The rats were initially familiarized to the radial maze for 3 trials per day for 3 days, with access to any two of the arms (baited with two 1 g chocolate pieces) that would be used in the first problem. Each successive problem was run for a minimum of 6 days with a one day break between problems. Testing continued until a rat reached a criterion of 10 out of 12 correct trials on each of two consecutive days or a maximum of 15 days testing for the first two problems and 18 days for the last problem. The second rat in each group used an arm allocation one

arm clockwise from the first rat's arm allocation, the third rat 2 arms from the first rat's arm allocation, and so on. At the start of each session, the rat was placed on the centre platform and, after a 20-25 second delay, the doors to the rewarded arm and to one of the non-reward arms were opened simultaneously. The first arm entered by the rat's hind legs was recorded as the rat's choice and the door to the non-entered arm closed. Once the rat had returned to the centre platform the second door was closed. For the second and all subsequent trials for that session, the maze was rotated according to a randomly determined sequence to minimize the use of intra-maze cues, while the rat was still present on the centre platform (20-25 second inter-trial interval). The randomly determined sequence of maze rotations ensured that each specific arm on the maze was the reward arm once in the daily block of 12 trials, but the spatial orientation of the rewarded arm remained constant.



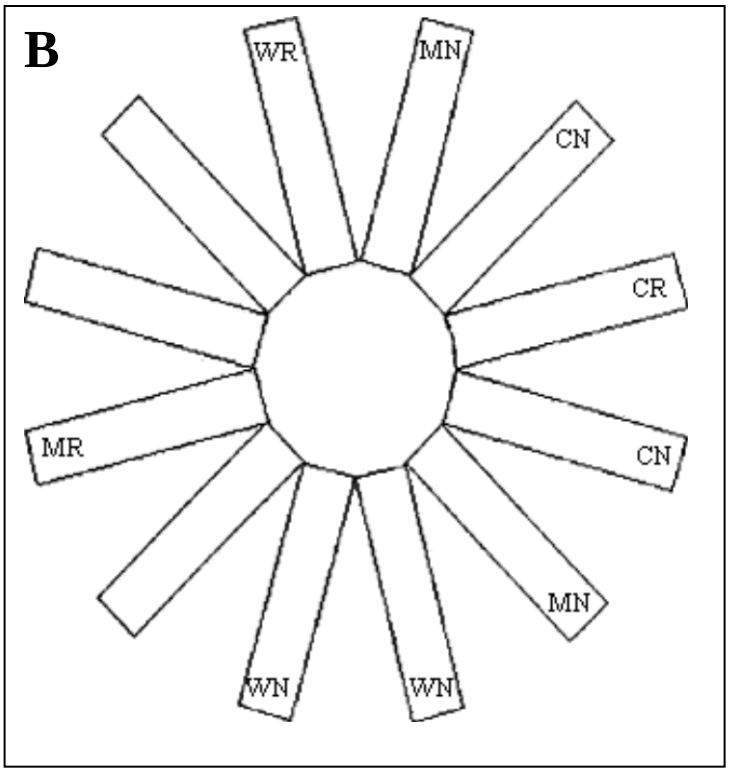


Fig 8. **A:** A photograph depicting the 12-arm radial maze apparatus. **B**: examples of positions of radial arm allocations for wide (W), medium (M) and close (C) separation tasks with rewarded (R) arms and non-rewarded (N) arms (for one rat).

6.2.5 General Histology Procedure

On completion of behavioural testing, all rats were transcardially perfused with cold saline followed by 4% formalin. The brains were post-fixed for two days, cryoprotected in 30% sucrose, and every coronal 50-µm section throughout the thalamic region was collected for cresyl violet staining of cell bodies. Lesion extent was replicated on electronic copies of the Paxinos and Watson (1998) atlas (see Mitchell & Dalrymple-Alford, 2005). Automated pixel counts of the estimated damage relative to the relevant intact brain region were used to generate percent lesion volumes by factoring in the pixel areas multiplied by the distances provided in the atlas. Acceptable lesions were defined as having more than 50% bilateral damage to the ATN, but not more than 40% damage to the corresponding adjacent lateral thalamic region (LT, which included the intralaminar and lateral mediodorsal nuclei) and posteromedial thalamic region (MT, which included the medial and central mediodorsal nuclei). The latter regions were of interest because they have also been suggested as possible causes of some aspects of diencephalic amnesia, but only ATN lesions of more than 50% damage size are consistently associated with severe spatial memory deficits (Bailey & Mair, 2005; Gibb et al., 2006; Mitchell & Dalrymple-Alford, 2005; 2006).

6.3 Results

6.3.1 Lesion Evaluation

The largest and smallest acceptable lesions in the two lesion groups are shown in Figures 9A & B. Only rats with ATN lesions meeting the Histology criteria (8 SC and 7 EE rats) were included in the behavioral analyses (see Table 5). All of the rejected lesion cases had ATN lesions that were too small and the median damage (and range) sustained in rejected cases was: for the 4 EE-ATN cases, 14.7% ATN (4.0-24.6%), 1.1% LT (0.1-18.1%) and 0.1% MT (0.1-2.4%); for the 4 SC-ATN cases, 18.5% ATN (5.3-25.5%), 2.6% LT (0.8-3.1%) and 0% MT (0.0-0.2%).

Table 5. Immediate enrichment and lesion size: Percent bilateral damage (volume) to selected brain areas for each of the rats in the immediate enrichment study.

	ATN and components				Other Nuclei								
RATS	AD	AM	AV	ATN	MT	LT	IAM	LD	PT	PVA	PV/ PVP	Re	Rh
EE-ATN													
31	59.6	44.6	29.9	54.2	1.8	0.1	0.0	0.0	3.0	0.1	0.0	0.1	0.0
32	96.0	78.2	79.9	85.4	2.9	0.8	18.0	1.0	17.7	0.2	0.0	1.9	0.0
33	88.9	85.4	44.0	87.4	3.5	1.4	28.9	0.0	9.9	0.0	0.0	0.0	0.6
48	82.2	78.7	74.2	79.4	9.4	4.7	13.6	1.1	16.4	0.0	0.0	0.0	0.0
51	82.6	76.1	79.2	78.1	4.1	1.5	0.3	4.5	6.3	0.0	0.0	0.2	0.0
52	96.6	69.8	59.7	81.3	2.8	0.8	4.2	6.7	4.3	0.0	0.0	0.0	0.0
54	76.8	62.2	38.2	71.0	1.8	0.1	0.0	0.0	2.4	0.0	0.0	0.0	0.1
EE-ATN Median	**82.4**	**76.1**	**59.7**	**79.4**	**3.2**	**0.8**	**1.8**	**1.0**	**2.9**	**0.0**	**0.0**	**0.0**	**0.0**
SC-ATN													
25	96.0	70.6	95.6	73.6	19.9	1.5	20.9	9.9	2.4	0.0	0.0	0.0	0.1
41	78.2	79.6	93.7	84.3	7.6	16.1	30.1	43.2	13.3	0.0	0.0	0.0	0.0
43	78.9	51.2	74.6	64.3	0.6	2.3	1.0	0.0	2.5	0.0	0.0	0.0	0.0
44	93.2	93.4	77.0	90.9	2.1	16.9	26.6	11.3	23.5	0.0	8.0	0.0	10.8
47	87.2	95.2	63.4	85.7	8.2	21.2	96.5	3.5	43.9	6.2	0.0	0.2	4.1
62	95.4	45.4	84.9	72.7	2.2	9.7	13.5	33.7	14.6	0.0	0.0	0.0	0.0
63	75.6	71.6	48.5	71.8	1.3	6.1	2.2	0.4	2.5	0.0	0.0	0.0	0.1
64	79.2	81.9	76.5	73.1	0.8	3.0	21.8	4.1	5.9	0.0	0.0	0.6	0.0
SC-ATN Median	**83.2**	**75.6**	**76.7**	**73.3**	**2.1**	**7.9**	**21.3**	**7.0**	**9.6**	**0.0**	**0.0**	**0.0**	**0.05**

Abbreviations: AD= anterodorsal nucleus; AM= anteromedial nucleus; ATN = anterior thalamic aggregate comprising the anterodorsal, anteromedial and anteroventral thalamic nuclei; ATN median= median percent damage for all included rats; AV= anteroventral nucleus; EE-ATN = rats with anterior thalamic lesions housed in enriched cages; IAM= interanteromedial nucleus; LT= lateral medial thalamic aggregate comprising the intralaminar nuclei (centrolateral, paracentral and rostral central medial nuclei) and lateral mediodorsal thalamic nuclei (lateral and paralamellar nuclei); LT median= median percent damage for all included rats; MT= posteromedial thalamic aggregate comprising the central and medial mediodorsal nuclei and the intermediodorsal nucleus; MT median= median percent damage for all included rats; PT= paratenial nucleus; PVA= anterior paraventricular nucleus; PV/PVP= paraventricular nucleus/posterior paraventricular nucleus; Re= reunions nucleus; Rh= rhomboid nucleus; SC-ATN = rats with anterior thalamic lesions housed in standard cages.

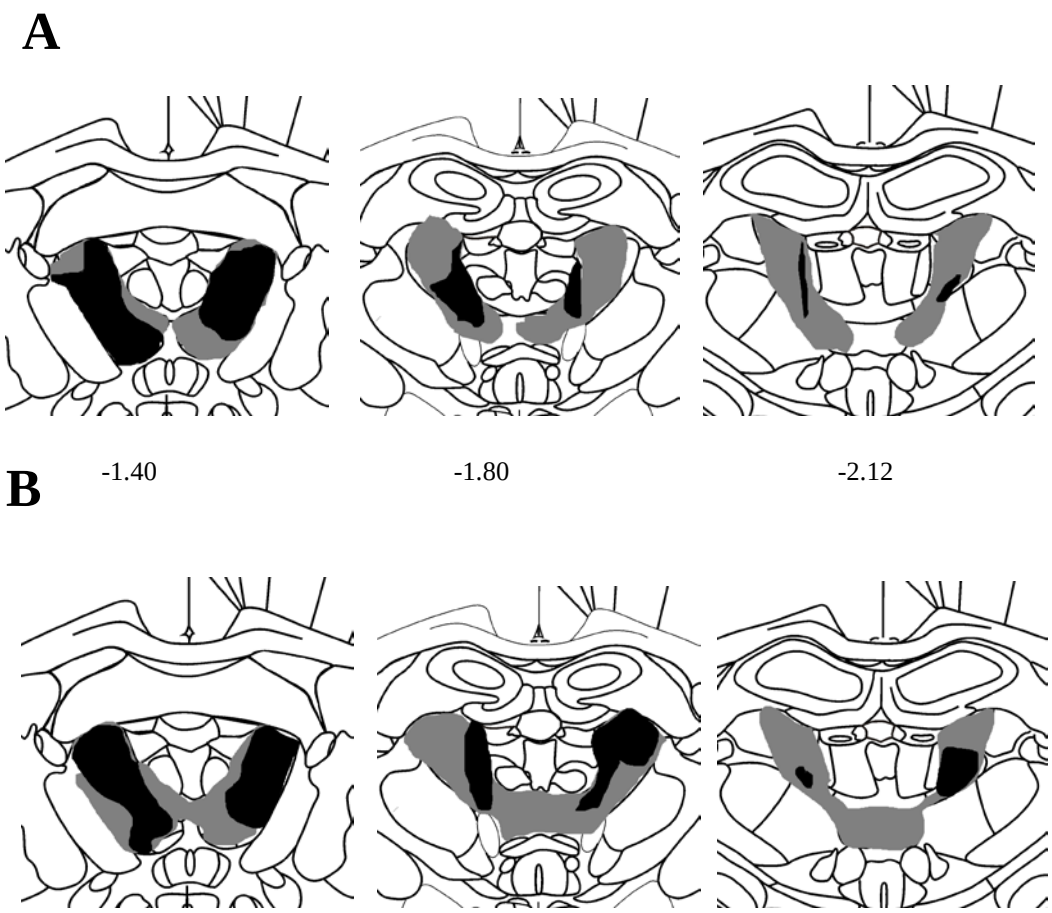


Fig 9. Immediate Enrichment and Lesion Size. Schematic representation of the largest (grey) and smallest (black) lesion in the **A**: ATN enriched group and **B:** ATN standard caging group. Numbers indicate distance from bregma in millimetres (from Paxinos & Watson (1998).

6.3.2 Non-matching to sample spatial working memory

Pre-surgery training - Figure 10 shows before surgery spatial working memory performance in a cross-maze configuration in terms of overall percent correct, combined across both trial types (i.e. irrespective of "same start position" and "opposite start position" trials) for the last 10 days of training taken back from the last 2 days of training when 85% criterion was reached. Performance in all four groups of rats was equivalent across sessions (F's < 1).

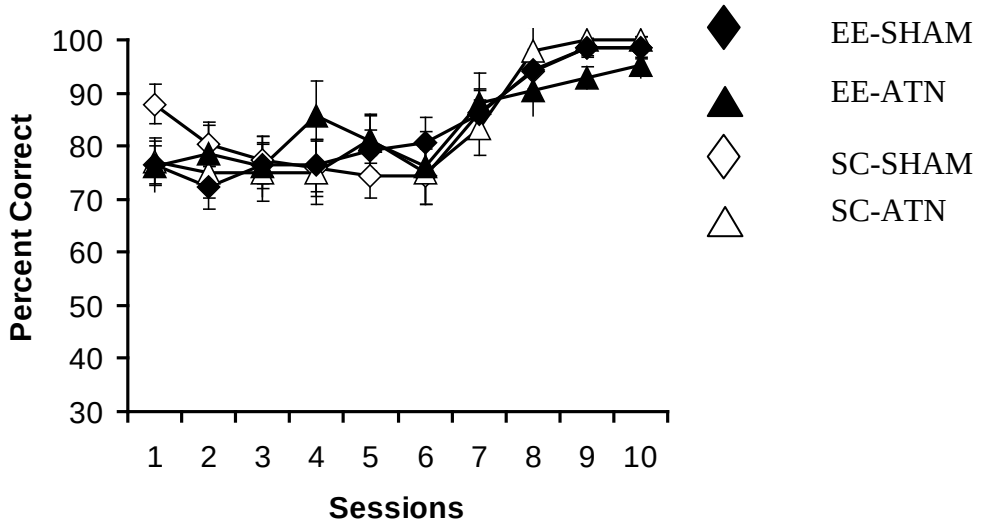


Fig 10. Immediate Enrichment and Spatial Working Memory performance (pre-surgery). Mean (± SEM) percent correct responses for the last 10 sessions of pre-surgery training taken back from the 2 last days of training when 85% criterion was reached on the spatial working memory task in the cross-maze configuration. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.

Post-surgery and enrichment training - Forty days post-surgery, the sham groups (especially the SC-SHAM) showed an initial reduction in performance, but they rapidly reacquired the task and achieved 80-90% correct performance (see Fig. 11). As expected the SC-ATN group displayed poor spatial working memory performance throughout the 10 post-operative test sessions, which was consistently around chance levels. The important finding, however, was that the EE-ATN group clearly displayed an overall performance that was superior to that of the SC-ATN group, although less accurate than that of the two sham groups. This improvement was more consistent during the latter part of testing when the performance of the EE-ATN group approached that of the SC-SHAM group.

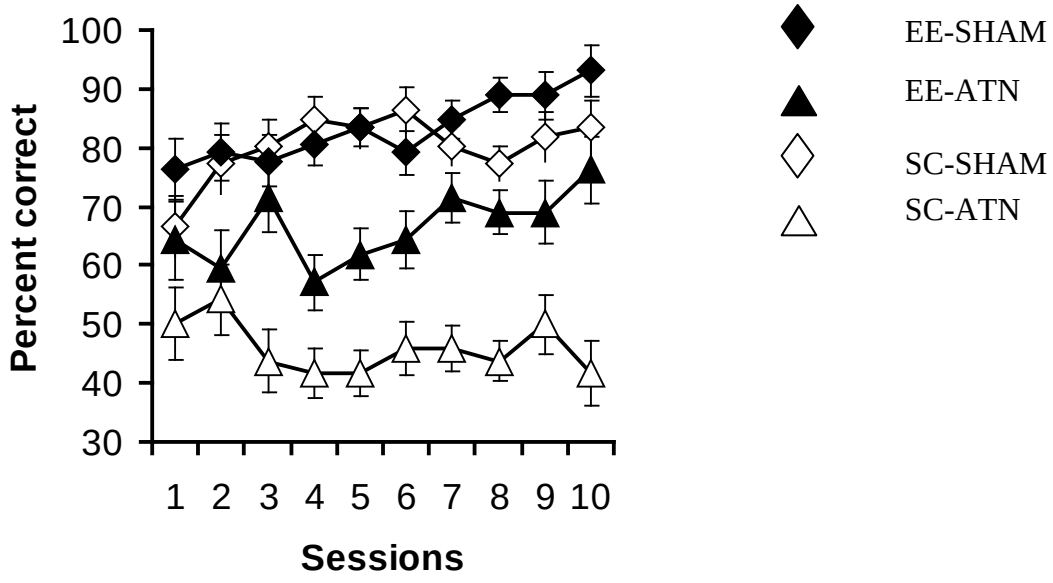


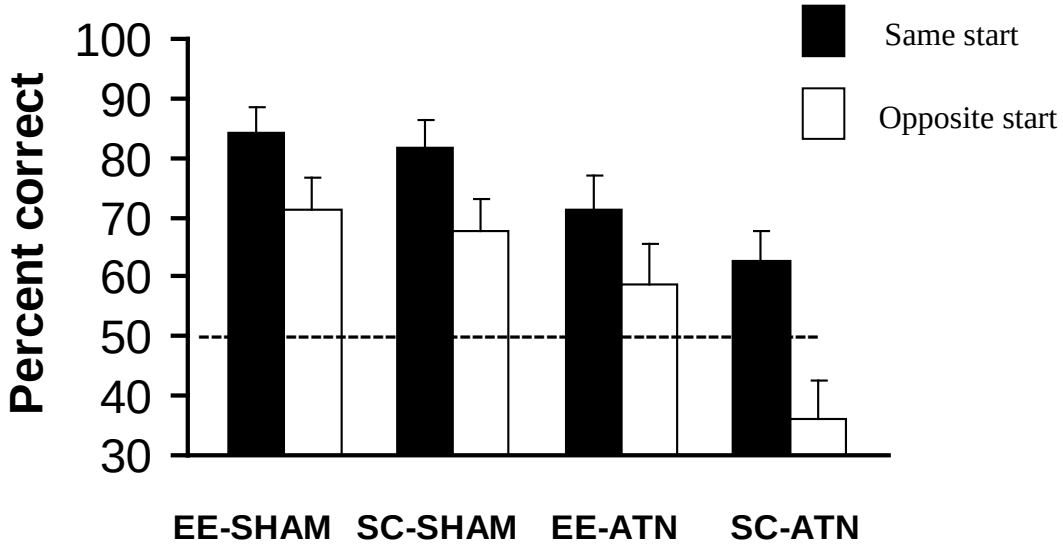
Fig 11. Immediate Enrichment and Spatial Working Memory performance. Mean (± SEM) percent correct responses on the 10 post-surgery, post-enrichment sessions on the spatial working memory task in the cross-maze. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.

A 4-way *ANOVA* (Group by Housing by Session by Trial Type) revealed highly significant effects for Lesion ($F_{(1,34)} = 194.9$; $p < 0.0001$), reflecting the severe ATN lesion deficit, and for Housing ($F_{(1,34)} = 41.73$; $p < 0.0001$), confirming that rats housed in enriched environments performed better than the standard-housed rats. However, the marked overall beneficial effects of post-operative enrichment on the ATN lesion deficit was confirmed by a highly significant Lesion by Housing interaction ($F_{(1,34)} = 23.01$; $p < 0.0001$). *Post-hoc Newman-Keuls* tests verified that, collapsed across the 10 sessions and across trial types, the EE-ATN group reliably performed far better than the SC-ATN group ($p < 0.001$). But the EE-ATN group still achieved lower percent correct scores than both sham groups (p's < 0.001), which did not differ ($p > 0.2$). All groups performed better when the "same start position" trials were used than when the "opposite start position" trials were used, resulting in a highly significant effect for Trial Type ($F_{(1,34)} = 137.48$; $p < 0.0001$). There was also a significant Session by Trial Type interaction ($F_{(1,306)} = 2.83$; $p < 0.01$), because overall performance on the "same start position" trials

generally improved across sessions whereas that for the "opposite start position" trials did not change markedly when the mean performance was assessed across all rats combined.

To provide a clearer perspective on the effects of trial type, ATN lesions and enrichment, performance for the two trial types was assessed on the first three sessions and then the last three sessions (see Figures 12A & 12B respectively). For the first three sessions EE-ATN rats achieved better performance when compared to the SC-ATN group on both trial types (Housing main effect, $F_{(1,34)} = 5.09$; $p < 0.05$; Housing by Lesion interaction, $F_{(1,34)} = 2.33$; $p > 0.10$; Figure 12A). On the first three sessions, the EE-ATN group performed above chance (50%) for the "same start position" trials ($t_{(6)} = 4.49$; $p < 0.01$). The mean performance for the EE-ATN group on the "opposite start position" trials was above chance but was not significantly so for first three sessions ($t_{(6)} = 1.22$; $p = 0.26$). For the last three sessions there was even clearer evidence of improved performance in the EE-ATN group compared to the SC-ATN group, with a significant Lesion by Housing interaction ($F_{(1,34)} = 8.13$; $p < 0.01$). On these last three sessions, the EE-ATN group was significantly above chance on the "same start position" trials ($t_{(6)} = 9.84$; $p < 0.01$). As more EE-ATN rats were now above chance on the "opposite start position" trials for the last three sessions, the mean of this group was significantly better than chance ($t_{(6)} = 2.51$; $p < 0.05$). By contrast, the SC-ATN group was at chance levels for the "same start position" trials ($t_{(7)} = 1.24$; $p > 0.25$) and below chance for the "opposite start position" trials ($t_{(7)} = -3.32$, $p < 0.02$). In summary, the main finding was that enrichment markedly improved performance after ATN lesions, particularly on the easier "same start position" trials, and there was also some evidence of improvement on the more difficult "opposite start position" trials.

A: First three sessions



B: Last three sessions

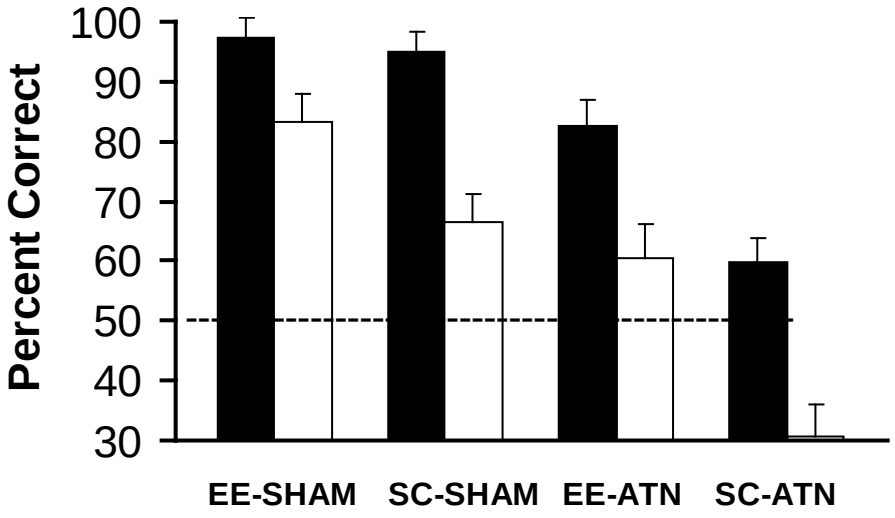


Fig 12. Immediate Enrichment and Spatial Working Memory performance by trial types. Mean (± SEM) percent correct responses for the first three (**A)** and last three (**B)** post-surgery and post-enrichment sessions of the cross-maze task expressed separately for the "same start position" trials (same start position used for both sample and test run per trial) and "opposite start position" trials (opposite start position used for the test run). ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment. --- = 50% chance line

6.3.3 Pattern Separation for Reward Magnitude

Using the approach suggested by Gilbert and Kesner (2002) the lick frequency data on the lickometer apparatus were converted into ratio scores (number of licks on the first bottle (2%) divided by a number of licks on the second bottle (32%, 16% or 8%)) to normalize the scores and control for individual differences in lick frequency. This lick ratio score was then subtracted from "1" so that scores near "1" were indicative of high anticipated discriminability and scores approaching "0" were indicative of low anticipatory discriminability.

Figure 13 shows the intake ratio scores of the sham and lesioned groups as a function of the relative difference between the sample and test phase sucrose concentration on successive blocks of the anticipatory discrimination task. Across conditions (2%-32%; 2%-16% and 2%-8%), the discriminability between the sample and test phase solution was more difficult and hence the need for a pattern separation mechanism increased. The order of testing of the paired solutions was fixed, but the order used means that "general learning" would predict improved performance across conditions.

The data was analyzed using an *ANOVA* with Lesion and Housing as the between group factors and Concentration condition (2%–32%, 2%–16%, or 2%–8%) as the within factor. The main effect for Concentration condition was highly significant ($F_{(2,68)} = 165.85$; $p < 0.001$) and *post-hoc Newman–Keuls* pair-wise comparisons of Concentration condition revealed that the intake ratio scores on the 2%–32% and 2%-16% trials were both significantly different ($p < 0.05$) from the intake scores on the 2%–8% trials. No main effects for Lesion, Housing or Lesion by Housing interaction were observed (all F's < 1) but a 3-way interaction between Concentration, Lesion and Housing just reached significance ($F_{(2,68)} = 3.81$; $p < 0.05$). Figure 13 suggests that this interaction emerged because the SC-ATN group demonstrated a different pattern of performance across conditions in comparison to the other three groups. The main difference appears in the performance of the SC-ATN group on the 2%-16% discrimination. However, the absence

of main effects or Lesion by Housing interaction and the presence of variability in response in other groups (e.g. lower intake ratios demonstrated by SC-SHAM on 2%-8% discrimination) suggest that the observed interaction may not be robust. The presence of the negative ratio values for the 2%-8% concentration in the SC-SHAM group and the both enriched groups indicate that the animals regarded the two solutions as of similar reward value and hence did not reduce their intake of the 2% in anticipation of the 8% solution, but drunk more of the 2% solution.

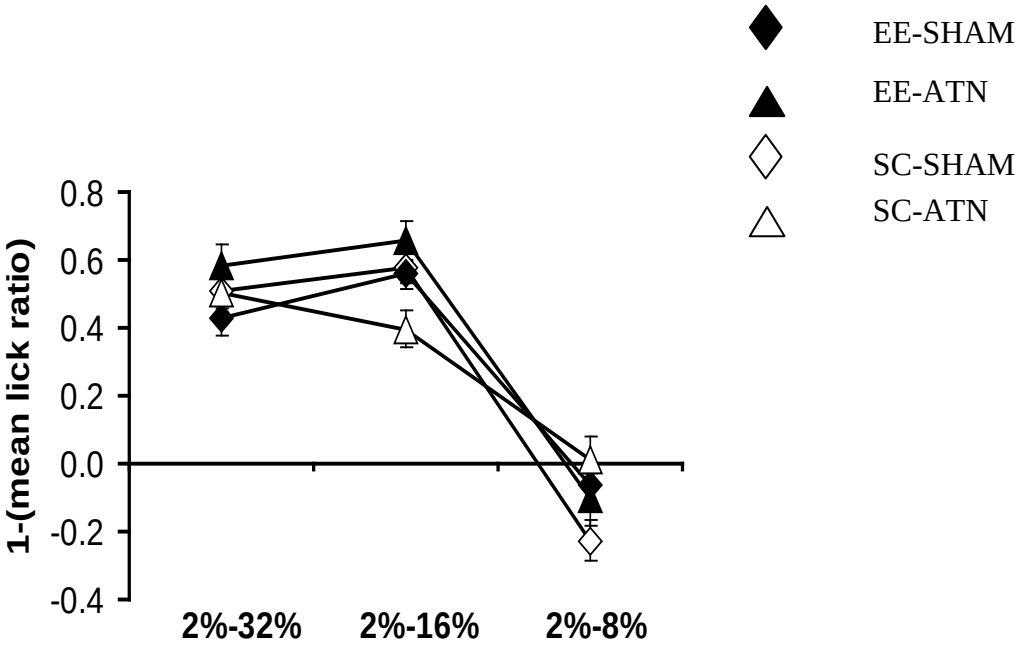


Fig 13. Pattern Separation for Reward Magnitude. Normalised Mean (± SEM) lick ratio scores of experimental groups as a function of concentration difference of sample phase water solution containing 2% sucrose followed by a test solution containing 32% sucrose (2%-32%), a 2% sample phase solution followed by a 16% sucrose test phase solution (2%-16%) and a 2% sample phase solution followed by an 8% test phase solution (2%-8%). Each condition consisted of 10 days of testing. The data were converted to ratio scores as described in the text. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.

A *post-hoc Newman–Keuls* comparison test revealed that there were no significant differences among all the groups in terms of intake ratio scores on the 2%–32%, as well as 2%-16% and 2%-8% anticipatory discriminations, despite the SC-ATN demonstrating a somewhat lower intake ratio score on the 2%-16% discrimination. The analysis also revealed that for all groups the ratio scores on the 2%–32% discriminations were not significantly different from their scores on the 2%–16% discriminations. When each discrimination was analysed separately no Lesion or Housing effects or interactions emerged for any of the discriminations (all F's < 1).

Overall, the analysis shows that when the reward values are very large and anticipatory discriminability is high, such as in 2%-32% discrimination, all groups show increased lick ratio scores. Even when the reward values are more similar and presumably the discriminability is lower, as in 2%-16%, all rats continued to demonstrate high lick ratios. All groups demonstrated markedly decreased lick ratios on the 2%-8% discrimination, indicating that they found it difficult to discriminate in memory between similar rewards.

6.3.4 Spatial pattern separation problems

This task commenced 110 days post-surgery. Performance was expressed as the trials to criterion for the four groups in each of the three spatial discrimination problems (Fig 14). Rats that failed to achieve criterion within 15 days for the wide and medium spatial separation problems, and 18 days for the close separation problem, were given a 'trials to criterion' score equal to the maximum number of trials used for that task plus two days (204 trials for the wide and medium separation problems; 240 trials for the close separation problem). This addition of two days extended the trials to criterion score for a rat by the minimum number of (24) trials that would be theoretically needed to attain criterion. The number of rats per group that failed to reach criterion on each problem was as follows: the wide separation, 3 (of 8) SC-ANT rats, 2 (of 7) EE-ATN rats, 1 (of 11) SC-SHAM rats; the medium separation, 1 (of 12) EE-SHAM rats, 4 SC-ATN rats, 3 EE-ATN rats; close separation, 3 SC-SHAM rats, 7 SC-ATN rats, 3 EE-SHAM rats, and 5 EE-ATN rats.

Overall, the rats made more errors when learning the third problem that used adjacent arm locations than during acquisition of the first two problems in the radial-arm maze (Problem main effect, $F_{(2,68)} = 19.28$; $p < 0.001$) (Fig 14C). The rate of acquisition did not differ between the wide (Fig 14A) and intermediate (Fig 14B) spatial separations (*Newman-Keuls*, $p > 0.5$), but the difference between these tasks and the third problem (close separation) was highly significant ($p < 0.001$). There was also a highly significant effect for Lesion ($F_{(1,34)} = 45.35$, $p < 0.0001$), with ATN rats being clearly impaired relative to sham rats. There was, however, no evidence that the acquisition of the fixed rewarded locations by ATN rats was minimized by the use of a "wide" (easier) spatial pattern separation (Lesion by Problem interaction, $F_{(2,68)} < 1$). In addition, prior history of post-operative enrichment did not change the rate of acquisition on these spatial discrimination problems (Housing, $F_{(1,34)} < 1$; Housing by Lesion interaction, $F_{(1, 34)} < 1$; Housing by Lesion by Problem interaction, $F_{(2,68)} = 1.54$; $p > 0.2$). The same conclusions were evident using errors across sessions or the proportion of rats achieving criterion on each problem.

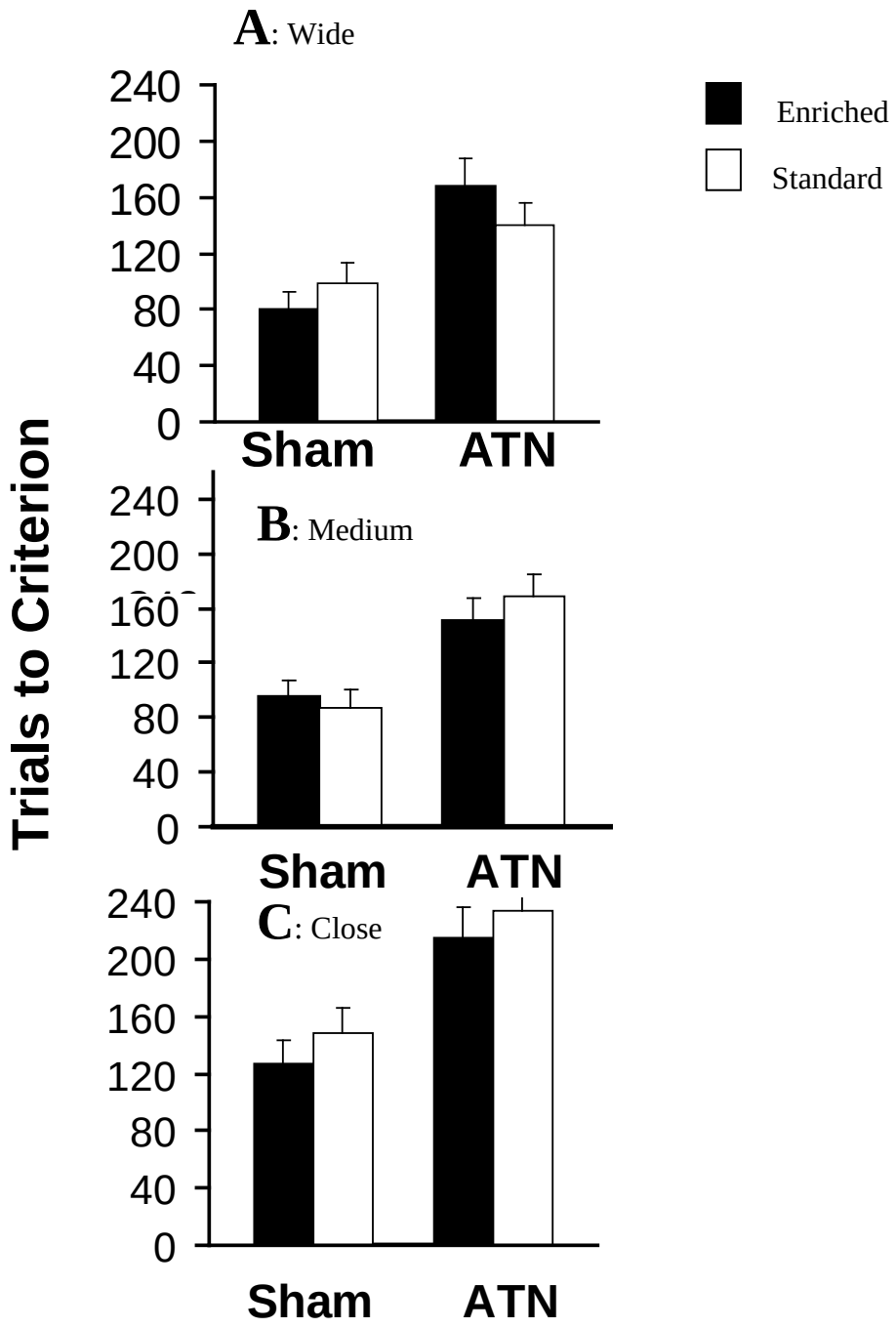


Fig 14. Spatial Pattern Separation. Mean (±SEM) number of trials to criterion achieved by each group on the spatial pattern separation task in a 12-arm radial maze, expressed separately for (**A**) wide, (**B**) medium and (**C**) close separations. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.

6.3.5 Individual performance, lesion size and spatial working memory

Figure 15 shows the individual spatial working memory performance as a function of lesion size for the EE-ATN (n = 7) and SC-ATN rats (n = 8) across 10 sessions of the spatial working memory test (as well as separately for the first and last 3 sessions of training) conducted immediately after the 30 day period of enrichment. Clearly, there was little overlap in scores for the ATN rats that experienced post-operative enrichment and those that only experienced standard housing conditions. There was no evidence that the benefits of post-operative enrichment varied as a function of lesion size. The performance-lesion size correlation was as follows; for the SC-ATN group (for the first three sessions: r = -0.16; last three sessions: r = -0.05; for 10 sessions: r = -0.172, df = 6, all p's > 0.5) and the EE-ATN group (for the first three sessions: r = -0.66; last three sessions r = -0.34 and for 10 sessions: r = -0.76, df = 5, all p's > 0.5). Inspection of Fig 15 suggests that the apparent correlation in the EE-ATN group was due to one rat with relatively small lesion. As noted in Table 5 the EE-ATN group median percent damage sustained to the AV was smaller (59.7%) than that sustained by the SC-ATN group (76.7%) no significant correlations were detected between performance and AV size in both lesion groups. The size of the lesion to the adjacent thalamic regions also did not correlate significantly with performance in either group.

6.4 Discussion

This study provides the first evidence that some deficits associated with brain injury to the ATN, which are strongly implicated in diencephalic amnesia, may be amenable to therapeutic intervention. A deficit in non-matching-to-sample spatial working memory represents one of the core findings in the rat ATN lesion model (e.g., Aggleton, et al., 1995a; Warburton & Aggleton, 1999; Warburton, et al., 1999; Warburton, et al., 2001; Ward-Robinson, et al., 2002). In the present study, near chance-level of performance on this reinforced spatial alternation task was evident in the standard-housed rats after highly localised ATN lesions. Such lesions are not subject to the interpretation confounds that arise when the ATN lesions extend to the adjacent thalamic regions (Warburton, et al., 1999).

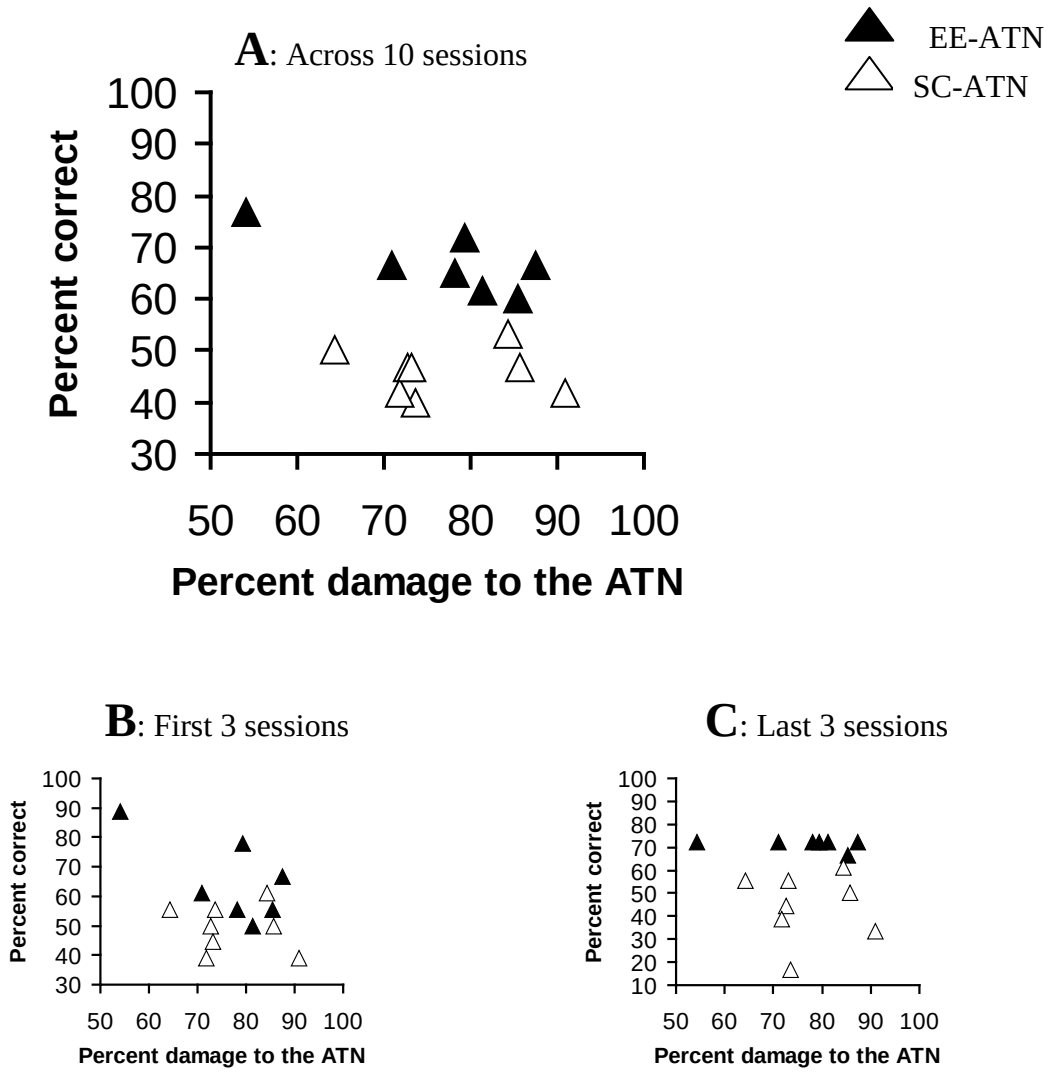


Fig 15. Immediate Enrichment, Spatial Working Memory performance and Lesion Size. Scatterplot depicting Mean correct percent responses across (**A**): 10 sessions, (**B**): for first 3 sessions and (**C**) for the last three sessions on the spatial memory task in the cross-maze versus percent of bilateral damage sustained to the ATN. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.

As with previous reports, the severity of the deficit probably reflected at least in part the cross-maze procedure that was employed, which restricted the benefits of an egocentric strategy (Aggleton, et al., 1996; Warburton, et al., 1999).

Exposure to enrichment substantially ameliorated the deficits on the spatial working memory tasks observed in ATN rats. This finding adds significantly to previous

literature that enrichment can promote recovery of function in some instances of acute brain injury (Dalrymple-Alford & Kelche, 1985; Johansson, 2003; Will & Kelche, 1992; Will, et al., 2004), as no previous evidence exists to our knowledge on the effects of enrichment after lesions to the limbic thalamus.

The potential value of enrichment or similar therapy as a means to obtain gains in diencephalic amnesia depends on the type of learning improvements achieved, so it is important to consider the nature of the spatial working memory improvements that were found. Rats appear to rely on more than one strategy during forced alternation working memory testing, including the use of spatial (allocentric; direction) cues and non-spatial cues (egocentric response) (Dudchenko, 2001; Futter & Aggleton, 2006; Skinner, et al., 2003, see Chapter 3). As in previous reports (Aggleton, et al., 1996; Warburton, et al., 1999), the intact rats in the current study found the "opposite start position" trials more difficult than the "same start position" trials. The latter trial type could be solved by alternating body turns (egocentric cues). Standard-housed ATN rats were disproportionately impaired on the "opposite start position" trials, which replicates reports that ATN lesions produce a weakness in using non-egocentric cues and /or a preference in using egocentric cues (Aggleton, et al., 1996; Sziklas & Petrides, 1999; Warburton, et al., 2001). The enriched rats demonstrated improved performance on the easier, egocentric-type trials at the start of post-enrichment re-testing as well as showed further improvements on these trials with training. One possibility is that enrichment in ATN rats improved spatial working memory by the use of one or more strategies, flexibility in switching from one type of preferred strategy to another or simply the inhibition of non-optimal strategies across training. However, the enriched ATN group demonstrated better performance than the SC-ATN group on the "opposite start position" trials that depend on the utilisation of spatial/directional cues, with the difference in performance becoming more evident by the end of testing.

The experiment also showed that ATN lesions produced clear deficits for the acquisition of spatial pattern separation problems based on fixed locations in a 12-arm maze, irrespective of the pattern separation between the correct and incorrect arms. The

fact that enrichment did not reduce these deficits suggests caution in assuming that spatial memory in general may be ameliorated by enrichment of rats with ATN lesions. All groups found the adjacent arm discrimination, which provided overlapping spatial information, more difficult to solve than the wide or intermediate separation problems. There is little demand for pattern separation in the intermediate and wide problems because there would be little or no overlap of relevant spatial information in those tasks. Hence the existence of a general impairment in ATN rats irrespective of the separation between arms suggests that the ATN are involved in more general aspects of spatial memory, such as consolidation, rather than spatial pattern separation. One important difference between the radial-arm maze task and the cross-maze task was that the latter examined working memory, which implies that the benefits of enrichment on spatial tasks after ATN lesions may be restricted to working memory processes. Alternatively, subtle procedural differences and variations in the cognitive and behavioural demands of the radial-arm and cross-maze tasks could explain the failure of enrichment to ameliorate acquisition of the simultaneous discrimination problems in the radial-arm maze. For example, the rats were already at the choice point (the central hub) for the discrimination problems, whereas the cross-maze procedure afforded the rats the opportunity to approach a choice point by traversing a start stem. The latter procedure would more readily encourage path integration and the use of directional and allocentric cues to guide spatial behaviour (Whishaw, Cassel, & Jarrad, 1995; Whishaw, McKenna, & Maaswinkle, 1997). Another difference was that the radial-arm maze was rotated between trials while the rat remained in the central hub and this procedure would introduce additional distraction and vestibular stimulation, which is known to impair the ability to use spatial information (Kirwan, Gilbert, & Kesner, 2005). Clearly, evidence from more tasks is required before concluding that post-operative enrichment in ATN rats does not improve spatial reference memory in general. The reason for doubting such a conclusion is supported by evidence that beneficial effects of enrichment have been found in the Morris water maze in other examples of brain injury, including hippocampal system lesions, transgenic Alzheimer's disease models and neonatal hypoxia-ischemia models (Frick & Fernandez, 2003; Frick, et al., 2003; Galani, et al., 1997; Jankowsky, et al., 2005; Pereira, et al., 2007). The water maze differs from the radial-arm maze spatial

discrimination problems used here in that the former encourages continuous navigation in an “open” arena and place acquisition that is based on many differing trajectories and viewpoints, the use of which may benefit from exposure to an enriched environment (see Chapter 10 for further discussion).

On the task of pattern separation for reward magnitude the ATN lesioned animals had no difficulty in displaying a reliable anticipatory discriminability when a low rewarding substance (2% sucrose solution) was followed by a highly rewarding substance (32% sucrose solution), suggesting that the ATN animals can learn to anticipate reward value when the discriminability between substances is high. Similarly, when the reward values were changed to 2% vs 16%, and presumably the need for the discrimination increased all rats were able to demonstrate a negative contrast effect. All rats found it difficult to discriminate between 2%-8% solutions suggesting that a threshold exists beyond which reward discrimination in memory becomes difficult. Current findings support previous reports that the ATN are not involved in memory for reward value (Mitchell & Dalrymple-Alford, 2005), and that damage to the ATN does not impair motivation, further supporting the inter-relationship between the ATN and the hippocampus (Aggelton & Brown, 1999).

Overall, the current findings on beneficial effects of enrichment on spatial working memory after ATN lesions are most encouraging, and represent the first evidence that ATN induced deficits can be ameliorated by exposure to enrichment. The lack of beneficial effects of enrichment in the fixed location problems in the radial-arm maze indicate that additional evidence from animal models is required before clear conclusions can be drawn with regard to the prospects of therapeutic interventions for diencephalic amnesia. The results also further support the notion that ATN involvement is not essential for optimal memory functioning for reward value.

Chapter 7

Effects of delayed post-operative enrichment on recovery of function after anterior thalamic lesions

7.1 Introduction

The experimental findings detailed in Chapter 6 provided the first evidence that exposure to environmental enrichment can promote recovery of functioning on the spatial working memory task in the cross-maze after ATN lesions. However, task specificity was observed. While spatial working memory performance improved with the introduction of post-surgery enrichment, no enrichment effect was observed on the reference memory task of spatial pattern separation. As discussed in Chapter 6 different behavioural demands of the spatial working memory and reference memory tasks could have contributed to the task specificity observed. However, procedural differences were also present, as the pattern separation task was administered 75 days after the initial 30-day continuous enrichment period. It is possible that the enrichment effect was time-dependent and had worn off by the time of the spatial pattern separation testing. That is, enrichment may have had an effect on tasks performed relatively soon after the period of enrichment and/or when the enrichment was imposed immediately after surgery, but not on tasks performed later.

Very few studies have examined delayed-enrichment effects and/or the long-term effects of prior enrichment and the outcomes have been contradictory. Two studies have investigated the effects of delayed enrichment on motor function recovery after a MCA occlusion. Johansson (1996) reported that the performance of MCA rats on a rotating pole, prehensile traction, limb placement, and postural

reflexes improved substantially after the rats were transferred to enriched environments 15 days following initial insult, suggesting that delayed postoperative environmental enrichment can improve outcome in experimental stroke. A later study, by Biernaskie and colleagues (Biernaskie, Chernenko & Corbett, 2004) assessed the impact of 30-day enrichment on locomotor activity of the MCA rats, at three different delay intervals 5, 14 or 30 days post-insult. Rats exposed to enrichment 5 days after insult significantly improved in skilled forelimb reaching and on tests of coordinated forelimb use during locomotor activity (narrow-beam and ladder-rung walking) relative to animals that received the same therapy beginning 30 days later. When the introduction of enrichment was delayed by 30 days the animals did not differ from the socially housed rats on any task, with the exception of the ladder-rung walking. The shorter delay to therapy (i.e. 14 days) provided moderate functional gains in reaching ability and beam walking compared with social housing. The researchers concluded that a 30-day treatment delay may be too long to induce functional gains.

Two other studies evaluated the impact of delayed enrichment on memory function after insults to the hippocampal system. Paban and colleagues (2005) found that after 192 IgG-saporin cholinergic forebrain lesion (which depletes the choline acetyltransferase in the cortex and hippocampus and supposedly mimics the effects of aging on the brain) 3 months of enrichment starting 9 months after injury markedly compensated for age-related non-matching to position in the T-maze and object-recognition deficits. These workers suggested that environmental enrichment had the greatest effect on the least cognitively capable animals at the time when the neurodegenerative processes were superimposed on an early lesion. An earlier study by Pacteau and colleagues (1989) examined behavioural responses in a set of spatial and cue tasks in adult rats that had been given ibotenic acid lesions of the dorsal hippocampus at weaning. The lesions or sham operations were immediately followed by 30-day differential rearing, either in enriched, social or isolated housing environments. The differential rearing was followed by standard (social) housing conditions until behavioural testing began at 4 months of age. Compared to sham-operated rats, the rats with early cytotoxic lesions showed substantial impairments in learning and efficient strategy formation in the radial arm maze and as well as in the retention of a spatial location, but not of a cue-marked location and alternation in a plus maze. The researchers concluded that differential rearing had some long-term

effects depending on the task. Enrichment, alleviated lesion deficits only in a spontaneous alternation task in a plus-maze where the variety and salience of proximal cues was maximised, but not in the radial maze task where only distal cues could guide performance. The authors suggested that the hippocampus may play an important role in the use of contextual information and that behavioural recovery after early hippocampal damage is limited to situations in which cues are highly salient.

To address the possible time-course dependent effects of enrichment in the current study the introduction of the 30-day enrichment phase was delayed 40 days post-surgery, which was seen as sufficiently long for the lesion associated processes to be well established. Prior to the animals being exposed to enrichment a post-surgery period of re-testing on the cross-maze was conducted to establish the ATN lesion effects and ensure pseudo-random assignment to groups in a fashion that the enriched and standard caging groups were likely to be equivalent in the mean level of behavioural impairment. Moreover, to examine the robustness of the possible enrichment effect all rats were re-tested 120 days post-surgery on the cross-maze after a 30-day time when no further enrichment occurred.

7.2 Materials and Methods

7.2.1 Animals

Forty-five female PVGc hooded rats were used (6-7 months old, weighing between 152-198 g at surgery). Body weights were restricted to 85-90% of free-feeding weight throughout the experiment, except free food access around the period of surgery, during immediate recovery, subsequent delay and the period of differential housing.

7.2.2 Surgery

The same surgery procedures to achieve ATN damage as described in Chapter 6 were used, with minor adjustments to the lesion coordinates in attempt to improve lesion accuracy. The anterior–posterior coordinates were moved 0.1 mm posterior for both AV and AM lesions sites. The AV lesion was also now ± 1.50 mm lateral from the midline and −5.55 mm ventral from dura and the AM lesion was ± 1.20 mm lateral

from the midline and −5.80 mm ventral from dura. The volumes of NMDA injected were as per Chapter 6.

7.2.3 Housing

Initially, all rats were housed in standard housing (group) conditions of 3 or 4 rats per opaque plastic cage as detailed in Chapter 6. The introduction to enrichment was delayed until 40 days post-surgery and after the initial confirmation of equal deficits in ATN lesioned rats (see Fig. 16 for experimental time line). This was done on the basis of rats' post-surgery performance on the cross-maze conducted at 14 days post-surgery. Following pseudo-random allocation into experimental groups the rats in the EE group were housed in groups of 11 or 12 in an enrichment cage, while the SC group remained housed in standard cages. Following the 30 days of continuous enrichment the rats in the EE group were re-housed in standard conditions of 3 or 4 rats per cage, with cage mates from the same enrichment cage, and all rats were placed under restricted food access for the second period of post-surgery testing (starting at 75 days post-surgery). Rats from the enriched cages were returned daily to the enriched environment for a period of 1.5 to 2 hours at the end of each session of this second post-surgery period of spatial working memory testing, followed by their daily food ration on return to the standard cages. After completion of this second period of post-surgery testing, however, all rats were returned to standard caging and no further enrichment was provided. Rats were retested for the third and final post-surgery time on the spatial working memory test starting at 120 days post-surgery. After confirmation of accurate lesions, the final group numbers were: SC-SHAM n = 11; SC-ATN, n = 8; EE-SHAM, n = 10; and EE-ATN, n = 11 (4 SC-ATN rats and one EE-ATN rat were excluded for details see Lesion Evaluation).

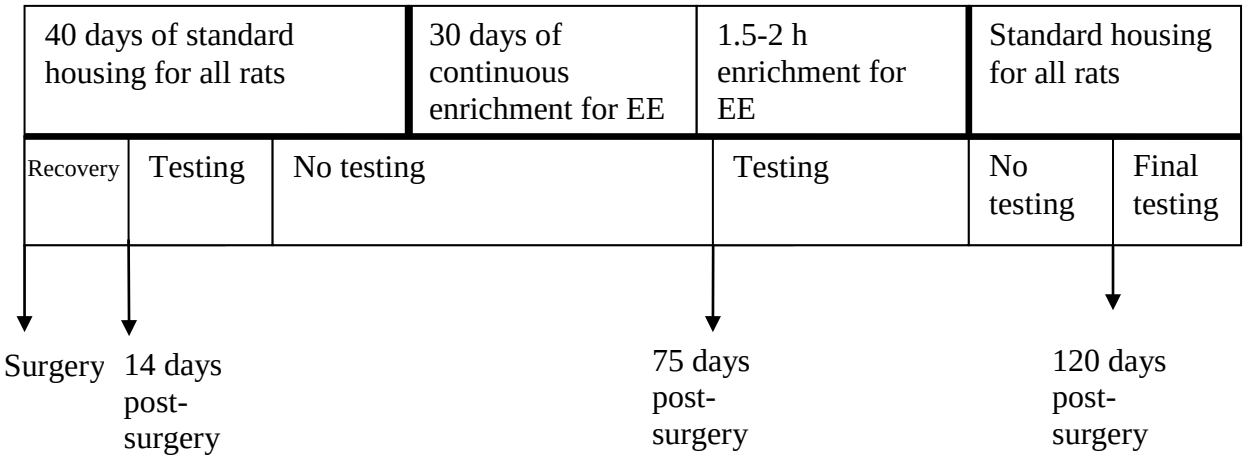


Fig 16. Post-surgery time line for the Delayed Enrichment experiment. Testing = Spatial Working Memory in the cross-maze. EE = enriched environment group.

7.2.4 Apparatus and behavioural testing

The same cross-maze apparatus and test room were used as described in Chapter 6. All rats were trained to criterion on the spatial working memory task prior to surgery and then re-tested on the same task for 10 consecutive sessions on each occasion at 14 days, 75 days and 120 days post-surgery. The only modification to the cross-maze procedure described in Chapter 6 was that preoperative training began from the start of testing with the "opposite start position" for the sample and test runs for half of the 6 trials per session, which continued until the rats reached the criterion of 85% correct for each of two consecutive sessions (requiring between 10-16 sessions). The initial post-surgery testing provided a behavioral assay for the lesion deficit during a post-operative delay prior to enriched housing and thus also enabled matched pairs of sham and ATN rats to be allocated to the subsequent housing environments. The third, and final, post-surgery test evaluated the durability of any enrichment and lesion effects.

7.2.5 Histology

The same histology procedure as detailed in Chapter 6 was used.

7.3 Results

7.3.1 Lesion Evaluation

The largest and smallest acceptable ATN lesions are shown in Figure 17. Eight SC-ATN and 11 EE-ATN rats met the Histology inclusion criteria. The lesions were similar to these described in Chapter 6 except that there was now slightly more damage to the LT region observed. The percent of damage to the ATN and components and other adjacent nuclei is depicted in Table 6. The extent of damage to the ATN and its components was highly similar across SC-ATN and EE-ATN groups. With the exception of the interanteromedial nucleus, the median damage (and range) to the other adjacent structures was again generally minimal in both groups. Five rats were excluded from the study. The mean percent damage sustained to the ATN and adjacent structures for excluded animals was for one EE-ATN No: 86 (ATN =12.4; LT = 5.3; MT = 0.3 small lesion); for each SC-ATN rat No: 88: (ATN =13.8; LT = 20.5; MT = 5.9; small lesion plus damage to the fornix); No: 107: (ATN = 42.1; LT = 35.2; MT = 5.3); No: 132: (ATN = 31.9; LT = 65.9; MT = 20.2); No: 89: (ATN = 45.1; LT = 49.7; MT = 28.4).

Table 6. Delayed Enrichment and Percent bilateral damage (volume) to selected areas for each of the rats in the study.

	ATN and components				Other Nuclei								
RATS	AD	AM	AV	AT	MT	LT	IAM	LD	PT	PVA	PV/ PVP	Re	Rh
EE-ATN													
106	73.6	86.5	72.0	69.6	13.9	32.3	72.4	14.9	1.2	0.2	1.0	0.1	1.4
90	96.6	99.5	93.0	97.9	8.8	24.2	73.6	30.1	18.5	1.2	0.0	0.0	1.0
96	96.3	71.9	68.4	77.6	2.4	10.8	36.0	3.1	12.8	0.2	0.0	0.0	0.8
98	81.7	92.2	55.5	74.8	2.5	21.2	83.4	10.0	5.5	0.5	0.0	5.0	15.2
110	94.8	80.4	45.9	83.4	8.1	8.0	47.5	2.1	3.5	0.2	0.0	0.1	0.6
112	79.2	58.1	55.1	53.7	5.5	36.6	65.3	17.0	2.5	0.2	0.0	0.3	18.1
117	95.0	97.0	87.6	93.3	4.3	25.0	86.3	22.6	6.3	0.0	0.0	0.6	18.5
118	95.5	93.4	78.0	87.7	2.8	27.1	83.4	21.4	7.0	0.2	0.0	0.7	9.2
111	96.1	88.3	39.4	80.3	0.7	8.5	65.2	1.8	5.0	0.2	0.0	0.1	5.4
114	92.4	95.4	30.1	78.2	3.1	32.1	3.6	11.6	8.8	1.9	0.0	0.1	4.4
129	90.1	88.8	74.4	78.7	4.7	29.3	51.0	20.8	3.5	0.2	0.0	0.1	0.7
EE-ATN median	**94.8**	**88.8**	**68.4**	**78.7**	**4.3**	**25.0**	**65.3**	**14.9**	**5.5**	**0.2**	**0.0**	**0.1**	**4.4**
SC-ATN													
100	99.2	91.8	46.0	73.3	8.2	36.4	91.9	23.3	8.2	0.8	0.0	0.1	0.7
92	79.6	72.7	71.2	72.4	2.6	12.4	35.1	8.2	28.2	1.7	0.0	2.2	7.3
97	95.6	95.4	76.0	83.8	0.2	25.6	88.5	21.3	9.3	11.4	0.0	1.6	7.2
116	96.4	95.2	68.4	88.7	2.1	17.7	86.0	10.1	13.3	1.2	0.0	0.1	4.5
128	74.8	88.0	52.9	72.7	3.5	34.7	60.6	11.7	7.1	0.2	0.0	0.6	18.5
119	89.5	96.5	83.3	93.7	3.7	21.6	70.5	10.0	10.4	0.7	0.0	10.5	12.3
127	86.9	83.3	86.9	84.4	2.0	15.1	28.9	16.9	9.8	0.2	0.0	0.1	1.2
123	60.5	76.8	46.3	59.0	6.3	25.0	58.6	7.3	4.1	0.2	0.0	0.1	2.7
SC-ATN median	**88.2**	**88.9**	**69.8**	**78.6**	**3.1**	**23.3**	**65.5**	**10.9**	**9.5**	**0.7**	**0.0**	**0.3**	**5.8**

Abbreviations: AD= anterodorsal nucleus; AM= anteromedial nucleus; ATN = anterior thalamic aggregate comprising the anterodorsal, anteromedial and anteroventral thalamic nuclei; ATN median= median percent damage for all included rats; AV= anteroventral nucleus; EE-ATN = rats with anterior thalamic lesions housed in enriched cages; IAM= interanterodorsal nucleus; LT= lateral medial thalamic aggregate comprising the intralaminar nuclei (centrolateral, paracentral and rostral central medial nuclei) and lateral mediodorsal thalamic nuclei (lateral and paralamellar nuclei); LT median= median percent damage for all included rats; MT= posteromedial thalamic aggregate comprising the central and medial mediodorsal nuclei and the intermediodorsal nucleus; MT median= median percent damage for all included rats; PT= paratenial nucleus; PVA= anterior paraventricular nucleus; PV/PVP= paraventricular nucleus/posterior paraventricular nucleus; Re= reunions nucleus; Rh= rhomboid nucleus; SC-ATN = rats with anterior thalamic lesions housed in standard cages.

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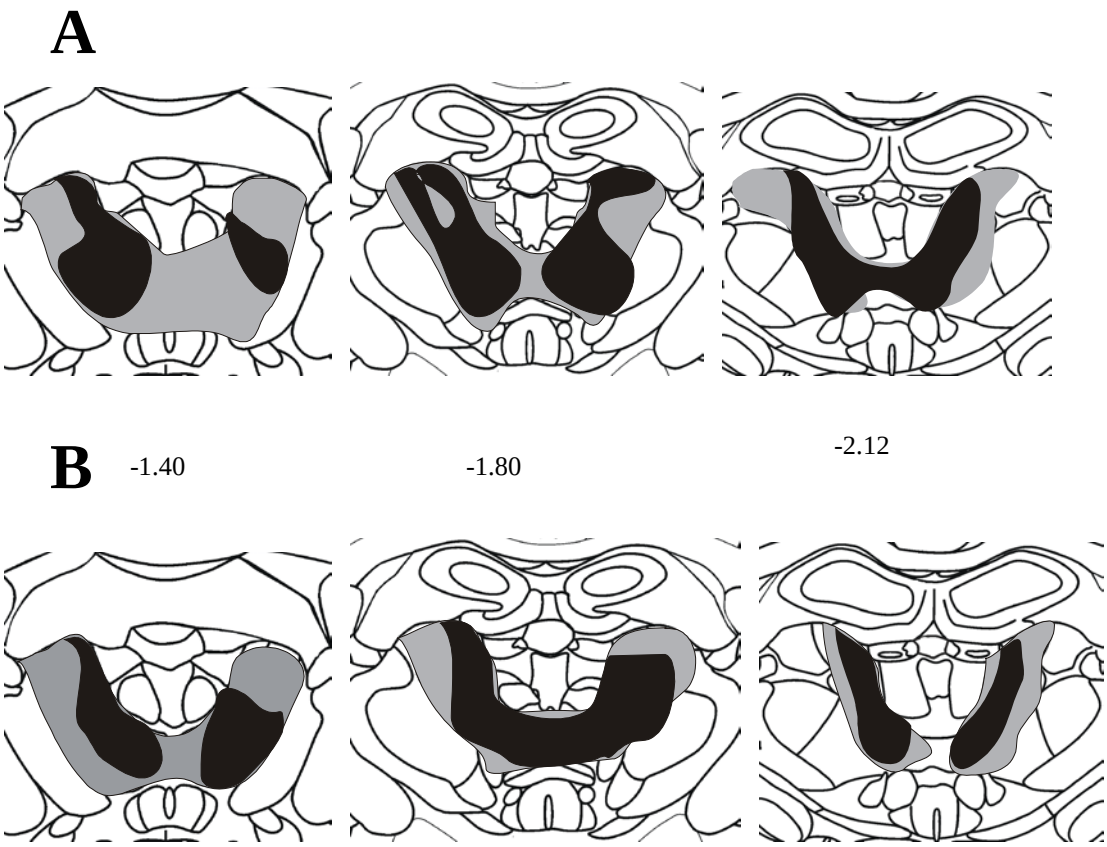


Fig 17. Delayed Enrichment and Lesion size. Schematic representation of the largest (grey) and smallest (black) lesion in the **A**: ATN enriched and **B:** ATN standard caging group. Numbers indicate distance from bregma in millimetres (from Paxinos & Watson, 1998).

7.3.2 Non-matching to sample spatial working memory

Pre-surgery training - Figure 18 shows spatial working memory performance in terms of overall percent correct, combined across both trial types (i.e. irrespective of the "same start position" and "opposite start position" trials) before surgery for 10 sessions taken back from the last 2 days of training when 85% correct criterion was reached. As seen in Fig 18 performance in all four groups was equivalent (all *F*'s < 1).

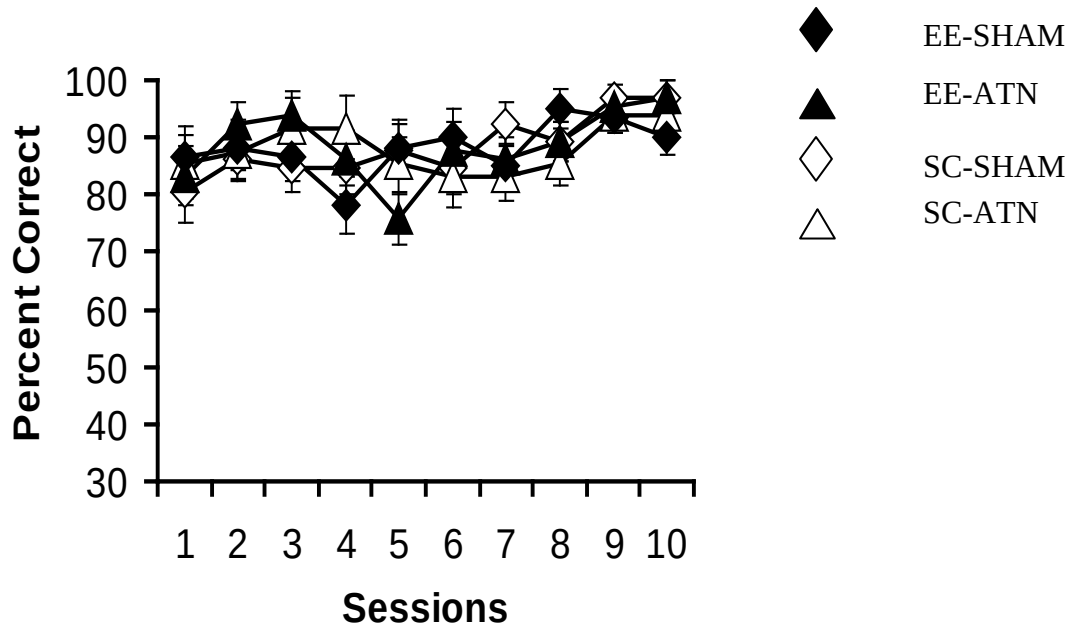


Fig 18. Delayed Enrichment and Spatial Working Memory Performance (pre-surgery). Mean (± SEM) percent correct responses for the last 10 sessions of pre-surgery performance, taken back from the 2 last days of training when 85% criterion was reached, on the spatial working memory task in the cross-maze configuration. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.

Initial post-surgery test - The initial spatial working memory test, conducted 14 days post-surgery and prior to enrichment, revealed the expected severe deficits after ATN lesions ($F_{(1,36)} = 605.01$; $p < 0.0001$; Figure 19). The matching procedure produced two groups of rats with ATN lesions that showed equally severe impairments in this first test. The only other significant finding for this pre-enrichment test was that all groups showed some improvements across the ten sessions ($F_{(9,324)} = 9.82$; $p < 0.001$). There was a main effect for Trial Type, with rats experiencing greater difficulty in solving the working memory task when the "opposite start position" was used on the test run ($F_{(1,36)} = 132.25$; $p < 0.0001$).

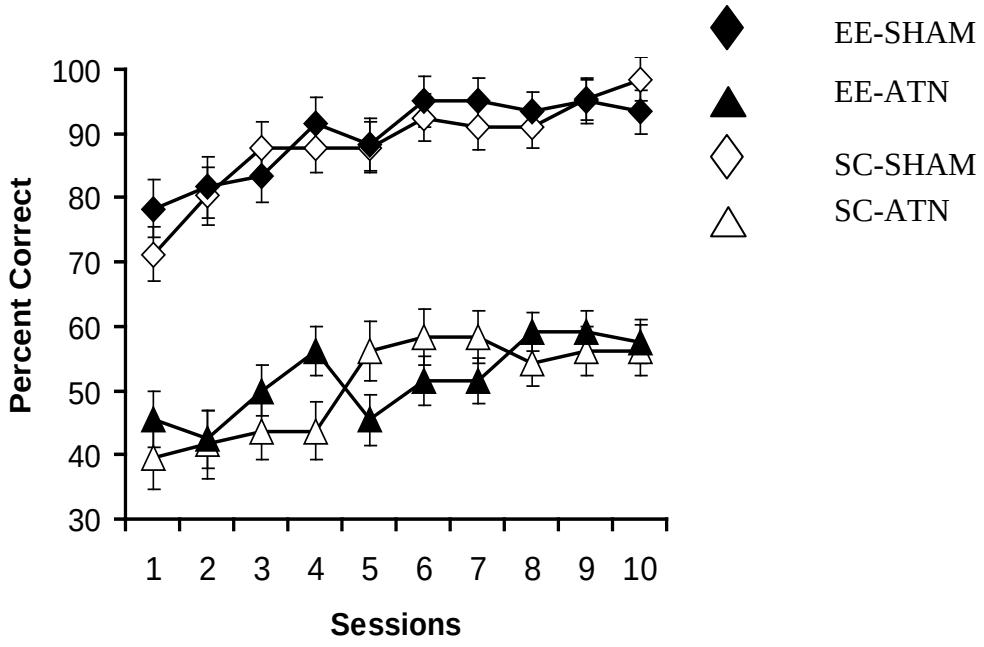


Fig 19. Delayed Enrichment and Spatial Working Memory performance at 14 days post-surgery and prior to enrichment. Mean (± SEM) percent correct responses for 10 sessions of post-surgery, pre-enrichment training on the spatial working memory task in the cross-maze configuration. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group. conditions; EE = enriched environment.

The last three sessions were examined more closely to assess the effects of ATN lesions with respect to Trial Type when overall performance was relatively stable (Figure 20). For these sessions, the severely impaired overall performance of the ATN rats (Lesion effect, $F_{(1,36)} = 255.81$; $p < 0.0001$) was relatively worse in terms of the "opposite start position" trials (Lesion by Trial Type, $F_{(1,36)} = 25.20$; $p < 0.001$), in both the to-be SC-ATN group and the to-be EE-ATN group. On the last three sessions the performance of both SC-ATN and EE-ATN groups was significantly above 50% chance on the "same start position" trials ($t_{(7)} = 4.73$; $p < 0.01$) and ($t_{(10)} = 6.86$; $p < 0.01$) respectively. On the "opposite start position" trials the SC-ATN's performed significantly below chance ($t_{(7)} = -3.05$; $p < 0.05$), while the EE-ATN performance was not significantly below chance ($t_{(10)} = -1.89$; $p = 0.09$).

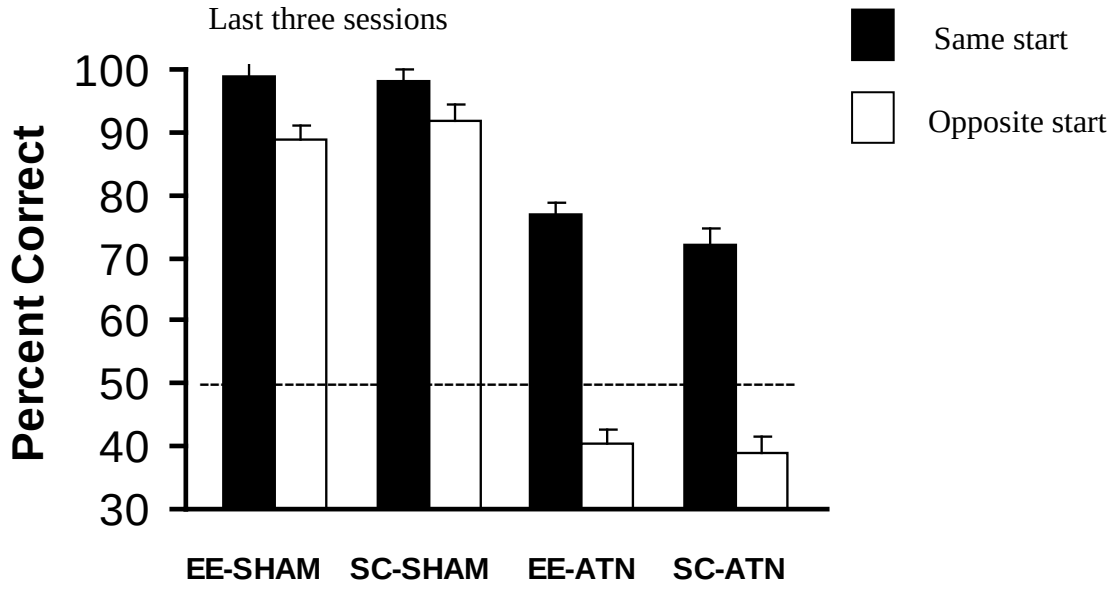


Fig 20. Delayed Enrichment and Spatial Working Memory performance by trial type on the last three sessions of 14-post-surgery, prior to enrichment testing. Percent correct responses for the last three sessions of the cross-maze task 14 days post-surgery but pre-enrichment expressed separately for the "same start position" trials (same start position used for both sample and test run per trial) and "opposite start position" trials (opposite start position used for the test run). ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment. --- = 50% chance line.

Initial post-enrichment performance - For the test conducted at 75 days post-surgery, immediately after the 30 day enrichment period, the two sham groups continued to perform at a high level, while the SC-ATN group remained at its previous, near-chance level of performance (Figure 21). The important finding, however, was that the EE-ATN group now demonstrated a reduced spatial working memory deficit. At the start of testing the EE-ATN group performed no differently to the SC-ATN group, but the performance of the EE-ATN group then improved towards that of the sham groups by the end of testing. These observations were supported by a highly significant Lesion effect ($F_{(1,36)} = 108.92$; $p < 0.0001$), Housing effect ($F_{(1,36)} = 13.28$; $p < 0.001$), and Lesion by Housing ($F_{(1,36)} = 5.55$; $p < 0.05$) and Lesion by Housing by Session interactions ($F_{(9,324)} = 2.13$; $p < 0.05$). A highly significant main effect for Trial Type was also evident ($F_{(1,36)} = 90.01$; $p < 0.0001$) with "opposite start position" trials again being more difficult to solve for all groups.

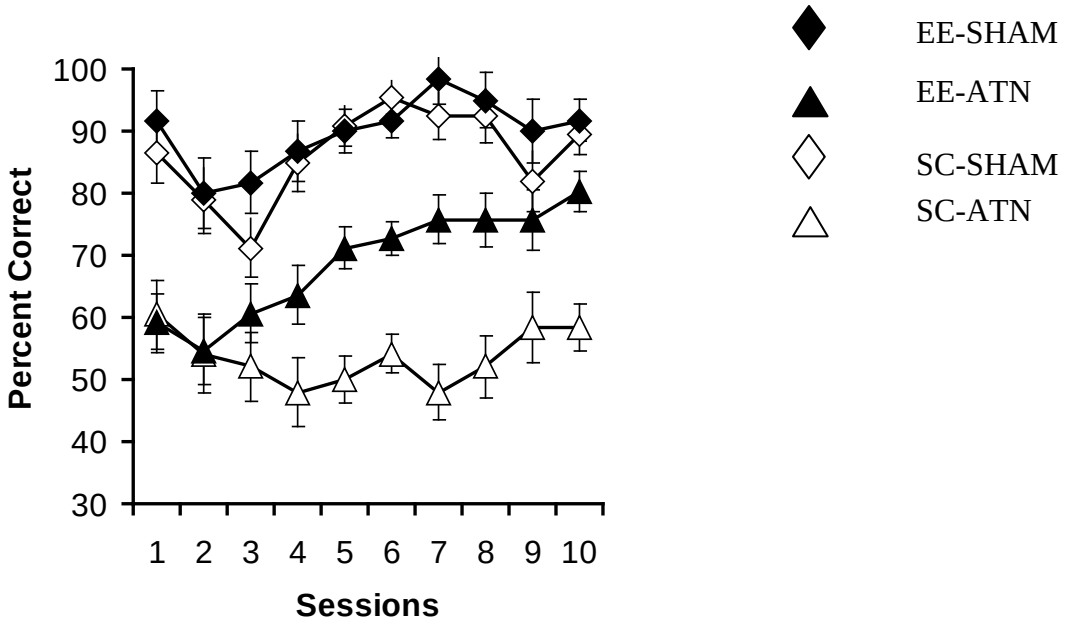


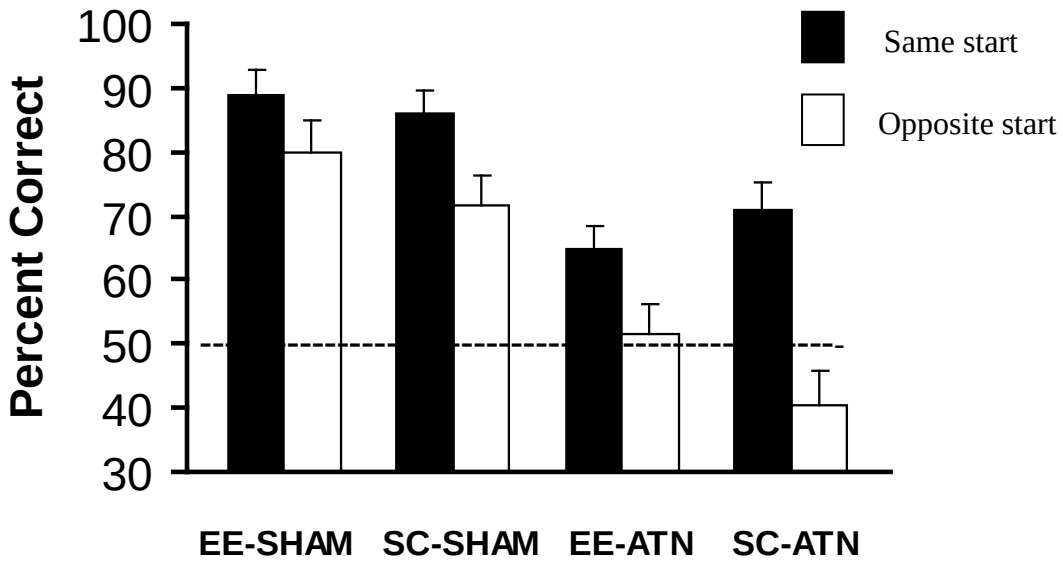
Fig 21. Delayed Enrichment and Spatial Working Memory performance at 75 days post-surgery, post-enrichment. Mean (± SEM) percent correct responses for 10 sessions on the spatial working memory task in the cross-maze configuration starting at 75 days post-surgery and after a period of enrichment. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.

To provide a clearer perspective on the effects of trial type, ATN lesions and enrichment, performance for the two trial types was assessed on the first three sessions, and then on the last three sessions (see Figures 22A & 22B respectively). For the first three sessions both lesion groups found it difficult to solve either types of trials with a significant effect for Lesion being detected (Lesion main effect, $F_{(1,36)}$ = 67.83; $p < 0.001$) but no Housing or Lesion by Housing interaction. Both the SC-ATN and the EE-ATN groups performed at above chance levels on the "same start" trials ($t_{(7)} = 10.24$; $p < 0.001$); ($t_{(10)} = 2.66$; $p < 0.005$) resepectively, but at chance levels on the "opposite start" trials ($t_{(7)} = -1.90$; $p = 0.09$) and ($t_{(10)} = 0.23$; $p = 0.82$). However, the marked benefits of enrichment emerged over sessions.

The analysis of the three last sessions of training revealed a significant Housing effect ($F_{(1,36)} = 11.83$; $p < 0.02$) and significant interactions for Housing by Lesion ($F_{(1,36)} = 5.11$; $p < 0.03$) and Housing by Lesion by Trial Type ($F_{(1,36)} = 6.44$; $p < 0.02$). The EE-ATN group displayed improved performance compared to the SC-ATN group for both types of trials. Specifically, the EE-ATN group achieved a significantly better than chance level of performance for both the "same start

position" trials ($t_{(10)} = 6.50$, $p < 0.0001$) and the "opposite start position" trials ($t_{(10)} = 4.73$, $p < 0.001$). The SC-ATN group displayed above chance performance for the "same start position" trials ($t_{(7)} = 3.52$, $p < 0.01$) and performance that was non-significantly below chance for the "opposite start position" trials ($t_{(7)} = -1.52$, $p = 0.17$).

A: First three sessions



B: Last three sessions

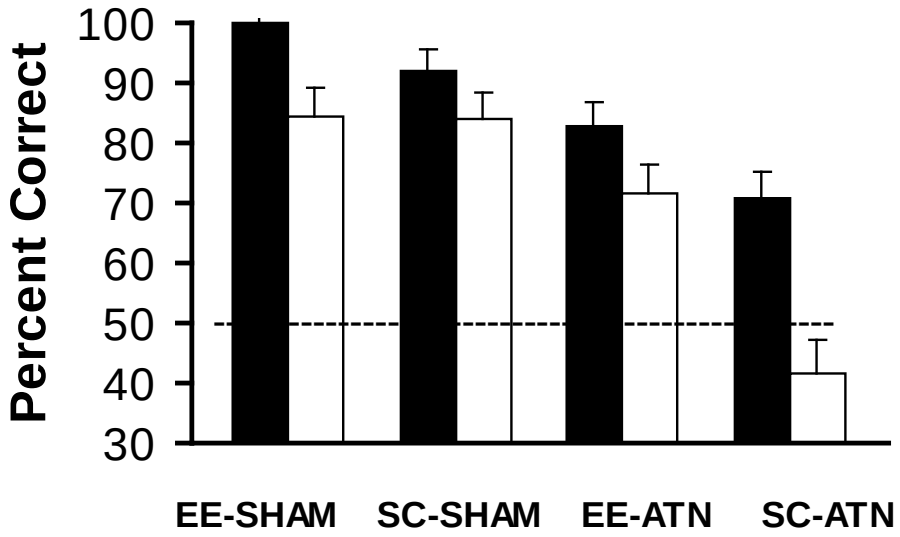


Fig 22. Delayed Enrichment and Spatial Working Memory performance at 75 days post-srugery, post-enrichment by trial type. Mean percent correct responses for the first three (**A**) and last three (**B**) sessions of the cross-maze task starting at 75 days post-surgery and after a period of enrichment expressed separately for the "same start position" trials (same start position used for both sample and test run per trial) and "opposite start position" trials (opposite start position used for the test run). ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment. --- = 50% chance line

Performance at 120 days (4 months) post-surgery - Overall performance on this final test replicated in large part the earlier findings (Figure 23). The SC-ATN group showed a sustained severe deficit in spatial working memory. The two sham groups maintained a high level of performance, although accuracy was lower at the start of testing and improved thereafter. Again, the important finding was that the EE-ATN group displayed an intermediate level of performance, which on this occasion did not approach that of the sham groups at the end of testing. These observations were supported by significant main effects for Lesion ($F_{(1,36)} = 97.92$; $p < 0.0001$), Housing ($F_{(1,36)} = 12.95$; $p < 0.001$) and Session ($F_{(9,324)} = 9.01$; $p < 0.0001$), and a Lesion by Session interaction ($F_{(9,324)} = 2.19$; $p < 0.05$). As before, overall performance was better for the "same start position" trials (Trial Type, $F_{(1,36)} = 81.16$; $p < 0.0001$). Most relevant, the Lesion by Housing interaction was again significant ($F_{(1,36)} = 11.87$; $p < 0.01$). Thus, the overall effect of enrichment on spatial working memory was resilient over time, at 4 months post-lesion, despite the enrichment period being introduced for a restricted period of 30 days that started at only 40 days post-surgery.

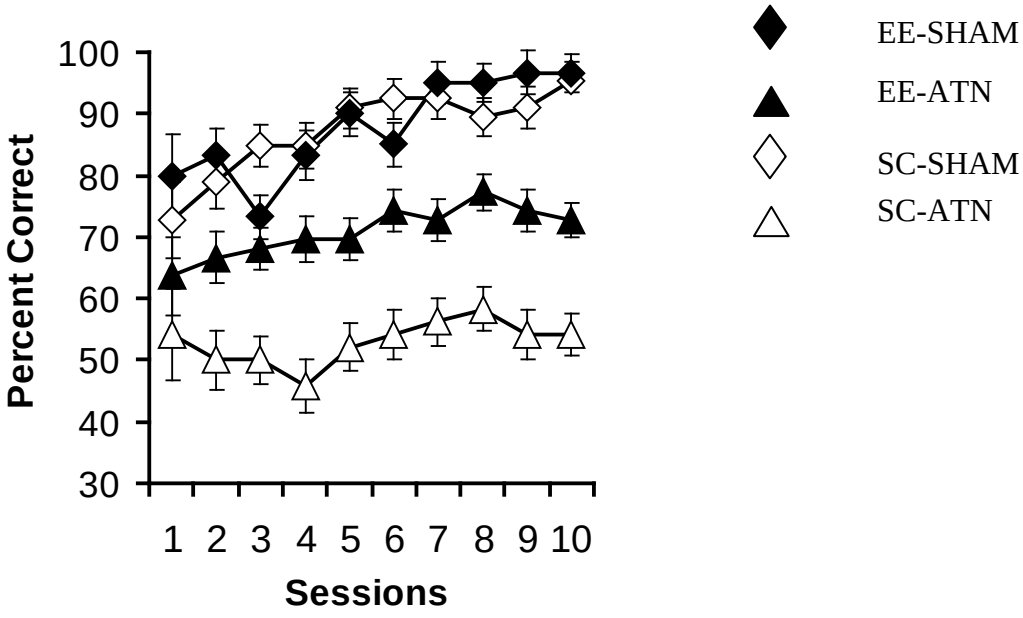


Fig 23. Delayed Enrichment and Spatial Working Memory performance at 120 days post-surgery. Mean (± SEM) percent correct responses for 10 sessions of training on the spatial working memory task in the cross-maze configuration starting at 120 days post-surgery after a period of no further enrichment. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.

The effect of Trial Type on performance is depicted in Figure 24. On the first three days of training at 120 days post-surgery the previously enriched lesioned rats continued to demonstrate superior performance on both types of trials in comparison to the SC-ATN rats (Fig 24A). There was a significant effect for Lesion ($F_{(1,36)}$ = 24.77; $p < 0.001$), but not Housing or Lesion by Housing interaction (all F's < 1). There was also an effect for Trial Type ($F_{(1,36)} = 52.77$; $p < 0.001$) and Lesion by Trial interaction ($F_{(1,36)} = 6.99$; $p < 0.05$) with the two sham groups demonstrating improved performance on the "opposite start position" trials in comparison to the lesioned rats.

On the last three sessions (Figure 24B) the beneficial effect of enrichment was confirmed, with a significant Housing effect ($F_{(1,36)} = 21.31$; $p < 0.001$) and Housing by Lesion interaction ($F_{(1,36)} = 8.77$; $p < 0.01$). At the end of testing the two sham groups showed improvements on the "opposite start position" trials relative to the lesion groups at the end of testing, which produced a Lesion by Trial Type interaction for the last three sessions ($F_{(1,36)} = 10.52$; $p < 0.01$). As before, the EE-ATN group displayed improved performance compared to the SC-ATN group on both trial types. Specifically, the EE-ATN group displayed above chance level of performance for both the "same start position" trials ($t_{(10)} = 11.25$, $p < 0.0001$) and the "opposite start position" trials ($t_{(10)} = 3.12$, $p < 0.02$). By contrast, the SC-ATN group displayed above chance performance for only the "same start position" trials ($t_{(7)} = 5.78$, $p < 0.001$) and were not different to chance on the "opposite start position" trials ($t_{(7)} = -1.09$, $p > 0.30$).

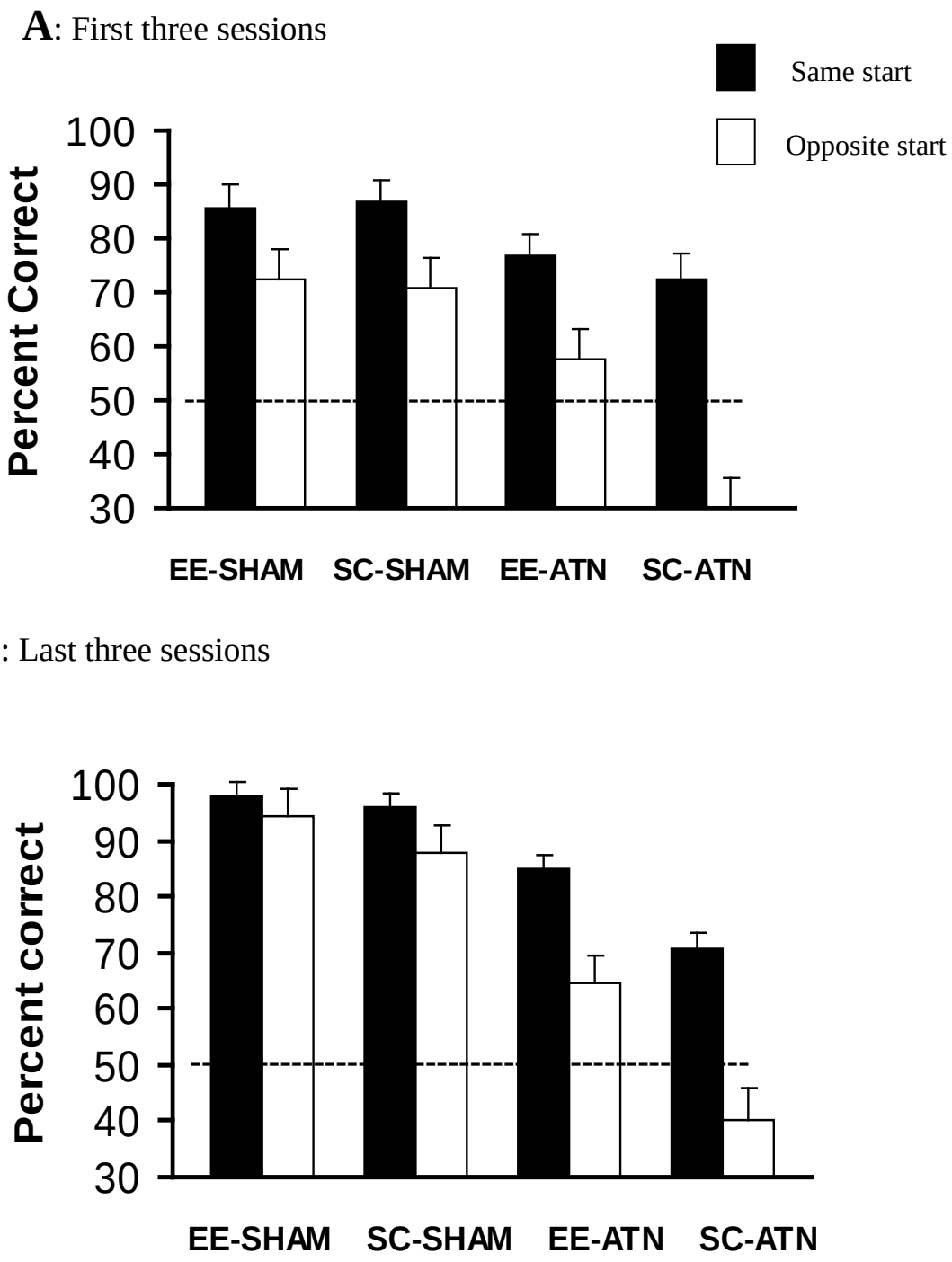


Fig 24. Delayed Enrichment and Spatial Working Memory performance at 120 days post-surgery by trial type. Mean percent correct responses for the first three (**A**) and last three (**B**) sessions of the cross-maze task at 120 days post-surgery and after a period of no further enrichment expressed separately for the "same start position" trials (same start position used for both sample and test run per trial) and "opposite start position" trials (opposite start position used for the test run). ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment. --- = 50% chance line.

7.3.3 Individual performance, lesion size and spatial working memory

Figure 25 depicts the relationship between lesion size and performance on the cross-maze across all 10 testing sessions as well as separately for the start (first 3 sessions) and end of testing (last 3 sessions). As evident, post-surgery and prior to enrichment all rats in both lesion groups performed poorly irrespective on the lesion size (the minimum lesion size was set at > 50%). Both post-enrichment and at 120 days re-test again no associations were observed between performance and lesion size (see Table 7 , except a significant negative correlation between the size of the lesion sustained by the SC-ATN rats and the performance of these animals on the first 3 sessions of the cross-maze conducted at 120 days post-surgery. Overall, there was no indication that enrichment effect could just be explained by the different distribution of lesion sizes across groups.

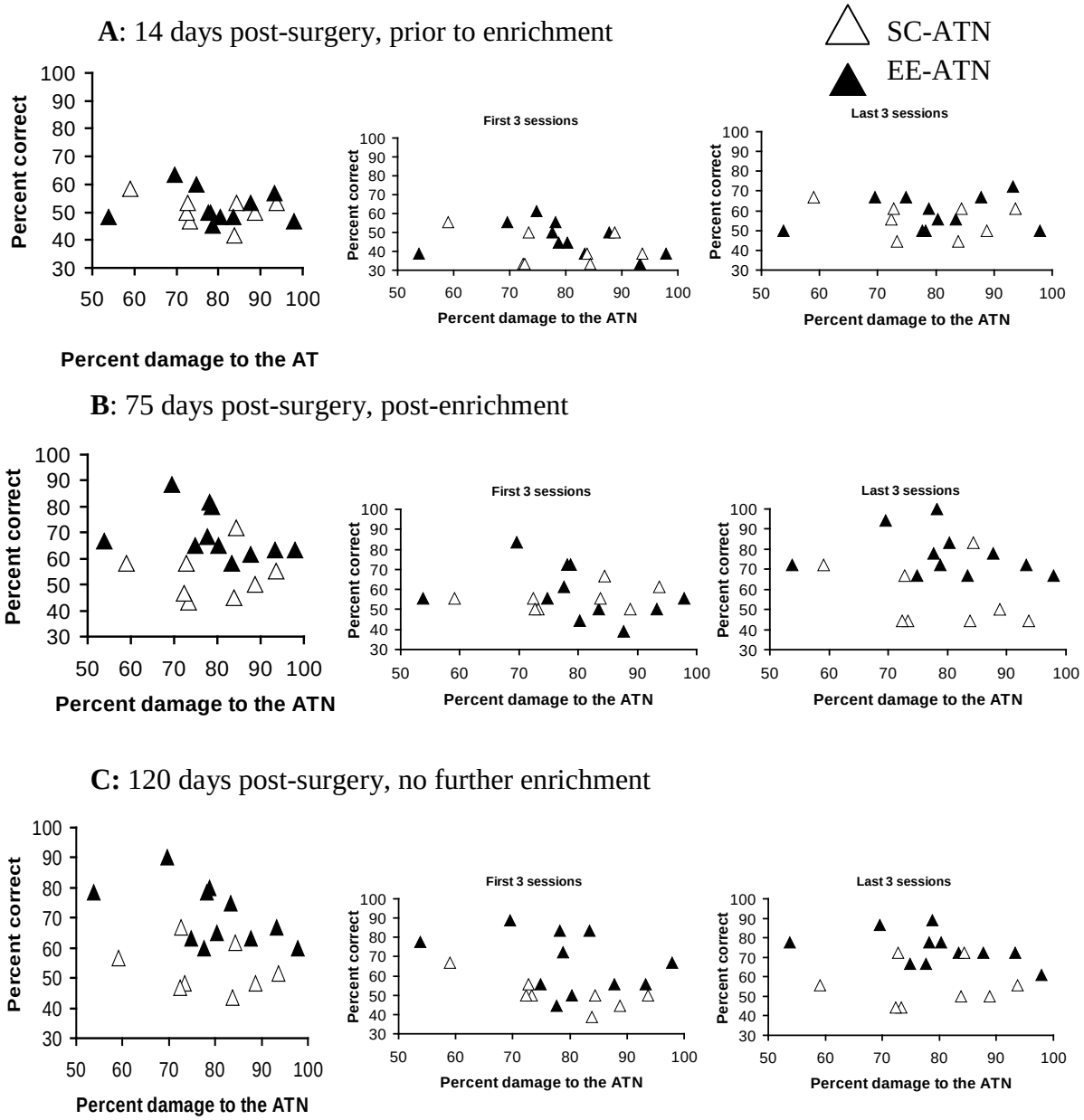


Fig 25. Delayed Enrichment, Spatial Working Memory performance and Lesion Size. Scatterplots depicting the mean percent correct responses on the cross-maze as a function of the extent of the bilateral damage sustained to the ATN at three time intervals **A**: at 14 days post-surgery testing across all 10 sessions and separately for the first and last three sessions; **B**: at 75 days post-surgery and post-enrichment testing across all 10 sessions and separately for the first and last three sessions and **C**: at 120 days post-surgery and after a period of no further enrichment across all 10 sessions and separately for the first and last three sessions. ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.

Mean percent correct responses on the cross-maze	Correlation with SC-ATN lesion size *df* = 6	Correlation with EE-ATN lesion size *df* = 9
14 days post-surgery sessions	-0.35	-0.12
First 3 sessions	-0.33	-0.29
Last 3 sessions	-0.28	0.21
75 days post-surgery and post-enrichment sessions	0.02	-0.37
First 3 sessions	0.33	-0.36
Last 3 sessions	-0.31	-0.23
120 days post-surgery and after no further enrichment sessions	-0.24	-0.55
First 3 sessions	**-0.74**	-0.34
Last 3 sessions	0.04	-0.47

Table 7. Delayed Enrichment, Spatial Working Memory and Lesion Size correlation matrix. Correlation matrix between the average lesion size for the SC-ATN and EE-ATN groups and Mean percent correct responses across all 10 sessions of 14 days post-surgery; 75 days post-surgery post-enrichment and 120 days post-surgery with no further enrichment performance on the cross-maze provided separately across all 10 testing sessions as well as for the first and last three sessions of each testing period. Bold highlights significant correlations at $p < 0.05$.

7.4 Discussion

The current experiment provided new evidence of the long-term nature of spatial working memory impairment when rats are housed in standard conditions, despite preoperative training and repeated post-operative testing on three occasions over a 4-month period. It is, therefore, of considerable interest that 30 days of enriched housing, despite it being delayed until 40 days post-surgery, markedly ameliorated severe and otherwise permanent spatial working memory impairment. It was especially remarkable that, on the spatial working memory tests conducted after the enrichment period, there was little overlap for the last three sessions between the performance of individual rats with ATN lesions that were housed in standard conditions and that of rats with ATN lesions that experienced an enriched environment. It is also important that treatment gains for spatial working memory were maintained at four months post-surgery, despite no further enrichment. This evidence is particularly encouraging, especially as surprisingly few studies have examined similar delayed-enrichment effects. Our data show that a 40-day delay before enrichment still produced substantial functional gains in spatial working

memory after neurotoxic ATN lesions. Moreover, these benefits remained at 4 months post-surgery, despite a period of no further enrichment. While the ATN rats housed in standard conditions remained close to chance performance overall, the ATN group with prior enrichment continued to show impressive gains, although the spatial working memory performance in this enriched ATN group was below that of the sham groups, which continued to improve towards optimal performance.

The current experiment provided evidence of recovery from an otherwise prior and sustained deficit, as opposed to sparing of the emergence of a deficit. However, the enriched ATN rats were clearly impaired at the start of testing, so that treatment and task-relevant training were required in combination for optimal functional gains. In the current experiment, the enriched ATN group achieved significantly better than chance levels of performance on both types of trials on the cross-maze, including the "opposite start position" trials that depend on the utilisation of spatial/directional cues, so the effect of enrichment was not based simply on an improved use of an egocentric strategy or just the inhibition of this non-spatial strategy.

In the previous enrichment experiment (see Chapter 6), it was speculated that the failure of ATN animals to demonstrate any enrichment induced recovery on the task of spatial pattern separation in the radial maze was partially due to the enrichment effect "wearing off" since the radial-maze testing was conducted 75 days following a period of continuous enrichment. Here, the rats were tested 35 days after a period of continuous and when no further (even 1.5 to 2 hours enrichment occurred), yet, clear enrichment effects were still observed. Although the enriched ATN rats did not reach the level of performance of controls, they were still significantly superior in their abilities to solve the maze on both allocentric/direction type and engocentric trials in comparison to the standard housed ATN rats. While the period of delay post-continuous enrichment was shorter (35 in the current experiment versus 75 days in the first experiment) the fact that no enrichment at all (versus limited enrichment) occurred during that time and behavioural effects were still observed suggests that the failure to observe enrichment effects on the previous radial-arm maze task was probably not entirely due to enrichment protocol. While a possible extension to the current study would be to implement even longer post-enrichment delay times, the impact of procedural task differences on disparent results between cross-maze and

radial-maze tasks should also be considered (see Chapter 10 for further discussion of procedural issues).

The current findings contribute to our understanding of the processes of brain plasticity. A dominant view in the field of recovery has been that unless some recovery is produced immediately after injury, little or no gains would be made with the passage of time (see Stein, 1994 for review). However, there is no principle of neuronal and behaviour organization that demands that functional and behavioural recovery must occur immediately post-injury or not at all. In fact, it seems that recovery may have a longer time course and gains can be made even when behavioural deficits are well in place. Several theories have been postulated in attempt to explain the mechanisms of brain recovery. A remote lesion effect or diathesis theory (Monakow, 1914 cited in Stein, 1994) argues that a lesion can produce a state of "depression" of function in other brain cites morphologically connected to the lesioned area. It is suggested that with the passage of time the suppressed state of functioning dissipates, and inhibited functions that are mediated by undamaged structures slowly re-emerged. The theory implies that behavioural recovery can be tied to the extent to which neural "shock" dissipates. The ATN damage is known to be associated with widespread limbic system dysfunction (Jenkins, et al., 2002b; 2004) and the recovery observed here encourages speculation that enrichment may influence one or more of the various interconnected neural systems that subserve spatial memory (Aggleton, et al., 2000). For instance, dramatic, ATN lesion-induced reductions in c-*fos* activity are found in the granular retrosplenial cortex (Jenkins, et al., 2004) and the same pattern can be seen after hippocampal lesions (Albasser, et al., 2007). Similarly, it has been suggested that the stimulation of acetylcholine release in the hippocampus is decreased following thalamic injury (Savage, et al., 2003, Savage, Roland & Klintsova, 2007) and even that the volume of the hippocampus itself may be subtly diminished following diencephalic damage (Kopelman, et al., 2003). Evidence that enrichment induces changes in the hippocampal formation (most notably in the recent literature, neurogenesis; Olson, Eadie, Ernst & Christie, 2006) and the prefrontal cortex (Del Arco, et al., 2007) is consistent with a hypothesis that the enrichment effects in this study may be promoting changes in multiple relevant brain regions, which is resulting in improved performance observed.

In recent years, some investigators (Stein & Hoffman, 2003) have taken a different position on recovery and suggested that recovery should not be evaluated on the basis of whether the goal is reached (end analysis) but rather than on the basis of how that goal is reached (means analysis). In their review of the impact of enrichment on recovery Will and colleagues (2004) pointed out that "means" analysis is rarely conducted in enrichment studies which raises the question whether there is clear evidence of real recovery (reappearance of behaviours identical to those in the premorbid states) exists. Stein and Hoffman (2003) suggest that an exaggerated appearance of recovery frequently occurs because the tests used may be insensitive to the nature of residual deficits. In the current experiment, we have been able to analyze in more detail the strategies the rats used to solve the cross-maze by separately examining the performance on the "same start position" and "opposite start position" trails. Enriched ATN rats improved on both egocentric "same start position" trials and allocentric/direction "opposite start position" type trials on the cross-maze. Egocentric learning might represent a compensation mechanism since egocentric abilities are seen to be independent of ATN function (Aggelton, et al., 1995a; Warburton, et al., 1997). However, allocentric-type ability is believed to be heavily reliant on the function of ATN and is seen as a reflection of hippocampal-type ability. The ability of enrichment to promote better performance on both types of trials may be indicative of reappearance of premorbid behaviour or recovery.

The suggestion that "enrichment-enhanced" performance on allocentric trials represents reinstitution of a premorbid state is based on the assumption that performance on the "opposite start position" trails reflects allocentric spatial memory specifically. It should be noted that the rats are able to solve the cross-maze by relying on a variety of strategies, such as response, place and direction, and ability to utilise a direction strategy or flexibly switch between the types of strategies may allow the rats to accomplish the task successfully (Skinner, et al., 2003). In fact, Skinner and colleagues (2003) argued that direction and place strategies may be difficult to separate and that conditional learning may underlie both direction and place learning. In both types of problems, rats learn "if at Position A, then make a right turn; if at Position B, then make a left turn" and hence relying on direction only strategies may be sufficient to allow the rats to solve the maze. It is also possible that direction and place learning share a common mapping mechanism. In fact, both direction and place

learning were found to be hippocampus mediated (Stringer, Martin, & Skinner, 2005) and both place cells (O'Keefe & Dostrovsky, 1971) and head direction cells (Taube, Muller, & Ranck, 1990) have been identified in the hippocampal formation (Cacucci, Lever, Wills, Burgess, & O'Keefe, 2004). Hence, it is possible that to some degree enrichment is promoting compensation by encouraging the rats to either effectively utilize direction strategies or switch direction and place strategies to solve the maze. The possible impact of enrichment on ATN animals' ability to utilise predominantly allocentric information and arguably promote re-institution of allocentric memory requires further investigation by using tasks that directly provide a measure of rats' ability to use overlapping spatial representations to guide novel allocentric responses (see Chapter 10 for further discussion).

In summary, the current study represents a second replication of the enrichment induced recovery on spatial working memory tasks following ATN lesions. The results also demonstrate that enrichment can still exert its beneficial effects even when its introduction is delayed post-surgery and the behavioural gains obtained continue to be maintained in the long-term, even when no further enrichment is occurring.

Chapter 8

Effects of Cerebrolysin on recovery of function after anterior thalamic lesions

8.1 Introduction

Chapters 6 & 7 presented strong evidence that environmental enrichment can enhance recovery of function after anterior thalamic lesions, at least on the spatial working memory task in the cross-maze. However, in both cases when enrichment was introduced either immediately post-surgery or delayed for 40 days after surgery, the treated rats remained impaired relative to the intact controls. Similar outcomes have been observed in other lesion studies which employed post-operative enrichment, and where improvement but not complete recovery of functioning has been noted (Dalrymple-Alfrod & Kelche, 1985; Dalrymple-Alford, et al., 1988; Donovick et al., 1973; Einon, et al., 1980; Galani, et al., 1997; Pacteau, et al., 1989; Will, et al., 1983). The absence of full restitution of function raises the question of whether other types of therapeutic intervention may be more effective.

Different potential pharmacological treatments that can promote recovery of functioning after brain damage are constantly being investigated, with most of the agents being investigated in connection with age-related cognitive deterioration. In contrast to enrichment, which may not be easily applied to humans, the pharmacological treatments would be easier to administer and afford strict control procedures. As was reviewed in Chapter 5 acetylcholinesterase inhibitors so far have been the most well-investigated compounds postulated to slow down the rate of cognitive decline in neurodegenerative disorders (Kaduszkiewicz & Hoffman, 2008). Other avenues of research have explored anti-oxidants, anti-inflammatory drugs and statins as potential compounds which can enhance the cognitive function of the ageing brain (Lleo,

Greenberg & Growdon, 2006). Drugs that exert neuroportection have also been suggested to have potential beneficial effects in neurodegenerative disorders (Becker & Greig, 2008). In Chapter 5 a review of the pharmacological properties of the drug Cerebrolysin was provided and it was discussed that it represents a potentially effective neuroprotective compound which can promote recovery of function after brain lesions. It has the advantage of being non-toxic and easy to administer. Of relevance to the current research are several reports of Cerebrolysin's effectiveness in promoting recovery of function on the water maze task following lesions to the components of the extended hippocampal system, such as the hippocampus and the fimbria-fornix (Francis-Turner and Valouskova, 1996; Koroleva, et al., 1999). The ability of Cerebrolysin to improve spatial memory after fimbria-fornix and hippocampal damage, as well as the observation that Cerebrolysin may delay cognitive decline in Alzheimer's dementia patients, who will also have anterior thalamic pathology (Braak & Braak, 1991), make it a viable candidate for therapy after anterior thalamic lesions

The purpose of the present study was to determine if Cerebrolysin influenced the deficits in a cross-maze performance caused by damage to the anterior thalamus. The spatial working memory performance of the animals was assessed following ATN lesions and Cerebrolysin administration. Cerebrolysin (2.5mg/kg) was administered intraperitoneally daily for 30 days post-surgery starting at 48 hours after surgery. Previous lesion studies have been able to demonstrate that longer periods of administration (30 days rather than 14 days) produce more robust behavioural changes (Francis-Turner, et al., 1996). The dose of 2.5mg/kg has been documented to be most effective in producing recovery when administered soon after the fimbria-fornix lesions (Valuskova & Francis-Turner, 1996).

8.2 Materials and Methods

8.2.1 Animals

Forty-four female PVGc hooded rats were used (5-7 months old, weighing between 158-201g at surgery). Body weights were restricted to 85-90% of free-feeding weight throughout the experiment, except free food access around the period of surgery, during immediate recovery and the period of differential treatment.

8.2.2 Housing

All rats were housed in standard housing (group) conditions of 3 or 4 rats per opaque plastic cage (see Chapter 6 for dimensions) throughout the experiment.

8.2.3 Surgery

The anaesthesia procedure was modified from that described in Chapter 6, in response to veterinary recommendations and with an aim to increase survival rates. The rats were anesthetised with ketamine (75 mg/kg i.p.) followed by domitor (1 mg/kg i.p.). This was supplemented by subcutaneous mepivacaine (3 mg/kg) and ketofen (0.50 mg/kg). To reverse the effects of domitor, antisedan was administered following completion of surgery (5 mg/kg i.p.).

Otherwise the same surgery procedure was followed as described in Chapter 6. The lesion coordinates and the volumes of NMDA injected were as per Chapter 7.

8.2.4 Drug Application

Commercially available drug Cerebrolysin (no dilution; EBEWE Arzneimittel, Austria; Cerebrolysin was kindly supplied by Dr Moessler of EBEWE) was used for treatment of experimental animals. Cerebrolysin was injected intraperitoneally at a rate of one injection (2.5ml/kg) per day (all rats were weighed daily prior to injection) for 30 days starting at 48 hours post-surgery. The control groups received an intraperitoneal injection of saline of 2.5ml/kg per day.

The animals were assigned randomly to Saline or Cerebrolysin groups following surgery. The group numbers were: SAL-SHAM n = 9; CERE-SHAM n = 8; SAL-ATN n = 13; CERE-ATN n = 14 (see Lesion Evaluation for details).

8.2.5 Apparatus and behavioural testing

Spatial working memory was tested using a cross-maze configuration as described in Chapter 6. All rats were trained to criterion on the spatial working memory task prior to surgery. The pre-

surgery testing started with testing with the “opposite start position” for the sample and test runs for half of the 6 trials per session, which continued until the rats reach the criterion of 85% correct for two consecutive sessions (requiring between 10-16 sessions). Following surgery and completion of 30 day drug administration the rats were re-tested on the same task for 10 consecutive sessions.

8.2.6 Histology

The same histology procedure as detailed in Chapter 6 was used.

8.3 Results

8.3.1 Lesion Evaluation

Analysis of lesion sizes (see Fig 26) revealed that none of the animals in both lesion groups reached the criterion of at least 50% damage to the ATN. The median and range for CERE-ATN was 28% (15-45%) and for SAL-ATN 31.5% (19-44%). As all rats failed to reached the lesion criterion none of the rats were excluded from the final sample. Evaluation of lesions revealed that in the majority of lesions the placement of the infusion was in the correct position, however the size of the lesion was too small to meet inclusion criteria. It is suspected that exitotoxic effects of NMDA were counteracted by the presence of Ketamine which is the NMDA receptor antagonist, and which was used for anaesthesia in the present experiment. On pilot surgery it was noted that the size of the lesion was smaller than ordinarily observed when pentobarbitone was used as an anaesthetic, but still within the acceptable margin, this however was not the case when all lesions were examined at the completion of the experiment. Later experiments in our laboratory revealed that lager volumes of NMDA infusion and a higher concentration of NMDA were able to counteract the effects of Ketamine and produce acceptable lesions.

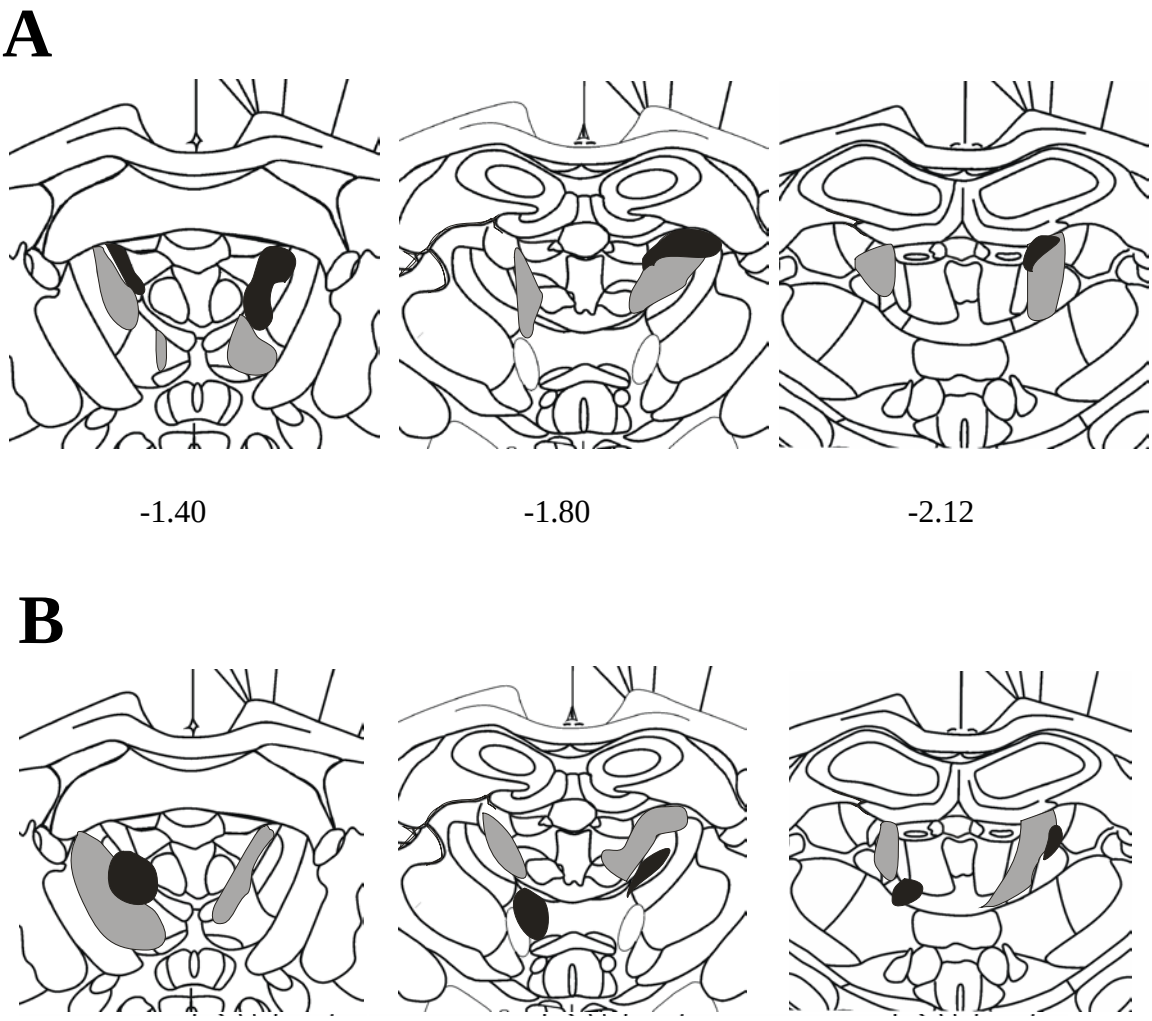


Fig 26. Cerebrolysin and Lesion size. Schematic representation of the largest (grey) and smallest (black) lesion in the **A**: Cerebrolysin ATN group and **B**: Saline ATN group. Numbers indicate distance from bregma in millimetres (Paxinos & Watson, 1998).

8.3.2 Non-matching to sample spatial working memory

Pre- surgery training - Figure 27 shows spatial working memory performance in terms of overall percent correct, combined across both trial types (i.e. irrespective of the "same start position" and "opposite start position" trials) before surgery. Performance in all four groups of rats was equivalent at the end of 10 days of training taken back from the last 2 days when 85% criterion was reached (F's < 1).

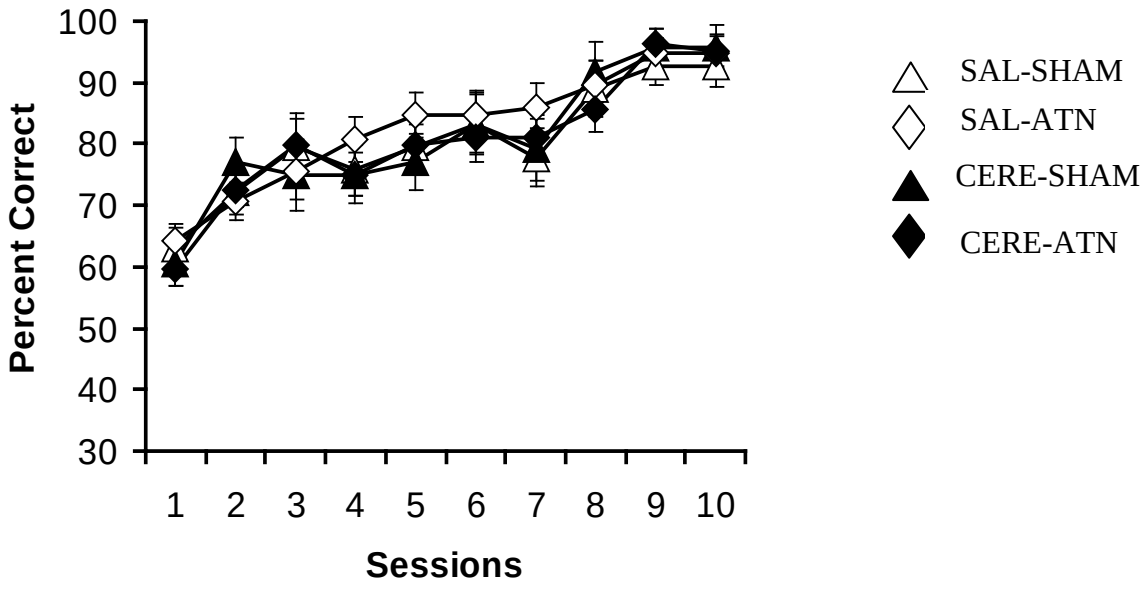


Fig 27. Cerebrolysin and Spatial Working Memory performance (pre-surgery). Mean (± SEM) percent correct responses for the last 10 sessions of pre-surgery performance taken back from the 2 last sessions of training when 85% criterion was reached on the spatial working memory task in the cross-maze configuration. ATN = neurotoxic lesion of the anterior thalamic nuclei; SAL = injected saline; CERE = injected Cerebrolysin.

Post-surgery and treatment testing – Post-treatment performance is depicted in Figure 28. Due to the small lesion size, the SAL-ATN lesion group did not demonstrate a typical spatial working memory impairment which had been observed in previous experiments. A mild significant effects for Lesion ($F_{(1,40)} = 4.64$; $p < 0.05$) was detected, which suggests that despite the lesions being small the performance of lesioned animals was still negatively affected, which seemed to be particularly evident on the first session of training (Lesion by Session interaction $F_{(9,360)} = 2.00$; $p < 0.05$). When the first session was analysed separately, a clear Lesion effect emerged ($F_{(1,41)} = 15.19$; $p < 0.001$). Despite the CERE-ATN demonstrating a slightly better performance than the SAL-ATN, no treatment effect was detected for the first session. As overall, across 10 sessions the SAL-ATN performed at a level comparable to the CERE-ATN and no treatment effect has emerged ($F < 1$). Cerebrolysin also had no affect on the performance of control rats, with no significant differences being observed between SAL-SHAM and CERE-SHAM groups. There was an effect for Sessions which reflects the rats' ability to acquire the task over time ($F_{(9,360)} = 6.43$; $p < 0.0001$). A significant effect for Trial

Type ($F_{(1,40)} = 5.86$; $p < 0.05$) reflecting that rats found the "opposite start position" trials more difficult to complete and a Session by Trial Type interaction also emerged ($F_{(2,80)} = 37.64$; $p < 0.001$).

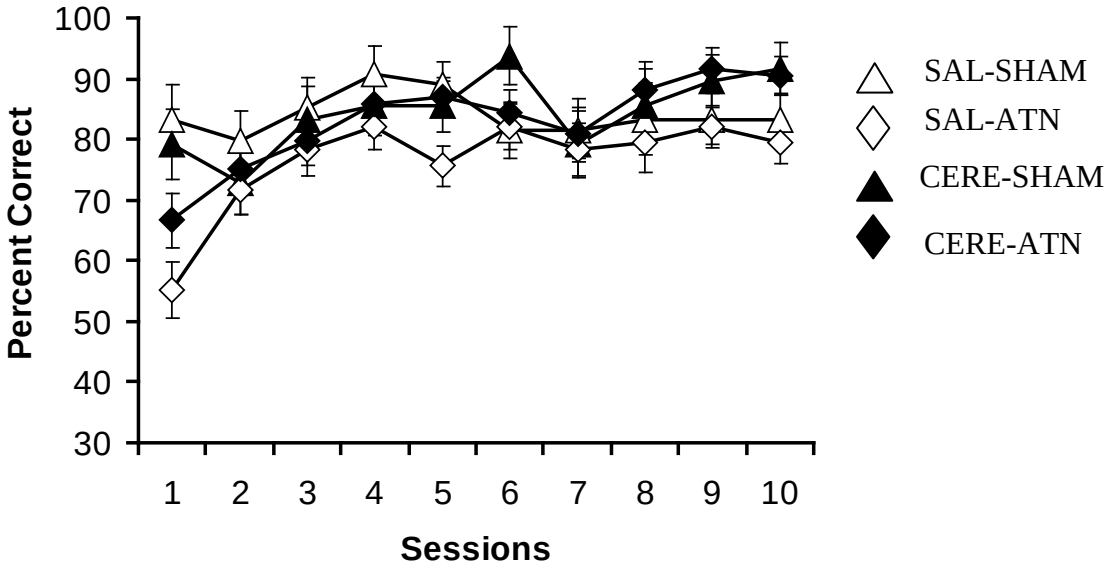


Fig 28. Cerebrolysin and Spatial Working Memory performance. Mean (± SEM) percent correct response on the 10 sessions of post-surgery, post-treatment training on the spatial working memory task in the cross-maze configuration. ATN = neurotoxic lesion of the anterior thalamic nuclei; SAL = injected saline; CERE = injected Cerebrolysin.

Differences between Cerebrolysin treated and un-treated groups emerged over the last three days of training, which suggested that drug administration had some beneficial effect on the spatial working memory performance, particularly, the ability to complete the more difficult "opposite start position" trials (see Fig 29). This was confirmed by the presence of mild significant effect for Treatment ($F_{(1,40)} = 5.73$; $p < 0.05$) and a Treatment by Trial Type interaction ($F_{(1,40)} = 5.12$; $p < 0.05$) when just the last 3 sessions were analysed. The Lesion effect did not reach significance ($p = 0.77$).

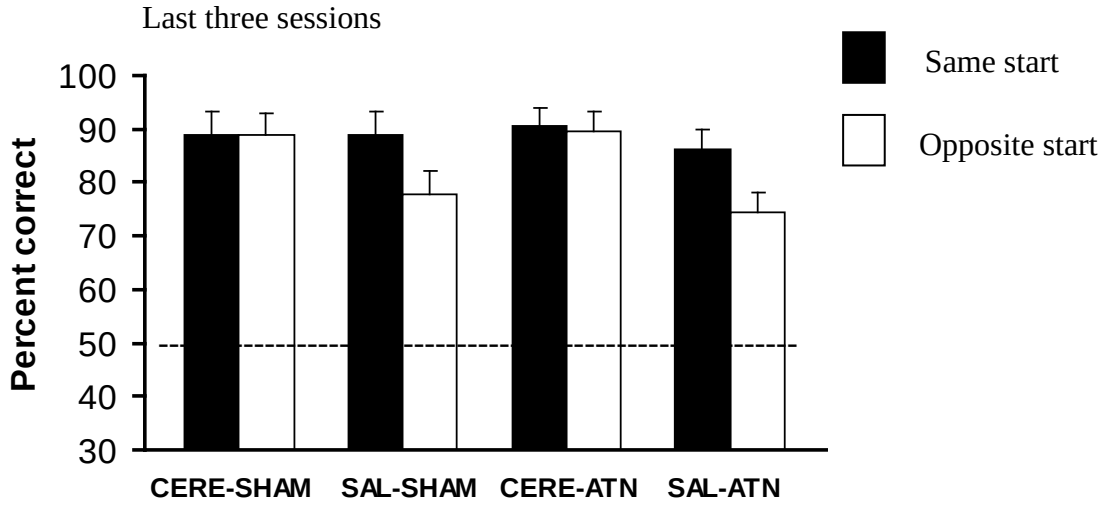


Fig 29. Cerebrolysin and Spatial Working Memory performance by trial type. Mean (± SEM) percent correct responses for the last three sessions of the cross-maze task post-surgery and following Cerebrolysin administration expressed separately for the "same start position" trials (same start position used for both sample and test run per trial) and "opposite start position" trials (opposite start position used for the test run); SAL = injected saline; CERE = injected Cerebrolysin. --- = 50% chance line.

8.3.3 Individual performance, lesion size and spatial working memory

Figure 30 shows the individual spatial working memory performance for the CERE-ATN (n = 14) and SAL-ATN (n = 13) rats across all 10 sessions, as well as separately for the first three and the last three days on the spatial working memory test conducted immediately after the 30 day period of drug administration as a function of lesion size. As evident from the scatterplot the variation in the lesion size had little effect on performance, with all animals performing well on the task. The correlations were as follows for the SAL-ATN: (for the first three sessions: r = 0.25; the last three sessions: r = 0.14 and for all 10 sessions r = 0.25, df = 11, all p's > 0.5) and for the CERE-ATN (for the first three sessions r = -0.35; the last three sessions: r = -0.41; and for all 10 sessions: r = -0.31, df = 12, all p's > 0.5).

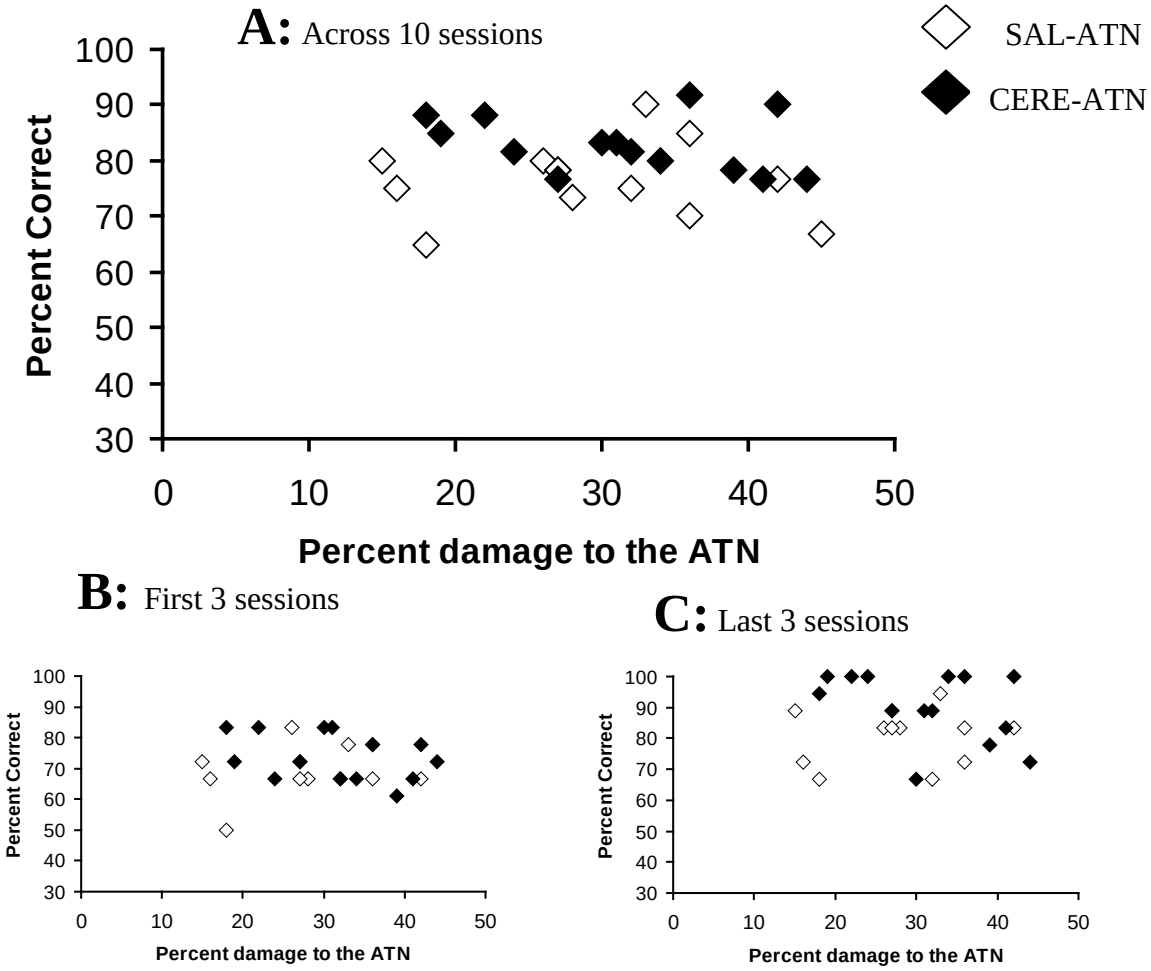


Fig 30. Cerebrolysin, Spatial Working Memory performance and Lesion Size. Scatterplots depicting the mean percent correct responses on the cross-maze as a function of the extent of the bilateral damage sustained to the ATN (**A**) across all 10 sessions; (**B**) for the first and (**C**) and last three sessions. ATN = neurotoxic lesion of the anterior thalamic nuclei; SAL = injected saline; CERE = injected Cerebrolysin.

8.4 Discussion

Unfortunately, due to the small lesion sizes, no clear conclusion as to the therapeutic effectiveness of Cerebrolysin could be drawn. Most of the ATN lesion animals were able to perform well on the spatial working memory task in the cross-maze demonstrating at least 60% correct response rate across both “same” and “opposite start position” trials and overall means

of 80-90% correct. Some valuable insights could still be gained from the data obtained. By the end of testing there was some indication that Cerebrolysin was able to promote better spatial working memory functioning in all rats (sham or lesion) that underwent drug treatment, with the treated animals being able to show equal levels of performance on both easy "same start position" and more difficult "opposite start position" trails. These data are valuable and suggest that to some degree Cerebrolysin is able to alter the behaviour of intact rats and rats with minimal lesions.

Also, only a limited number of studies in the past have employed a control-Cerebrolysin group, with majority of studies using a saline-control group only (Rockenstein, et al., 2003; Ren, Sietsma, Qui, Moessler, & Finklestein, 2007; Valouskova & Francis-Turner, 1998). Previously (Valouskova & Gschanes, 1999) reported that intact, Cerebrolysin-treated rats demonstrated higher motor activity as reflected in faster swimming speeds than the saline controls, but showed no differences in their ability to solve the reference memory task in the Water Maze as expressed in escape latencies or trajectory length. Masliah and colleagues (Masliah, Armasolo, Veinbergs, Mallory & Samuel, 1999) reported no differences in intact rats' ability to solve the water maze irrespective of whether the rats received Cerebrolysin or Saline. The current results suggest that Cerebrolysin may possibly influence the memory function of the rats and their ability to utilize allocentric/direction type cues to guide their responses; however the treatment effect seems to be training dependent as it was only evident by the end of testing. It would thus be interesting to examine whether Cerebrolysin may benefit the performance of animals with more severe, lesion-induced impairments.

In Chapter 6 the use of inclusion criteria of at least 50% damage to the ATN was advocated, based on the premise that lesions of smaller size may be insufficient to produce behavioural impairments associated with anterior thalamic damage. The outcomes of the current study clearly demonstrate that when thalamic lesions fall below the 50% cut-off mark only very small impairments are observed. These data further highlight the notion that substantial damage to the ATN complex is required in order to produce severe and permanent behavioural deficits (Aggleton, et al., 1996; Byatt & Dalrymple-Alford, 1996).

Overall, due to the unexpected failure of the surgical procedure and consequent small lesion sizes observed in the ATN groups, no clear conclusions with regard to Cerebrolysin effectiveness could be drawn. However, the ability of Cerebrolysin to benefit the performance of intact and minimally impaired rats on the more difficult “opposite start position” trials on the cross-maze warrants further investigations into the drug’s effectiveness with rats with more severely impaired spatial working memory.

Chapter 9

Enriched environments and Cerebrolysin: recovery of function after anterior thalamic lesions

9.1 Introduction

This chapter documents the last of the experiments in this thesis which examine whether recovery of function can be elicited after damage to the anterior thalamic nuclei. Rats were exposed post-operatively to different therapeutic interventions, environmental enrichment, Cerebrolysin administration or the combination of the two. Rats were subsequently tested on the spatial working memory task that had shown robust effects of enrichment in the previous studies (Chapters 6 & 7). The current experiment contrasted the effectiveness of different modes of treatment and examined whether the combination of the two therapeutic strategies can result in cumulative beneficial effects on memory after anterior thalamic lesions. The experiment also explored some of the neural changes that might underlie the behavioural changes observed, by examining patterns of c-*fos* neuronal activation in selected brain regions at the end of behavioural testing.

Complete recovery of functioning in terms of spatial working memory performance was not observed in the previous experiments described in Chapters 6 & 7. In both studies the ATN lesioned enriched rats continued to be relatively poor at the start of training and although their performance on the working memory task reached 60-80% accuracy by the end of testing, it was still significantly below the 90-100% accuracy demonstrated by sham controls. The absence of full recovery observed posed the question of whether enrichment can be further augmented by introduction

of another therapeutic strategy, such as pharmacological treatment, and whether the pharmacological treatment alone may be more effective than enrichment.

In the experiment detailed in Chapter 8, an attempt was made to examine the effectiveness of the drug Cerebrolysin in ameliorating memory impairment after ATN lesions. As reviewed in Chapter 5 Cerebrolysin represents a unique compound that has neurotrophic activity similar to that of naturally occurring growth factors. In comparison to the naturally occurring growth factors it has the important benefit of being effective when administered peripherally. Cerebrolysin has been used to promote recovery in various animal models of degenerative diseases (see Chapter 5) as well as after acute brain lesions. It has been shown to ameliorate anterograde memory deficits after damage to the fimbria-fornix (Francis-Turner & Valouskova, 1996; 1999) and CO_2 induced hippocampal damage (Koroleva, et al., 1997). This evidence may be of particular relevance to ATN lesions given the presumed role ATN plays in extended hippocampal system (see Chapter 3). Unfortunately, when the effectiveness of Cerebrolysin alone was examined after ATN lesions (Chapter 8) no clear conclusions could be drawn as the ATN lesion size was consistently too small, but some valuable insights were gained. The administration of Cerebrolysin to either sham or ATN lesioned rats resulted in improved performance of these animals on the more difficult “opposite start position” trials on the cross-maze by the end of training. The presence of beneficial Cerebrolysin effects in intact and only minimally affected rats suggests that it is worth investigating its effectiveness in ATN rats with complete lesions.

In the current experiment rats that sustained a lesion to the ATN were pseudo-randomly assigned to four groups. One group (SC-CERE) was housed in groups of 3 or 4 rats in standard cages and received daily intraperitoneal injections of Cerebrolysin (Cere) for 30 days post-surgery starting at 48 hours after surgery (as per the recommendation of Dr H. Moessler, EBEWE Pharmaceuticals Ltd., leading investigator of Cere, personal communication, 20 December, 2006). A second group was exposed to the combination of treatments (EE-CERE); these rats were housed in enrichment for 30 days starting 5 days after surgery and also received intraperitoneal Cere injections for 30 days starting at 48 hours after surgery. The third group (EE-SAL) began housing 5 days after surgery in an enriched environment for 30 days and

received only vehicle injections for 30 days starting at 48 hours after surgery. Finally, a group of ATN lesioned rats received no active therapeutic intervention and was housed in standard cages and was injected intraperitoneally daily for 30 days with saline only. Only one comparison control group was used, which received sham surgery and was housed in standard conditions, as well as received vehicle injections for 30 days at 48 hours after surgery. The design therefore focused on interventions in rats with ATN lesions (4 groups) and compared their performance to that of a single intact control group. Following completion of the 30-day therapeutic intervention (enrichment, Cere, or enrichment plus Cere) all rats were exposed to 10 sessions of re-testing on the spatial working memory task in the cross-maze.

The current study also examined the possible treatment effects when memory load was increased. Rats were tested over 4 sessions in which a 40-s delay interval was imposed between the sample and the test phase of the trial, rather than the usual 5 to 10 seconds interval. Previous studies suggest that the impairment of ATN rats in the standard T-maze task ("same start position" all trials) is increased as the delay interval increases (Aggleton, et al., 1995a; 1996; Warburton, et al., 1997).

In Chapter 4 evidence was discussed that enrichment increases gliogenesis, neurogenesis, synaptogenesis, cortical dendritic morphology and various biochemical and neurotrophic factors (Kempermann, et al., 1997; van Praag, et al., 2000), any of which may influence behaviour. In Chapter 5 it was noted that Cere administration can also promote neurogenesis, synaptogenesis and prevent apoptosis (Reinprecht, et al., 1999; Tatebayashi, et al., 2003; Windholz, et al., 2000). The brain regions responsible for the enrichment (or potentially Cerebrolysin) induced improvement after ATN lesions are not known. It is possible that enrichment influences changes in unrelated regions, for example, prefrontal cortex (Kesner & Rogers, 2004) or the neurocircuitry directly influenced by ATN lesions. As a first approach to this question, this study examined whether enrichment or Cerebrolysin influence some of the c-*fos* neural changes associated with ATN lesions.

In Chapter 3 evidence was presented that lesions to the ATN disrupt the pattern of c-*fos* neuronal activation most markedly in the retropslenial cortex (Albasser, et al., 2007; Jenkins, et al., 2002a; 2004). Hypoactivation was observed in

the superficial layer especially, as well as with the passage of time in the deep layers of granular and dysgranular cortex of the retrosplenial cortex (Poirier & Aggleton, 2009). Given the close interdependence of the ATN and retrosplenial cortex (Sutherland & Hoesing, 1993) one can speculate that enrichment, and potentially Cere, may exert an influence on the level of activation in these areas of "covert pathology". To examine this possibility changes in the activity of the immediate early gene c-*fos* were mapped after ATN lesions and treatment administration. This gene was selected because it provides a general maker of neuronal activity (Dragunow & Faull, 1989) and because it has been specifically linked to mnemonic processes, including spatial memory (He, Yamada, Nakajima, Kamei, & Nabeshima, 2002; Herdegen & Leah, 1998; Tischmeyer & Grimm 1999). This study provides a first attempt to assess c-*fos* changes after recovery in ATN rats. The Fos levels were measured after the completion of treatment and behavioural re-testing phases. Home-cage control groups were not included in the current study, as task related changes in Fos levels were the main interest this first investigation into the neurobiological correlates of behavioural recovery after ATN lesions. Evidence from previous studies that assessed ATN-lesion induced Fos changes suggests that home-cage controls show changes only in the caudal retrosplenial cortex as well as elevated levels of c-*fos* in the primary motor and somato-sensory areas following ATN lesions (Jenkins et al., 2002a; 2004). If variations in c-*fos* in retrosplenial cortex reflect memory dependent changes, then there may be a correlation between c-*fos* levels and behavioural performance across ATN rats in the current experiment.

9.2 Materials and Methods

9.2.1 Animals

The experiment was replicated using two cohorts, with the same procedure being implemented across the two cohorts. There were twenty seven PVGc rats in the first cohort and fifty-one rats in the second. All rats were 5-6 months old and weighed 149-210 g at surgery. Testing occurred during the dark phase of the reversed 12-hr light cycle. Body weights were restricted to 85-90% of free-feeding weight for all testing conducted, with free access to food during surgery, recovery and treatment phases.

Prior to surgery all rats were housed in standard conditions of 3 or 4 rats per cage (as per the methods described in Chapter 6). Following surgery all rats were randomly assigned to different housing and treatment conditions. The sample sizes post-surgery were: Cohort one: n = 5 SC-SAL ATN; n = 6 SC-CERE ATN; n = EE-SAL ATN; n = 6 EE-CERE ATN and n = 5 SHAM. Cohort two: n = 9 SC-SAL ATN; n = 10 SC-CERE ATN; n = 10 EE-SAL ATN; n = 10 EE-CERE ATN and n = 3 SHAM.

Following lesion verification the final group numbers were n = 7 SC-SAL ATN (2 from cohort I); n = 6 SC-CERE ATN (3 from cohort I); n = 7 EE-SAL ATN (2 from cohort I); n = 6 EE-CERE ATN (3 from cohort I); and n = 8 SHAM (5 from cohort I). The rats that did not meet criteria of at least 50% damage to the ATN were excluded from the final sample. Unexpectedly, a large number of lesion failures occurred in both cohorts (see Table 9). The reason for lesion failure was unclear but could have possibly been due to problems with a faulty operation of the micro-injection pump and blockages of the Hamilton syringe. No differences in post-operative performance of the two cohorts were observed. The mean percent correct (±SEM) responses on the cross-maze for cohort one was 77.61 ± 3.99 and for cohort two 75.25 ± 3.34. A repeated measures *ANOVA* across 10 days of post-operative training confirmed no effect for cohort ($F_{(1,32)} = 0.206$; $p = 0.65$) or cohort by day interaction $F < 1$, with no obvious differences between individual groups. Consequently, the data for each subgroup/cohort were combined for analysis.

9.2.2 Surgery

Similar surgical procedures as described in the Chapter 6 were used. The anaesthetic was changed on veterinary recommendation to a freshly prepared Sodium Pentobarbitone solution – Private Formula No.1 (100mg/ml; ProVet). The lesion coordinates were slightly adjusted. For cohort one the following coordinates were used. For the AV lesion: the AP coordinates from bregma were −2.5 mm, laterality was ±1.65 mm from the midline and ventrality was −5.55 mm from dura. For the AM lesions the AP measures were −2.3 mm from bregma, laterality ±1.10 mm from the midline and ventrality −5.85 mm from dura. The NMDA volumes were 0.12 μl (AV lesion) and 0.10 μl (AM lesion). For cohort two of the experiment the AM site but

not the AV site was slightly adjusted: the AP measure from bregma was −2.4 mm; laterality ±1.12 mm from the midline and ventrality −5.80 mm from dura; 0.105 μl of NMDA was infused.

9.2.3 Housing and Drug Administration

The rats in the SC groups were housed in groups of 3-4 in standard cages. The rats in the EE groups were housed in groups of 10-11 in enrichment cages. The cage set up and dimensions were as per Chapter 6. The rats were assigned to EE or SC housing 5 days post-surgery recovery. Following a 30-day period of enrichment the rats in the EE group were re-housed in standard conditions of 3-4 rats per cage, with their cage mates from enrichment cages. All subjects (from standard and enriched cages) were then food deprived to 80–85% of free-feeding weight and underwent behavioural testing. During the time of behavioural testing only the rats from enriched cages were returned to an enriched environment overnight when the lights were on. The rats had free access to water while in enriched cages but received food only in standard cages following behavioral testing for the day.

Drug or saline administration began 48 hours post-surgery and continued daily for 30 days. The rats with ATN lesions were randomly assigned to Cerebrolysin or saline groups (see above) and all sham rats received saline injections only. Cerebrolysin (EBEWE Arzneimittel, Austria; Cere was kindly supplied by Dr Moessler of EBEWE) was injected intraperitoneally at a rate of one injection (2.5ml/kg) per day. The vehicle-only groups received an intraperitoneal injection of saline of 2.5ml/kg per day. All rats were weighed daily prior to injection.

9.2.4 Apparatus and Behavioural Testing

The acquisition and testing on the cross-maze procedure was as described in Chapter 6. Pre-surgery training began from the start, however, with the cross-maze configuration. Performance in all groups of rats was equivalent at the end of training prior to surgery. Post-surgery, each animal received six trials a day of testing for 10 consecutive days (delay between sample and test phase was 5 to 10 seconds). Two days later, the animals were tested for further 4 sessions in an identical manner,

except a delay phase of 40 seconds was introduced between the sample and test runs. During the delay phase the rat remained in the start position for 40 seconds.

9.2.5 Immunohistochemistry

Following completion of the behavioral testing the animals were divided into 4 squads balancing the distribution the rats from each experimental group with each across squad as far as possible for Fos processing. After a three day break following the 40-second delay testing, the first squad of rats (8-9 animals per squad) was re-tested on the cross-maze for three consecutive days with the standard (5 to 10 seconds) delay between sample and test phases. Following each rat's completion of the 6 test trials each day it was placed individually in a standard cage in a dark, quiet room for a period of 90 min to allow for the habituation to this new post-testing procedure to occur. The animals were then returned to standard (group) cages for feeding, prior to being placed in enriched environment cage overnight for the relevant groups. On the third day of this procedure, each rat was re-tested on the cross-maze as per usual and then confined in the holding cage for 90 minutes before being sacrificed. A 90 min post-testing delay is necessary for c-Fos protein expression. The remaining squads were tested in a similar fashion, with each squad beginning testing a day after the immunostaining was completed for the previous squad and until all the animals were processed.

Three types of staining methods were used: c-Fos, NeuN and cresyl violet (for the cresyl violet procedure see Chapter 6). All immunohistochemical procedures were conducted with assistance from Dr Mathieu Wolff.

After perfusion (see Chapter 6) the brains were removed and post-fixed for 4 hours in 4% paraformaldehyde followed by overnight 30% sucrose, and 50µm coronal sections were obtained using a vibratome. Consecutive sections were processed for standard cresyl violet staining, throughout the relevant thalamic region, bar one-in-five that was stained for NeuN. For c-*fos* staining, three sections, one in every consecutive 5 sections, were obtained in each of the following regions: the prefrontal cortex at the level of the prelimbic/infralmibic cortex; the ATN region; the dorsal hippocampal/anterior retrosplenial region and the posterior hippocampal/posterior retrosplenial region.

For NeuN histochemistry (the NeuN antibody is neuron specific), free-floating sections were rinsed several times in 0.1M phosphate buffered saline (PBS) containing 0.3% Triton X-100 (PBST) and treated with 0.5% H_2O_2 in PBST to inhibit endogenous peroxidase. The sections were incubated overnight with gentle rotation at room temperature with a mouse monoclonal anti-NeuN primary antibody (Chemicon, MAB377) diluted at 1:5000 in PBST. The next day, the sections were rinsed several times and then incubated for two hours with biotinylated horse anti-mouse serum (Vector Laboratories, BA-2001) diluted at 1:2000 in PBST containing 1% Horse Serum (Vector Laboratories, S-2000). After further rinses, the sections were incubated for two hours in 1:200 avidin-biotin-horseradish peroxidase complex (ABC elite, Vector Laboratories) and visualised using 0.001% diaminobenzidine in 0.0004% H_2O_2 PB 0.5M Tris solution. The reaction was stopped after 30 minutes by cold PB rinses and sections mounted on gelatine-coated slides and allowed to dry overnight at room temperature before coverslipping. Lesion extent was established as per Chapter 6 except that both Cresyl violent and NeuN sections were used for analysis.

For c-*fos* immunohistochemistry, free-floating sections were processed using the same procedures as for NeuN, but with a Fos rabbit polyclonal primary antibody (1:5000, Santa Cruz) and a goat anti-rabbit secondary antibody (1:1000; Jackson Immuno-Research) in 1% normal goat serum. Sections for c-*fos* were processed in squads, with brains from eight rats per squad comprised from rats form each of the groups to minimise variation in visualisation.

The cells were counted by using an in-house custom-designed computer program from photomicrographs of the regions of interests taken by Nikon Eclipse E-800 microscope equipped with Nikon digital sight DS-U2 camera interfaced with a PC. Counts of Fos-positive cells were made in a standard frame sample area (0.84 x 0.63 mm) using a x 10 objective. The region of interest was first identified and manually drawn using a computer program. The number of Fos positive cells in the drawn region that met the threshold criterion of the mean plus 3 SD grater than the grey values for that section were automatically calculated. The final counts were expressed as number of Fos-positive cells / area in μm^2.

A total of 11 regions were analyzed (see Fig 31 for examples of regions analyzed). At least three sections from each region of interest were counted, unless fewer sections (but never less than 2) were available due to accidental damage to the sections. The number of Fos-positive cells was expressed as a mean across sections counted. Sites were selected either because they had been implicated previously (Jenkins, et al., 2002a; 2004) or because they served as control cortical areas.

The cortical control areas comprised the somatosensory cortex (primary somatosensory area, Ssp) and the motor cortex area (primary motor cortex area MOp). Counts were taken across all layers in these two cortical regions.

Cytoarchitectonic subfields within the antero-dorsal part of hippocampal formation provided separate counts of the dentate gyrus (external blade; the inferior blade revealed almost no Fos staining), CA1 and CA3. The postero-dorsal and postero-ventral hippocampal counts were taken from the mid-AP level of the hippocampus and corresponded to approximately AP level -5.20 mm from bregma in Paxinos and Watson (1998). The border between the dorsal and ventral hippocampus corresponded to approximately the dorsoventral level -5.0 mm from bregma (Moser, Moser, Forrest, Andersen, Morris, 1995). The postero-dorsal counts included separate counts for the external blade of the dentate gyrus and dorsal part of CA1; the postero-ventral counts included separate counts for CA3 and ventral part of CA1. The subiculum complex was not included.

The primary cortical areas of interest included the prelimbic and infralimbic cortex, the anterior cingulate cortex (at the genu of the corpus callosum) and the retrosplenial cortex. The retrosplenial cortex (see Fig 3) as defined by Van Groen & Wyss (1990) can be divided into the rostral granular cortex (Rgb), dysgranular cortex (Rdg) and caudal granular cortex (Rga). Separate counts were made for all three subregions. In addition, Fos cells were counted separately in the deeper layers (lower III to VI) and superficial layers (layer II and upper III) of the granular Rgb and Rga as per Jenkins and colleagues (2004). This border is signaled by an abrupt change in cell size and packing density.

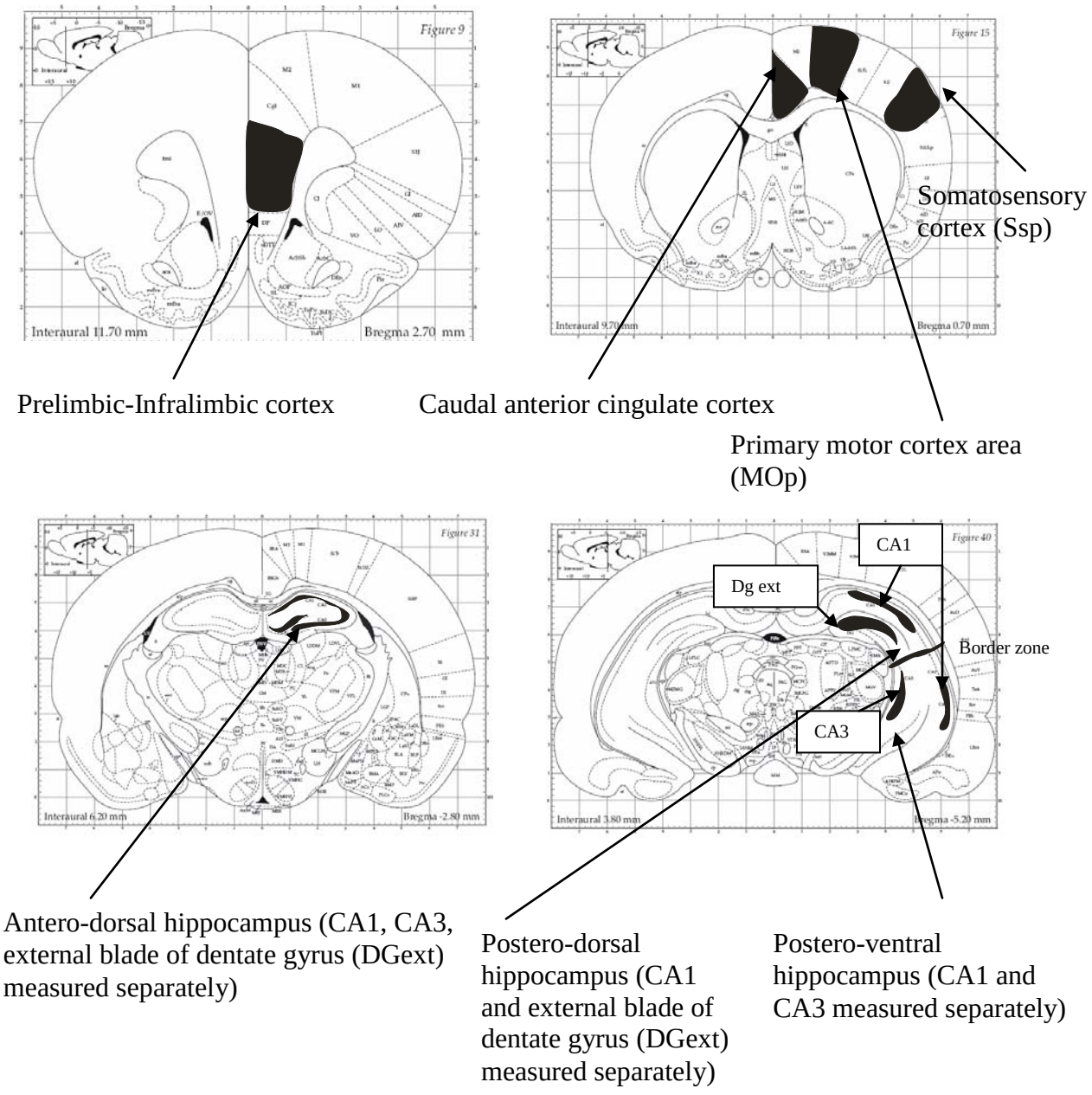


Fig 31. Diagrams of coronal sections indicating areas sampled for c-*fos* (from Paxinos and Watson, 1998).

9.3 Results

9.3.1 Lesion evaluation

Lesion criteria were as per described in Chapter 6. The largest and smallest acceptable ATN lesions across both cohorts are shown in Fig 32. Twenty-six ATN rats (7 SC-SAL ATN; 6 SC-CERE ATN; 7 EE-SAL ATN; 6 EE-CERE ATN) met the Histology inclusion criteria. The median lesion sizes (range) are depicted in Table 8. The lesions that failed to meet criteria are listed in Table 9. The volumes for these lesions were not calculated due to clear absence of damage to the ATN or presence of small unilateral damage only. The rejected lesions were evaluated visually by three independent raters (EL, MW & JDA) on the NeuN and cresyl violet slides. In successful lesions the damage sustained by the groups receiving an ATN lesion was comparable and no effects for Housing or Drug or Housing by Drug interaction were detected (all F's < 1). The groups also did not differ in the amount of damage sustained to the LT and the MT regions (no significant main effects or interactions, all F's < 1). Damage to other adjacent structures was minimal apart from damage to the interanteromedial nucleus (IAM). The lesion groups though did not differ in the degree of damage sustained to the IAM nucleus (all F's < 1).

9.3.2 Statistical analysis

The current experimental design included 4 lesion groups and one sham control group. A sequential approach to the analysis was employed. The aim of the first analysis was to provide an evaluation of each lesion group by comparison to the sham group. A 3-way *ANOVA* (Group x Session x Trial Type) across all 5 experimental groups was conducted, plus pair-wise planned comparisons between the control group and each of the four lesion groups were made, but using a Bonferroni adjusted p value of 0.0125 ($p < 0.05/4$). The main aim of this study was to compare the effects of two treatments (Enrichment and Cere) in rats with ATN lesions. This aim was addressed in the second analysis. For this second analysis the data from 4 lesion groups were analysed using a standard 2 x 2 design to examine the effects of Housing, Drug and their interaction.

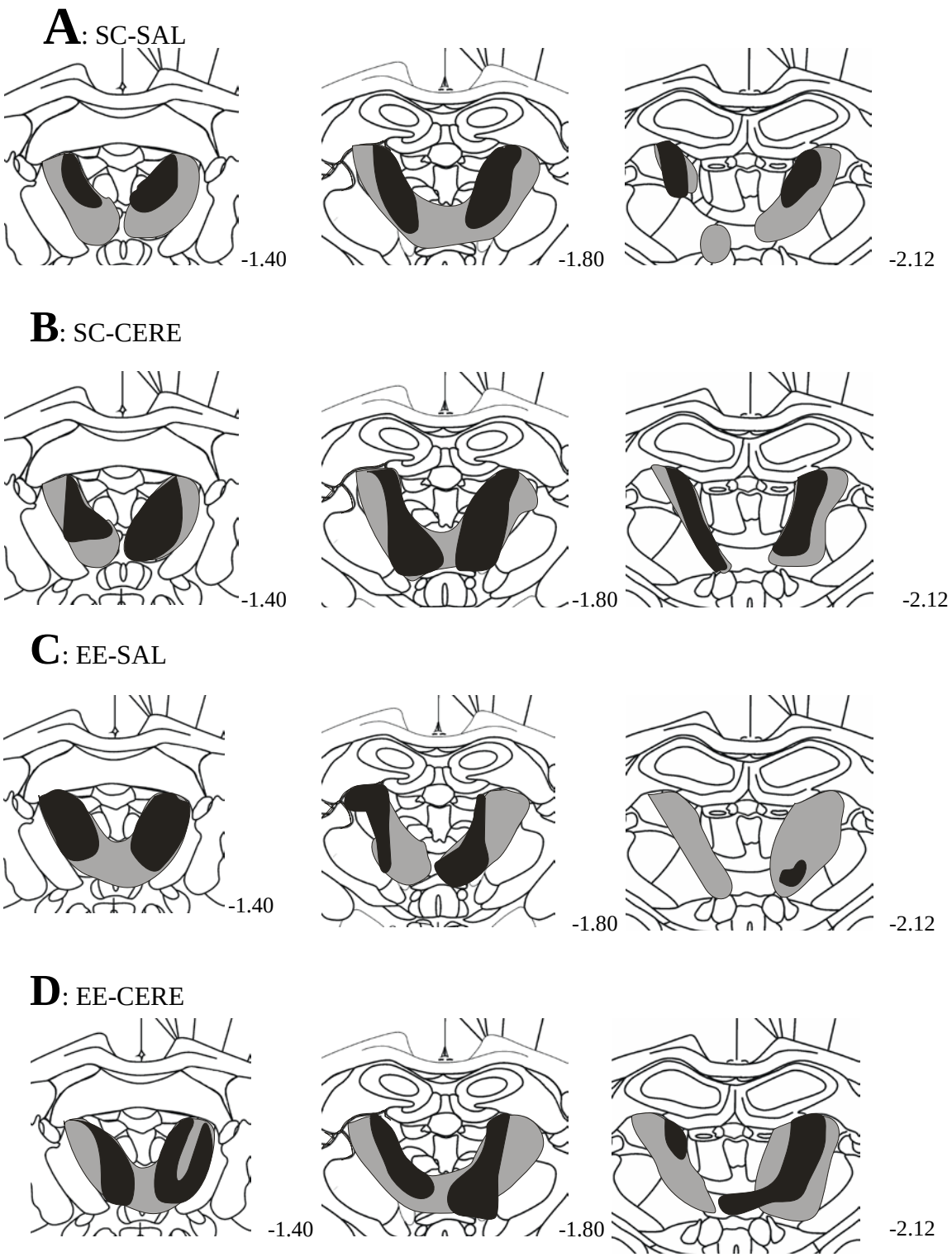


Fig 32. Enrichment, Cerebolysin and Lesion Size. Schematic representation of the largest (grey) and smallest (black) lesion in the four ATN lesion groups (**A**): Standard caging Saline ATN (SC-SAL); (**B**): Standard caging Cerebrolysin (SC-CERE) ATN; (**C**): Enriched environments saline ATN (EE-SAL); (**D**): Enriched environments Cerebrolysin ATN (EE-CERE). Numbers denote distance from bregma (Paxinos & Watson, 1998).

Table 8. Percent bilateral damage (volume) to selected areas for each of the rats in the Enrichment and Cerebrolysin study.

RATS	AT and components				Other Nuclei								
	AD	AM	AV	AT	LT	MT	IAM	LD	PT	PVA	PV/PVP	Re	Rh
SC-SAL ATN													
349	84.0	60.2	50.4	63.4	7.1	1.2	23.2	4.7	7.9	0.0	0.0	0.0	0.0
320	97.8	47.4	53.0	59.6	29.0	7.8	0.7	19.9	13.5	0.0	0.0	0.0	0.0
352	92.5	98.6	68.0	91.9	18.2	6.5	57.9	7.0	22.5	0.0	0.0	6.8	5.1
356	98.1	61.4	45.0	77.6	5.8	2.1	16.8	1.1	7.4	0.0	0.0	0.3	11.1
368	98.5	98.5	98.3	95.1	12.1	2.0	65.7	13.0	12.2	0.0	0.0	1.6	2.7
378	89.3	79.4	17.7	73.5	4.4	1.5	62.4	0.0	6.8	0.5	0.0	0.8	1.3
369	94.0	94.2	71.0	73.7	5.2	1.5	69.2	16.0	26.5	0.0	0.0	1.2	27.6
Median N=7	**94.0**	**79.4**	**53.0**	**73.7**	**7.1**	**2.0**	**57.9**	**7.0**	**12.2**	**0.0**	**0.0**	**0.8**	**2.7**
SC-CERE ATN													
379	96.0	80.1	87.9	85.0	9.8	1.2	49.3	10.0	24.5	0.0	0.0	0.0	0.0
382	94.8	87.3	54.9	86.0	14.4	5.6	24.5	5.8	4.9	0.5	0.0	0.6	0.8
374	98.9	97.9	97.0	92.3	35.6	7.2	74.7	23.6	11.0	0.7	0.0	0.4	10.2
344	85.3	76.3	18.0	73.5	4.4	1.5	15.5	0.0	3.7	0.2	0.0	0.3	2.1
335	94.3	84.2	53.0	69.2	34.0	6.0	29.7	18.1	13.6	0.0	0.0	0.0	0.0
324	98.2	82.0	84.1	72.9	14.4	3.1	91.8	8.6	11.4	0.0	0.0	0.4	8.6
Median N=6	**95.4**	**83.1**	**69.5**	**79.2**	**14.4**	**4.3**	**39.5**	**9.3**	**11.2**	**0.1**	**0.0**	**0.3**	**1.45**

RATS	AT and components				Other Nuclei								
	AD	AM	AV	AT	LT	MT	IAM	LD	PT	PVA	PV/PVP	Re	Rh
EE-SAL ATN													
316	94.0	88.1	58.8	79.9	23.3	13.7	91.1	10.7	24.5	0.7	0.0	0.2	13.7
325	93.0	73.7	66.0	73.5	23.6	9.3	44.8	8.2	24.0	0.0	0.0	0.0	0.0
373	67.4	60.5	44.6	61.4	3.7	0.8	10.6	0.0	5.4	0.2	0.0	0.0	0.0
389	91.8	91.4	93.2	92.0	14.9	14.3	60.5	9.8	37.2	1.5	0.0	3.3	0.1
380	95.1	83.0	85.2	82.5	13.9	2.2	48.2	7.0	10.2	0.0	0.0	1.2	1.6
375	80.6	90.0	65.2	85.1	10.2	0.7	46.2	0.0	9.4	0.0	0.0	2.5	2.0
386	96.5	98.6	69.8	91.0	27.6	6.4	53.1	25.1	12.2	0.0	0.0	1.1	1.7
Median N=7	**93.0**	**88.1**	**66.0**	**82.5**	**14.9**	**6.4**	**48.2**	**8.2**	**12.2**	**0.0**	**0.0**	**1.1**	**1.6**
EE-CERE ATN													
365	90.6	94.1	93.5	87.5	25.9	4.1	57.6	10.7	4.5	0.0	0.0	1.2	10.6
353	93.2	95.1	91.6	92.1	24.2	6.4	70.7	12.4	12.3	0.2	0.0	0.0	0.0
381	95.5	93.7	76.4	90.6	9.8	2.6	40.1	5.6	14.2	2.8	0.0	0.8	19.9
350	96.7	92.9	63.3	90.2	15.8	4.1	69.2	5.3	30.7	0.0	0.0	4.5	0.5
330	95.6	89.7	96.7	85.6	14.5	4.4	63.5	4.6	36.8	0.0	0.0	1.7	9.2
333	74.3	74.6	52.7	56.5	15.4	2.7	28.8	6.0	10.4	0.0	0.0	5.3	7.7
Median N=6	**94.3**	**93.3**	**84.0**	**88.8**	**15.6**	**4.1**	**60.5**	**5.8**	**13.2**	**0.0**	**0.0**	**1.4**	**8.4**

Abbreviations: AD= anterodorsal nucleus; AM= anteromedial nucleus; ATN= anterior thalamic aggregate; ATN median= median percent damage for all included ATN rats; AV= anteroventral nucleus; IAM= interanterodorsal nucleus; LD= laterodorsal nucleus; LT= lateral thalamic aggregate; MT= mediothalamic nucleus; PT= paratenial nucleus; PVA= anterior paraventricular nucleus; PV/PVP= paraventricular nucleus/posterior paraventricular nucleus; Re= reunions nucleus; Rh= rhomboid nucleus.

Table 9. Enrichment and Cerebrolysin and individual rats rejected from the lesion analysis.

RAT	**Reason for rejection**	**RAT**	**Reason for rejection**
SC-SAL		**EE-SAL**	
317	No lesion	327	No lesion
318	No lesion	328	No lesion
326	No lesion	340	Small unilateral lesion
348	No lesion	343	No lesion
359	Anterior to the ATN	377	No lesion
366	No lesion	383	No lesion
370	No lesion	385	Small lesion plus damage to the fornix
		387	No lesion
		392	No lesion
SC-CERE		**EE-CERE**	
323	Small unilateral lesion	338	Small unilateral lesion
336	Anterior to the ATN	339	Small unilateral lesion plus damage to the fornix
341	Posterior to the ATN	345	Small unilateral lesion
376	No lesion	347	Cortical lesion
384	Damage to MT, ATN intact	355	No lesion
388	No lesion	358	Anterior to the ATN
390	Too large plus damage to LT	362	Posterior to the ATN
391	No lesion	363	Small unilateral lesion
395	No lesion	364	Anterior to the ATN
396	Small unilateral lesion		

9.3.3 Non-matching to sample spatial working memory

Pre-surgery training: Figure 33 shows spatial working memory performance in terms of overall percent correct, combined across both trial types (i.e. irrespective of "same start position" and "opposite start position" trials) for the last 10 sessions of pre-surgery training taken back from the last 2 sessions when the 85% criterion was reached. Performance in all groups of rats was equivalent prior to surgery. None of the treatment groups differed from the sham group (all F's < 1) in the ability to perform the maze task. There was a significant effect for Sessions reflecting the rate of learning ($F_{(9,132)} = 31.91$; $p < 0.0001$). The 2 x 2 analysis also confirmed that there were no effects for Housing, Drug or Drug by Housing interaction (all F's < 1).

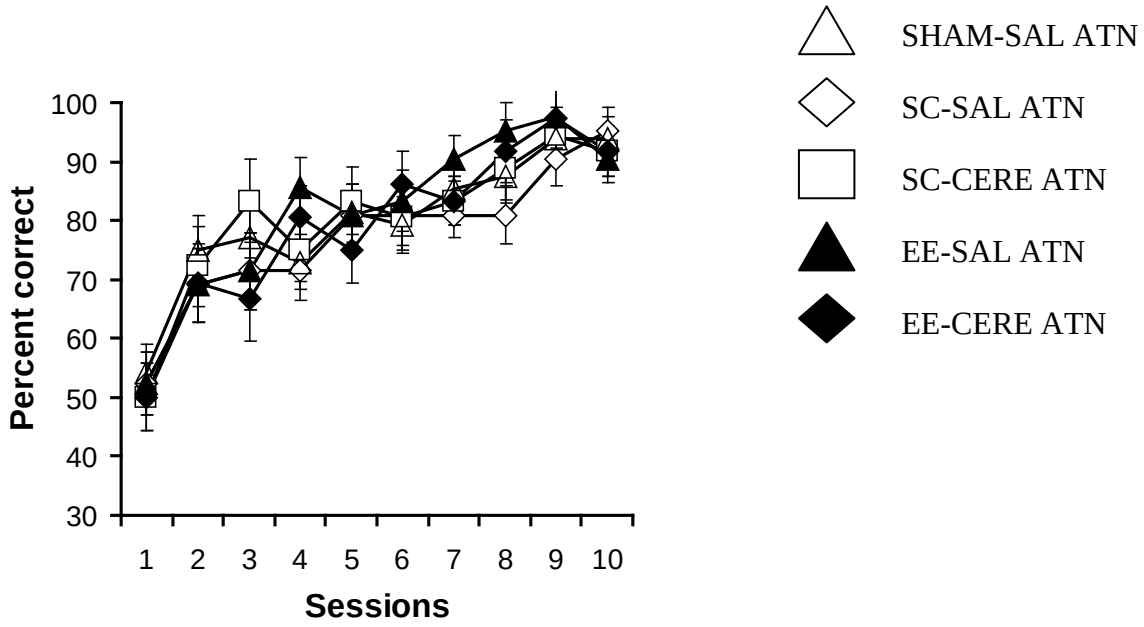


Fig 33. Enrichment and Cerebrolysin and Spatial Working Memory performance (pre-surgery). Mean (± SEM) percent correct responses for the last 10 sessions of pre-surgery training taken back from the 2 last sessions when 85% criterion was reached on the spatial working memory task in the cross-maze configuration. ATN = neurotoxic lesion of the anterior thalamic nuclei. SC = housed in standard group conditions; EE = enriched environment; CERE = administered Cerebrolysin; SAL= administered saline.

Post-surgery and treatment

Comparison to SHAM group across 10 days of training: As depicted in Figure 34, the sham group showed an initial reduction in performance after the post-operative differential housing/treatment period, but this group rapidly reacquired the task and achieved over 90% mean correct performance within 3 sessions. The SHAM group showed better mean performance than all four groups with ATN lesions. As expected the SC-SAL ATN group displayed poor spatial working memory performance throughout the 10 post-operative sessions, which was consistently near chance. None of the treatment groups were able to reach the level of performance of shams, but showed a superior performance to the non-treated ATN group. A 3-way *ANOVA* (Group x Session x Trial Type) performed across all 5 experimental groups revealed significant effects for Group ($F_{(1,29)} = 19.42$; $p < 0.0001$), Sessions ($F_{(9,261)} = 15.37$; $p < 0.0001$), and Trial Type ($F_{(9,261)} = 71.03$; $p < 0.0001$). No significant interactions were detected. Planned comparisons with Bonferroni adjustment across all ten

sessions and both trial types revealed that all lesion groups performed significantly below the level of sham controls (all *p*'s < 0.01).

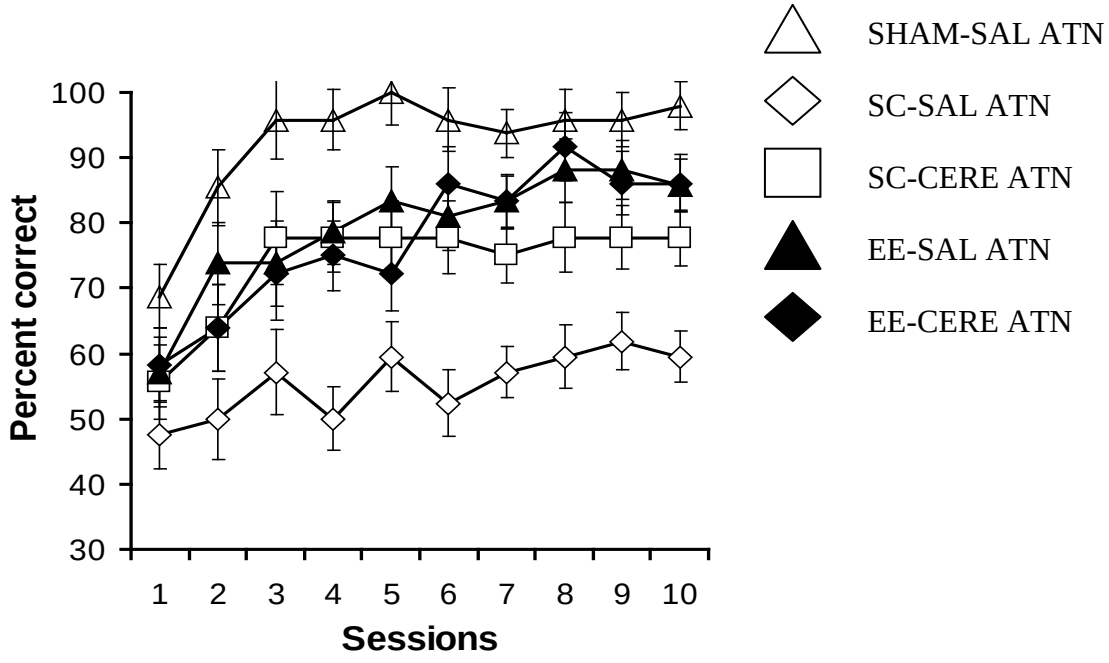


Fig 34. Enriched Environment and Cerebrolysin and Spatial Working Memory performance. Mean (± SEM) percent correct responses on the 10 post-surgery, post treatment sessions on the spatial working memory task in the cross-maze configuration. ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE = administered Cerebrolysin; SAL = administered saline.

Housing X Drug analysis in ATN groups across 10 days of training: The second analysis evaluated the contribution of different treatment modes to recovery. As depicted in Figure 34 exposure to any type of treatment, Cerebrolysin, enrichment or the combination of the two, resulted in improved performance of the ATN lesioned animals on the spatial working memory task. Living in enriched environment clearly had a marked positive effect on spatial working memory performance of the lesioned rats in comparison to living in the standard conditions (Housing $F_{(1,22)} = 14.44$; $p < 0.001$). A 30 day course of daily Cere injections also had a beneficial effect on behaviour of the lesioned animals in comparison to vehicle injections (Drug $F_{(1,22)} = 7.79$; $p < 0.05$). There was also however a significant Housing by Drug interaction ($F_{(1,22)} = 5.25$; $p < 0.05$) which reflected the fact that Cere administration benefited the standard but not enriched rats. *Post-hoc Neuman-Keuls* on data collapsed across all

sessions revealed that none of the three treatment groups differed significantly from each other (all p's > 0.05), although the rats that received Cere injections and housed in standard cages (SC-CERE) demonstrated an overall lower mean performance than the two enrichment groups towards the end of testing. There was a significant effect for Sessions reflecting an improved performance of all rats over time ($F_{(9,198)} = 9.72$; $p < 0.0001$) and a significant effect of Trial type ($F_{(1,22)} = 61.05$; $p < 0.0001$), reflecting the finding that all rats found the "same start position" trials easier to solve. No other interactions reached significance (all F's < 1.0).

Comparison to SHAM group across trial types: As in previous experiments, the performance of rats was also assessed across the two trial types on the first and last three sessions (see Figures 35 A and B, respectively). As previously observed, all rats found the "same start position" trials easier to solve. However, the standard housed lesioned rats given saline demonstrated a poorer level of performance on the "same start position" trials at the beginning of testing (Fig 35A). The analysis of the first three days of training across all 5 groups revealed a significant effect for Group ($F_{(4,29)} = 7.22$; $p < 0.001$) and Trial Type ($F_{(1,29)} = 29.34$; $p < 0.001$). Planned comparisons of the first three sessions with Bonferroni adjustment confirmed that, on the same start trials, the SC-CERE and especially the SC-SAL groups performed significantly worse than the sham group (both p's < 0.01). Interestingly, the two enrichment groups EE-SAL ($p = 0.04$) and EE-CERE group ($p = 0.1$) did not differ significantly in their performance on the "same-start position" trials compared to the sham group. All lesion groups showed poor mean performance in the first three sessions on the more difficult "opposite start position" trials, but variability in performance meant that on Bonferroni adjusted planned comparisons the SHAM-SAL group was significantly superior only by comparison to the performance of the SC-SAL and the SC-CERE groups, not the EE-SAL ($p = 0.12$) or the EE-CERE ($p = 0.02$). During the first three sessions the SC-SAL group performed at chance levels on both "same start position" and "opposite start position" trials ($t_{(6)} = 025$; $p = 0.25$) and ($t_{(6)} = 0.81$; $p = 0.81$) respectively. All three treatment groups performed significantly above chance on the "same start position" trials SC-CERE ($t_{(5)} = 6.84$; $p < 0.01$); EE-SAL ($t_{(6)} = 5.87$; $p < 0.01$); EE-CERE ($t_{(5)} = 5.33$; $p < 0.01$) but at chance level on the more difficult "opposite start position" trials SC-CERE ($t_{(5)} = 0.47$; $p = 0.65$); EE-SAL ($t_{(6)} = 0.93$; $p = 0.38$); EE-CERE ($t_{(5)} = -0.44$; $p = 0.67$).

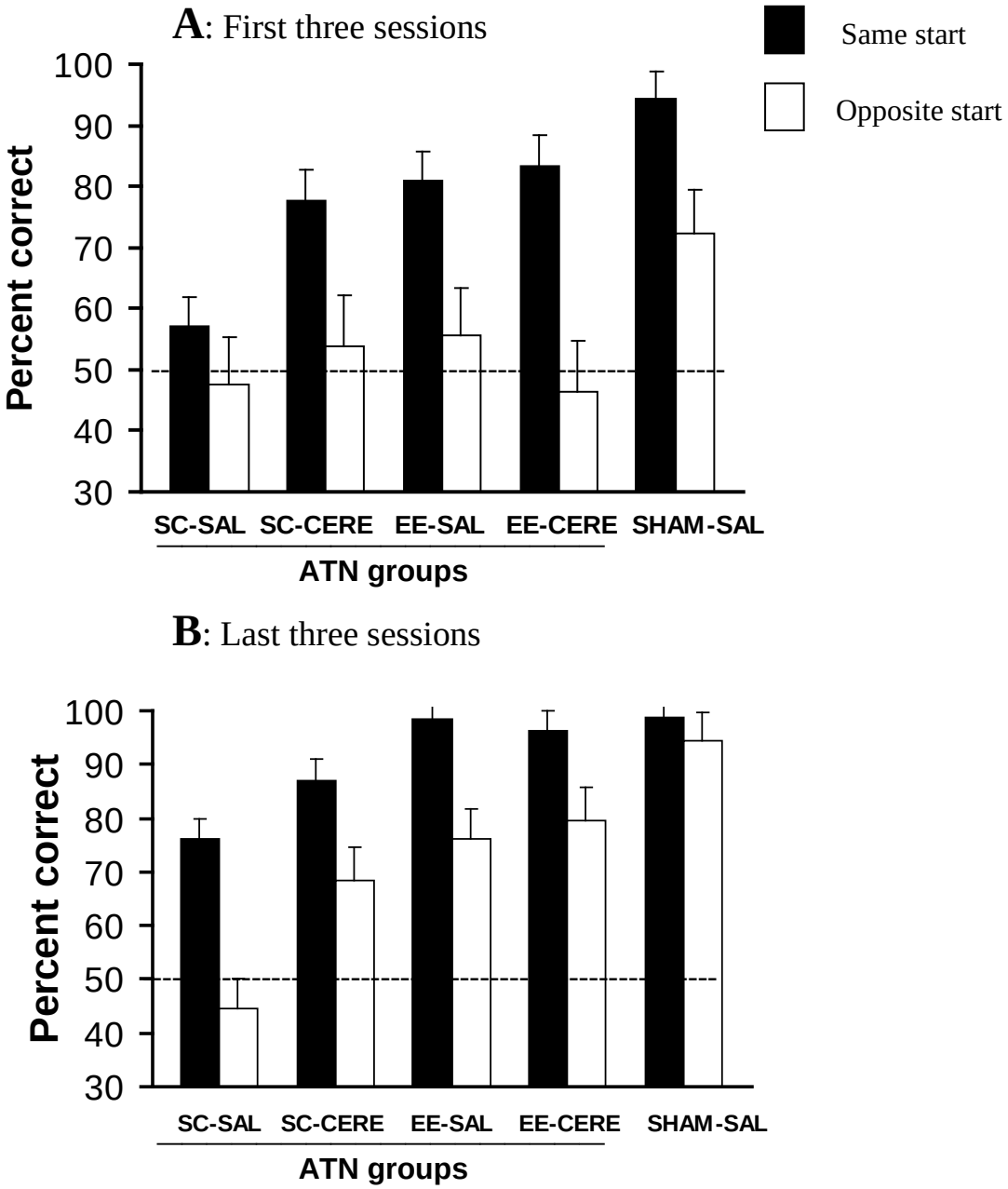


Fig 35. Enrichment and Cerebrolysin and Spatial Working Memory performance across trials. Percent correct responses on for the first three (**A**) and last three (**B**) sessions of the cross-maze task expressed separately for the "same start position" trials (same start position used for both sample and test run per trial) and "opposite start position" trials (opposite start position used for the test run). ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE = administered Cerebrolysin and SAL = administered saline. --- = 50% chance line.

At the end of training (last three sessions; Fig 35B) all animals improved in their ability to solve the easier "same start position" trials. More notably, shams as

well as all three lesion-treatment groups improved in their mean score on the more difficult "opposite start position" trials. In contrast, the SC-SAL group continued to show poor performance on the "opposite start position" trials despite their substantial improvement on the "same start position" trials. An *ANOVA* across all 5 groups for the last three sessions revealed a significant main effect for Group ($F_{(4,29)} = 17.67$; $p < 0.001$) and for Trial Type ($F_{(1,29)} = 39.52$; $p < 0.001$), but no other significant effects or interactions were detected. On the "same start position" trials, SC-SAL rats performed significantly worse than SHAM-SAL rats ($p < 0.001$), while all three treatment groups did not significantly differ from the controls. On the "opposite start position" trials, the SC-SAL ($p < 0.001$) and the SC-CERE ($p < 0.01$) groups performed significantly worse than controls, while the mean performance of both enriched ATN groups did not differ significantly from shams (*p* levels for the EE-SAL = 0.02; for EE-CERE = 0.07). On these last three trials all ATN groups performed significantly above chance levels on the "same start position" trials SC-SAL ($t_{(6)} = 4.26$; $p < 0.01$); SC-CERE ($t_{(5)} = 8.30$; $p < 0.001$); EE-SAL ($t_{(6)} = 30.50$; $p < 0.001$) and EE-CERE ($t_{(5)} = 19.76$; $p < 0.001$). All of the treatment groups also demonstrated a significantly above chance performance on the "opposite start position" trials by the end of testing SC-CERE ($t_{(5)} = 4.15$; $p < 0.01$); EE-SAL ($t_{(6)} = 5.13$; $p < 0.01$) and EE-CERE ($t_{(5)} = 5.58$; $p < 0.01$). However, the SC-SAL group remained at chance levels of performance on these more difficult trials ($t_{(6)} = -0.63$; $p = 0.54$).

Housing X Drug analysis in ATN groups across trial types: Analysis of the trial data during the first three sessions (Fig 35A) across just the lesion groups revealed that during the first three sessions all rats demonstrated better performance on the easier "same start position" trials in comparison to the more difficult "opposite start position" trials (Trial Type $F_{(1,22)} = 22.79$; $p < 0.01$). No other significant effects or interactions were detected (all *p*'s > 0.05), despite the poorer performance of the SC-SAL group on the "same start position" trails.

Analysis of the last three days of training (Fig 35B) showed that the standard saline ATN group (SC-SAL) was not significantly different from the three treatment groups on the "same start position" trials (all *p*'s > 0.05). However, the three treatment groups now showed superior performance on the "opposite start position"

trials, while the mean of the SC-SAL ATN group was below the chance level. Although, the CERE only group (SC-CERE) found it more difficult to solve both easy and difficult trials in comparison to the rats that were exposed to enrichment, the differences did not approach significance. The *ANOVA* for the last three sessions confirmed a significant effect for Housing ($F_{(1,22)} = 23.01$; $p < 0.0001$); Drug ($F_{(1,22)} = 5.47$; $p < 0.05$) and Trial Type ($F_{(1,22)} = 35.92$; $p < 0.0001$). A Housing by Drug interaction ($F_{(1,22)} = 4.70$; $p < 0.05$) supported the conclusion that Cerebrolysin improved performance in the standard housed ATN rats but was unable to further improve performance of the ATN rats housed in enrichment. No other significant interactions were detected.

Performance on the delay trials of the cross-maze

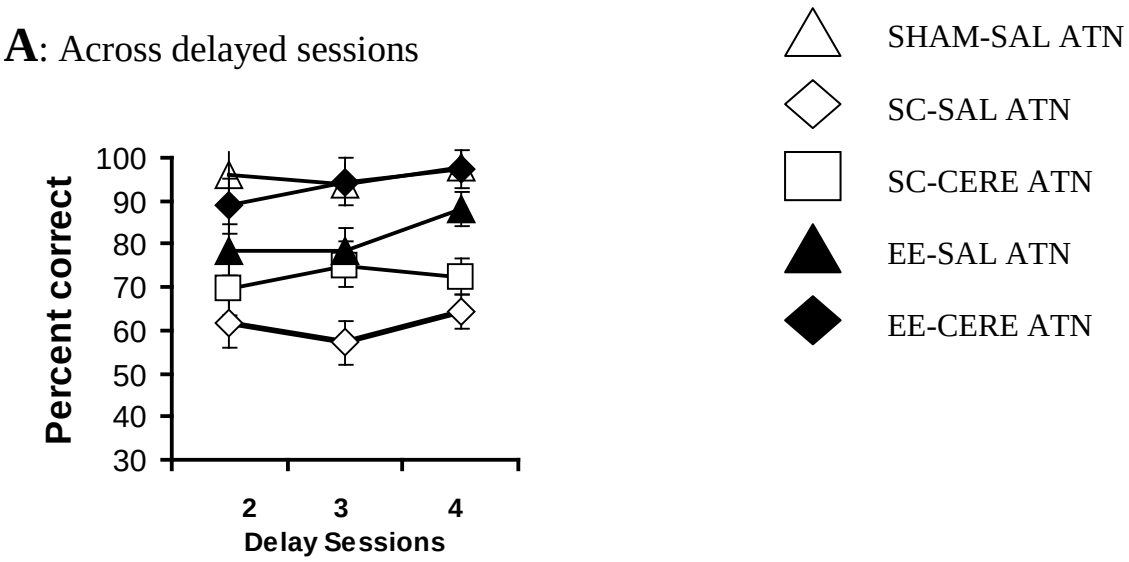
Comparison to SHAM group across delayed sessions: After completion of the standard 10 sessions of testing the animals underwent further 4 days of testing during which a 40-second delay was imposed between the sample and test runs. All groups showed a decline in performance on the first day of the 40-second delay introduction (SC-SAL mean = 50.0 ± 5.89; SC-CERE mean = 58.33 ± 6.37 EE-SAL mean = 76.19 ± 5.89; EE-CERE mean = 72.22 ± 6.37; SHAM-SAL mean = 85.41 ± 5.51) due to the change in testing procedure. Hence only the three last days of delayed testing were analysed to provide a clearer picture of between group differences (Fig 36A).

A beneficial cumulative effect of enrichment plus Cerebrolysin emerged. The mean performance of the EE-CERE group was equivalent to that of the SHAM-SAL group. A 3-way *ANOVA* (Group x Session x Trial Type) across all 5 groups revealed main effects for Group ($F_{(4,29)} = 18.07$; $p < 0.001$); Sessions ($F_{(4,58)} = 4.30$; $p < 0.05$) and Trial ($F_{(4,29)} = 51.92$; $p < 0.001$) and a Trial by Group ($F_{(4,29)} = 5.90$; $p < 0.01$) interaction. Both of the enriched groups did not differ from shams on both types of trials collapsed across three sessions (both p's > 0.05). On the contrary, the standard housing groups (SC-SAL and SC-CERE) performed significantly worse than the SHAM-SAL (both p's < 0.01) on both "same start position" and "opposite start position" trials (Fig 36B). All of the ATN treatment groups, including the SC-CERE group demonstrated the significantly above chance performance across both "same" and "opposite" start position trials (all p's < 0.05). The SC-SAL group performed

above chance levels on the "same start position" trials, ($t_{(6)}$ = 5.87; $p < 0.05$), but remained at chance levels on the "opposite start" trials ($t_{(6)}$ = - 1.86; $p = 0.11$).

Housing X Drug analysis in ATN groups of the delayed trails: Analysis of the lesion groups only across the 40-second delay trials (Fig 36A & B) showed that being exposed to any type of treatment, either Cerebrolysin or enrichment, improved rats' ability to solve the delayed trials, with significant effects for Housing ($F_{(1,22)}$ = 28.52; $p < 0.001$) and Drug ($F_{(1,22)}$ = 7.62; $p < 0.05$) being observed but no Housing by Drug interaction. There was a significant effect for Sessions ($F_{(1,22)}$ = 4.00; $p < 0.05$) reflecting a degree of task improvement across sessions. There was also a significant Trial type effect ($F_{(1,22)}$ = 42.05; $p < 0.001$) and a significant Trial type by Drug interaction ($F_{(1,22)}$ = 11.51; $p < 0.01$). *Post-hoc Newman-Keuls* comparisons revealed that although the standard housed groups performed somewhat worse than the enriched groups on the "same start position" trials the differences did not reach significance levels (all p's > 0.05). On the "opposite start position" trials, the SC-SAL group continued to be impaired and performed significantly below the level of all three treatment groups (all p's < 0.01). The most notable result was that with the introduction of the 40-s delay the effect of the Drug emerged with EE-CERE group now demonstrating relatively better performance on the "opposite start position" trials, primarily because the SC-CERE and the EE-SAL groups showed reduced performance on these trial types compared to their pervious performance, when the delay was 5-10 seconds. While the EE-CERE group performed better than the other two treatment groups on the "opposite start position" trials statistically the EE-CERE group was only significantly different from the SC-CERE group (p = 0.027).

In summary, exposure to either enrichment or Cerebrolysin on their own, soon after lesions to the ATN, resulted in improved performance of the rats on spatial working memory in the cross-maze. The improvement was evident on both "easy" "same start position trials" as well as more "difficult" "opposite start position" trials. On the more challenging task of 40-sec delayed spatial working memory, evidence of the possible beneficial cumulative effect of both treatment types emerged. On this task the EE-CERE group was able to demonstrate excellent performance on both "same" and "opposite start position" trials, performing at a level comparable to controls.



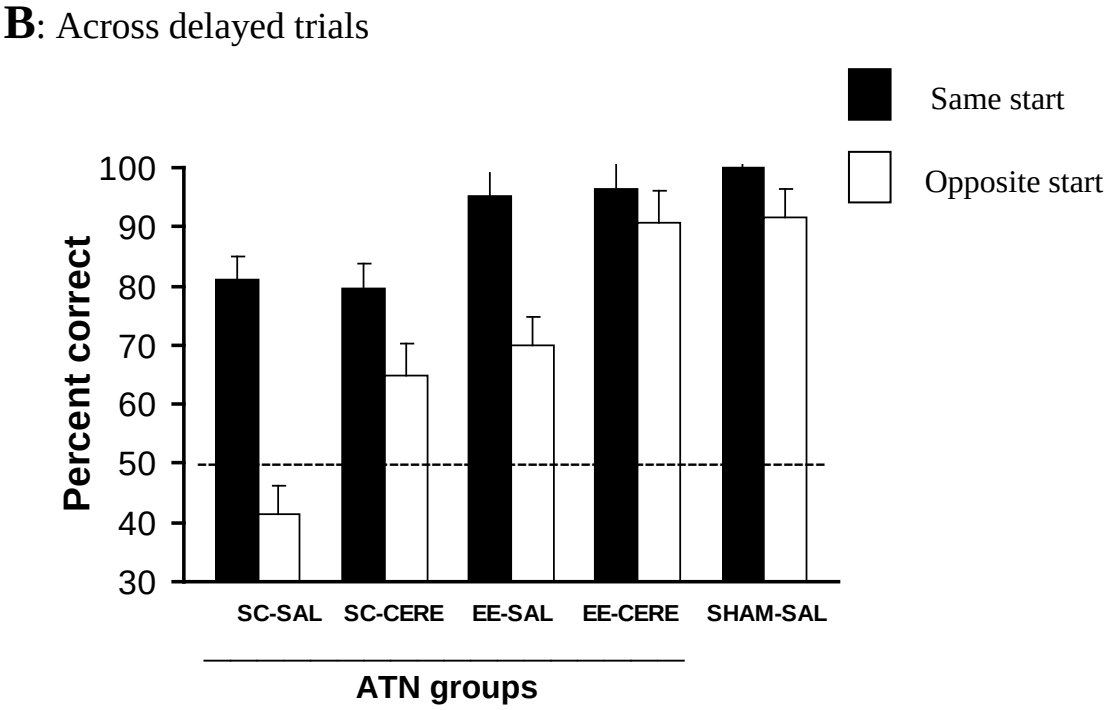


Fig 36. Enrichment and Cerebrolysin and Delayed Spatial Working Memory performance. **A**: Mean (± SEM) percent correct responses for the last 3 sessions of 40-s delay between sample-test runs on the cross-maze. **B**: Mean (+SEM) percent correct responses for the last 3 sessions of 40-s delay between sample-test runs expressed separately for the "same start position" trials (same start position used for both sample and test run per trial) and "opposite start position" trials (opposite start position used for the test run). ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE= administered Cerebrolysin; SAL= administered saline. ---- = 50% chance line.

9.3.4 Immunohistochemistry results

Prior to the c-*fos* procedure the animals were again re-tested over three days on the standard cross-maze task with 5 to 10-seconds delay between the sample and tests run prior to sacrifice on the last day. The performance of animals across these three days was very similar to that on the last three days of testing on the standard cross-maze task, despite the animals being exposed to delayed trials. The data for the 3 days of pre c-*fos* is presented in Fig 37A and B. Repeated measures *ANOVA* across all five groups revealed a significant Group effect ($F_{(4,29)} = 16.01$; $p < 0.001$), an effect for Trial Type ($F_{(4,29)} = 52.02$; $p < 0.001$) and Trial by Group interaction ($F_{(4,29)} = 3.20$; $p < 0.05$). As observed previously, all ATN treatment groups continued to perform significantly above chance levels on both types of trials (all p's < 0.05). The SC-SAL group performed above chance levels on the "same start position" trials ($t_{(6)} = 3.25$; $p < 0.05$), but remained not significantly different to chance in their performance on the "opposite start position" trials ($t_{(6)} = - 1.01$; $p = 0.34$). The immunohistochemistry results are presented by region.

Retrosplenial Cortex

Comparison to SHAM group: The ATN lesions produced a striking hypoactivity in parts of the retrosplenial cortex in comparison to that observed in sham animals, irrespective of whether or not the animals received any therapeutic intervention (see Fig 45 A; for examples of photomicrographs taken in the retrosplenial cortex. The micrographs presented for each given group are all taken from the same animal). As previous studies have highlighted that most marked changes in activation in the retrosplenial cortex following ATN lesions occur in the superficial cells layers of the Rgb and Rga regions the counts for superficial and deep layers of these regions (Jenkins, et al., 2002a; 2004; Poirier & Aggleton, 2009) were completed and analysed separately.

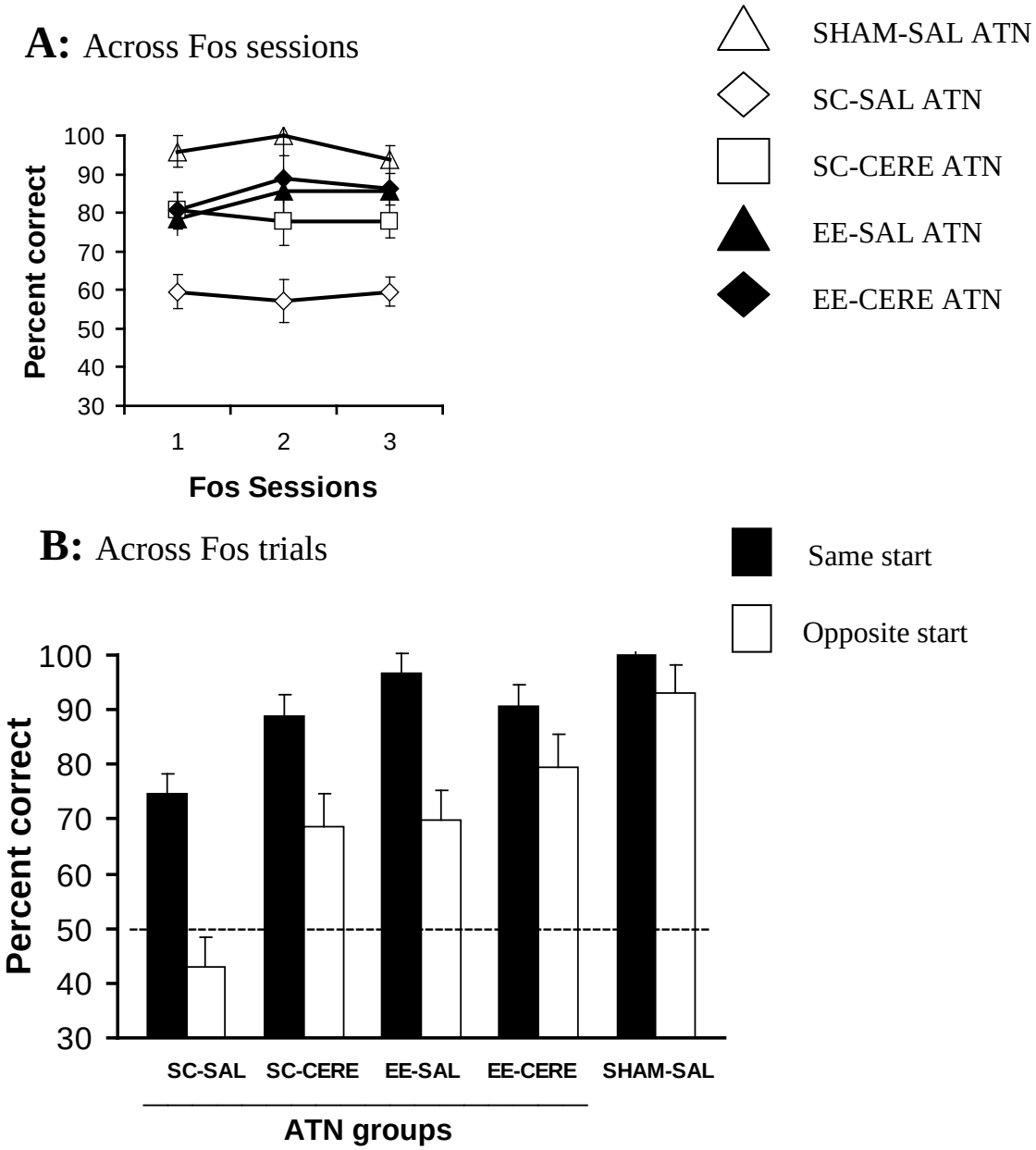


Fig 37. Enrichment and Cerebrolsyin and Spatial Working Memory performance on the pre-Fos sessions. **A**: Mean (± SEM) percent correct responses for the 3 sessions of Fos behavior activation on the cross-maze (sample-test delay of approximately 5 to 10-seconds). **B**: Mean (+SEM) percent correct responses for the 3 sessions of Fos behavior activation expressed separately for the "same start position" trials (same start position used for both sample and test run per trial) and "opposite start position" trials (opposite start position used for the test run). ATN = neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE= administered Cerebrolysin; SAL = administered saline. --- = 50 % chance line.

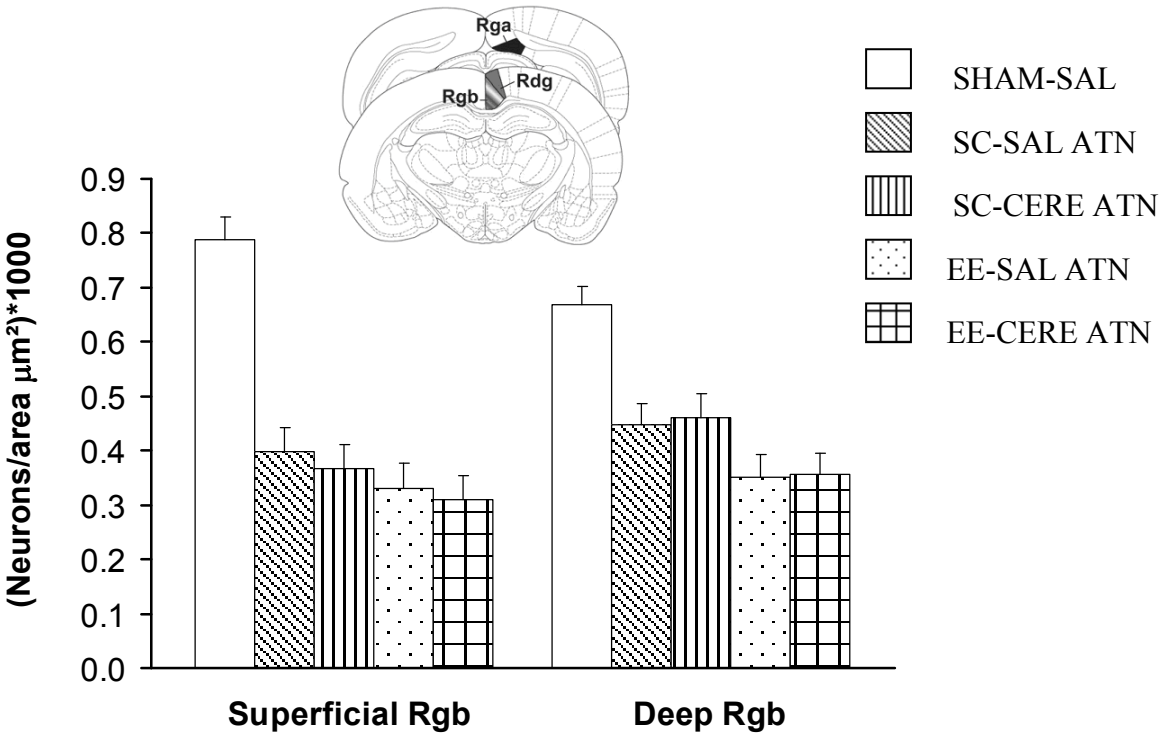


Fig 38. Enrichment and Cerebrolysin and Fos counts in the Rgb. Fos-counts (number of neurons/area µm²) in the superficial and deep cell layers of the rostral granular retrosplenial cortical area (Rgb). Data shown as mean (+SEM) number of neurons/area µm² counts multiplied by a 1000. ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE= administered Cerebrolysin; SAL= administered saline.

Striking cell loss was observed in the Rgb (rostral granular retrosplenial) in all lesion groups (Fig 38). ATN lesions induced hypoactivity in Rgb was detected in both superficial ($F_{(4,29)} = 21.90$; $p < 0.0001$) and deep layers ($F_{(4,29)} = 11.45$; $p < 0.001$) cell layers. Although the level of Fos activation in the Rgb of the SHAM-SAL group was slightly lower in the deeper layers than in the superficial layers, the sham rats still displayed comparatively much higher activation to the lesioned animals in both superficial and deep Rgb layers (all p's < 0.0125).

In the Rga (caudal granular retrosplenial) (Fig 39) region ATN lesions reduced Fos counts in the superficial layers ($F_{(4,29)} = 7.59$; $p < 0.001$). Planned comparison revealed that although the SC-SAL ATN's demonstrated lower counts this difference relative to the SHAM-SAL group did not reach the specified level of significance ($p = 0.02$). However, all three ATN-lesioned treatment groups demonstrated significantly reduced levels of activation in comparison to the SHAM-SAL (all p's < 0.001). No group differences were detected in the deep Rga layers ($F < 1$). It was notable that for the SHAM-SAL group the c-*fos* counts in the deep layers of the Rga were markedly lower than in the superficial cell layers of the Rga.

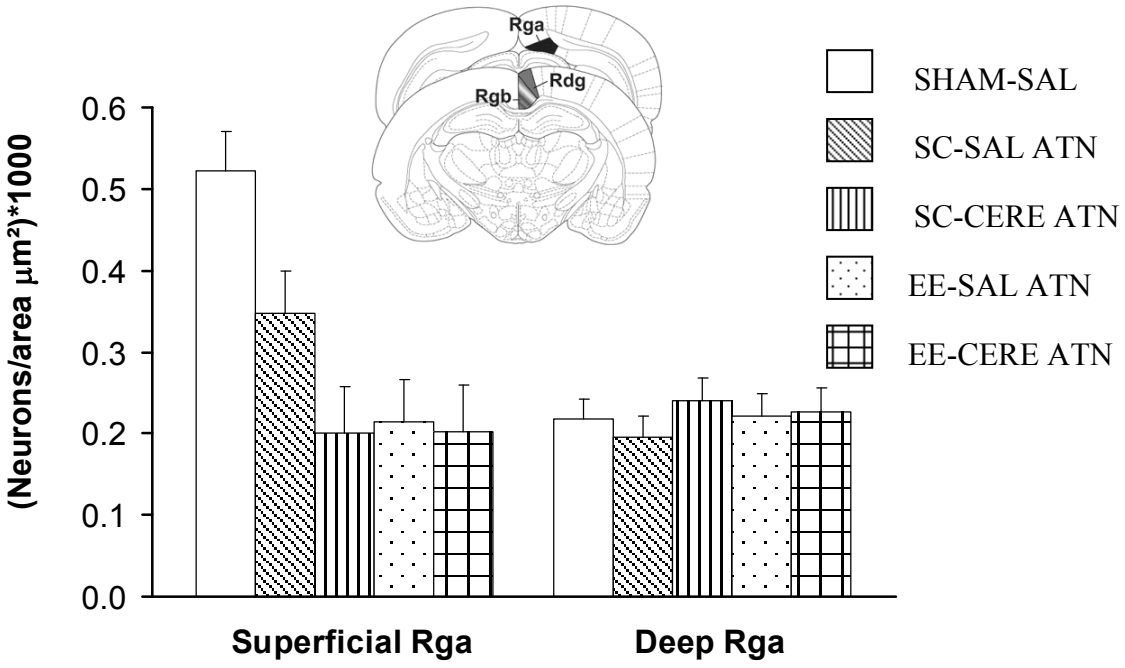


Fig 39. Enrichment and Cerebrolysin and Fos-counts in the Rga. Fos-counts (number of neurons/area µm²) in the superficial and deep cell layers of the caudal granular retrosplenial cortical area (Rga). Data shown as mean (+SEM) number of neurons/area µm² counts multiplied by a 1000. ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE= administered Cerebrolysin; SAL= administered saline.

The counts in the dysgranular cortex (Rdg) were taken across all cell layers (Fig 40). The group effect when the dysgranular cortex (Rdg) counts were analysed reached significance ($F_{(4,29)} = 3.51$; $p < 0.05$) (Fig 38) and planned comparisons revealed a presence of a significant difference between EE-SAL and SHAM-SAL ($p = 0.011$) while the difference between EE-CERE and SHAM-SAL did not reach the specified level of significance ($p = 0.046$).

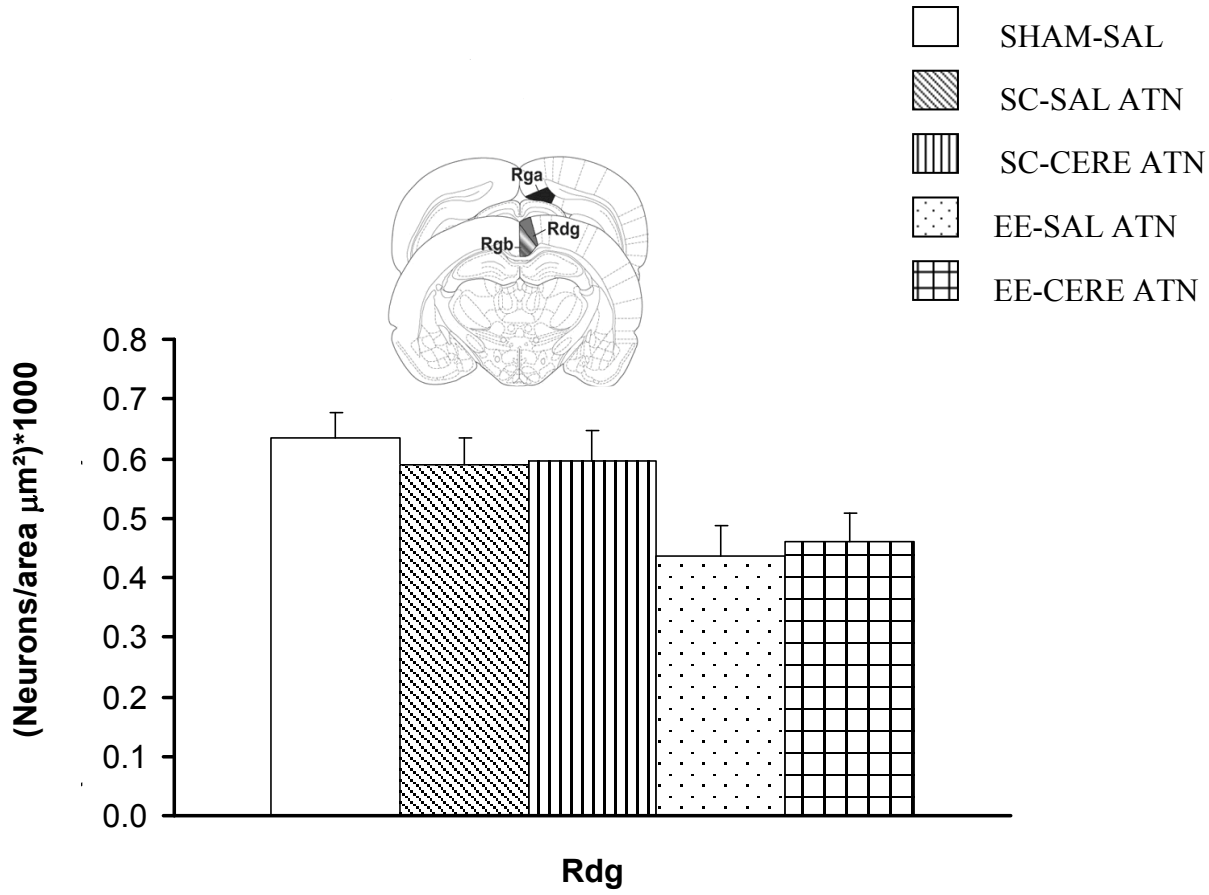


Fig 40. Enrichment and Cerebrolysin and Fos-counts in the Rdg. Fos- counts (number of neurons/area µm²) in the dysgranular retrosplenial cortical area (Rdg). Data shown as mean (+SEM) of neurons/area µm² counts multiplied by a 1000. ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE= administered Cerebrolysin; SAL= administered saline.

Housing X Drug analysis of the retrosplenial cortex counts: Analysis of just the lesion groups revealed that exposure to enrichment was associated with a relatively reduced level of cell activation in the deep cell layer of the Rgb (Fig 38) which was confirmed by a significant Housing effect ($F_{(1,22)} = 6.83$; $p < 0.05$). No effects for Housing, Drug or Housing by Drug interaction were detected when counts in the superficial cell layer of Rgb (see Fig 38) were analysed. Similarly no effects or interactions were detected when superficial and deep laminae of the Rga (all F's < 1) were analysed (see Fig 39) even though the three treatment groups demonstrated lower counts in the superficial Rga. In the Rdg (Fig 40), both enrichment groups demonstrated low Fos counts in comparison to the ATN lesioned animals housed in standard conditions with significant effect for Housing being detected ($F_{(1,52)} = 9.57$; $p < 0.01$), but no Drug effect or Drug by Housing interaction were observed (all F's < 1).

Frontal Cortex

Comparison to SHAM group: A significant group effect was obtained ($F_{(4,29)} = 6.54$; $p < 0.001$) in the anterior cingulate area, which reflected a reduced level of activation in both enriched groups (Fig 41, left panel, also see Fig 45 B for examples of photomicrographs taken in the frontal cortex). The enriched animals EE-SAL and EE-CERE were significantly different from SHAM-SAL (both p's < 0.01), while the standard-housed (SC-SAL and SC-CERE) groups were not (p's > 0.05). A significant group difference in cell counts was also observed for the prelimbic/infralimbic areas ($F_{(4,29)} = 3.23$; $p < 0.05$); (Fig 41, right panel), with enriched rats again demonstrating reduced levels of activation in comparison to the shams. However on planned comparison analyses none of the group differences for the prelimbic-infralimbic cortex reached the specified level of significance of $p <$

0.0125.

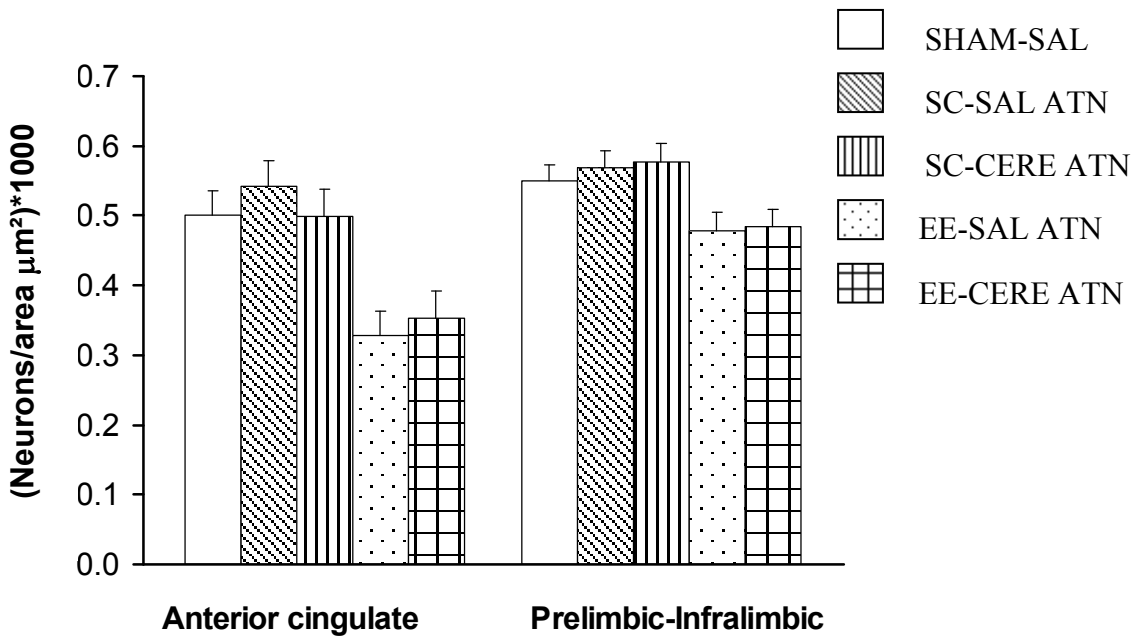


Fig 41. Enrichment and Cerebrolysin and Fos-counts in the Frontal Cortex. Fos- counts (number of neurons/area µm²) in the anterior cingulate and prelimbic-infralimibic cortical areas. Data shown as mean (+SEM) number of neurons/area µm² counts multiplied by a 1000. ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.; CERE= administered Cerebrolysin; SAL= administered saline.

Housing X Drug analysis of the frontal cortex counts: Examination of just the lesion groups (see Fig 41) revealed that enrichment resulted in reduction in activation in the anterior cingulate area with a highly significant main effect of Housing ($F_{(1,22)} = 20.27$; $p < 0.001$) being detected. There was no Drug effect or Drug by Housing interaction (all F's < 1) detected. In the prelimbic-infralimbic area analysis of the lesion groups again only revealed a main effect for Housing ($F_{(1,22)} = 11.14$; $p < 0.01$) with both enriched groups demonstrating lower counts than the standard housed groups. No other main effects or interactions were detected (all F's < 1).

Hippocampus

Analyses of the groups of rats using counts taken across various subfields of the hippocampus: Antero-dorsal hippocampus (CA1, CA3 and external blade of the dentate gyrus); Postero-dorsal hippocampus (external blade of dentate gyrus and CA1) and Postero-ventral hippocampus (CA3 and CA1) did not reveal any significant group effects. For the antero-dorsal hippocampus (Fig 42) no group differences were found in each individual region CA1 ($F_{(4,29)} = 1.00$; $p > 0.1$); dentate gyrus ($F_{(4,29)} = 0.66$; $p > 0.1$) and CA3 ($F_{(4,29)} = 0.92$; $p > 0.1$).

Analysis of only the lesion groups also did not reveal any differences across all the individual subregions. For the postero-dorsal hippocampus (Fig 43) no group differences were detected in counts taken from the external blade of dentate gyrus ($F_{(4,29)} = 1.30$; $p > 0.1$) and CA1 ($F_{(4,29)} = 0.3$; $p > 0.1$). In the postero-ventral hippocampus (Fig. 41), again no group differences were observed in CA3 ($F_{(4,29)} = 0.3$, $p > 0.1$) or CA1 $F_{(4,29)} = 0.71$; $p > 0.1$). When just the counts of treatment groups were analysed no group differences were detected in any of the hippocampal subregions analysed.

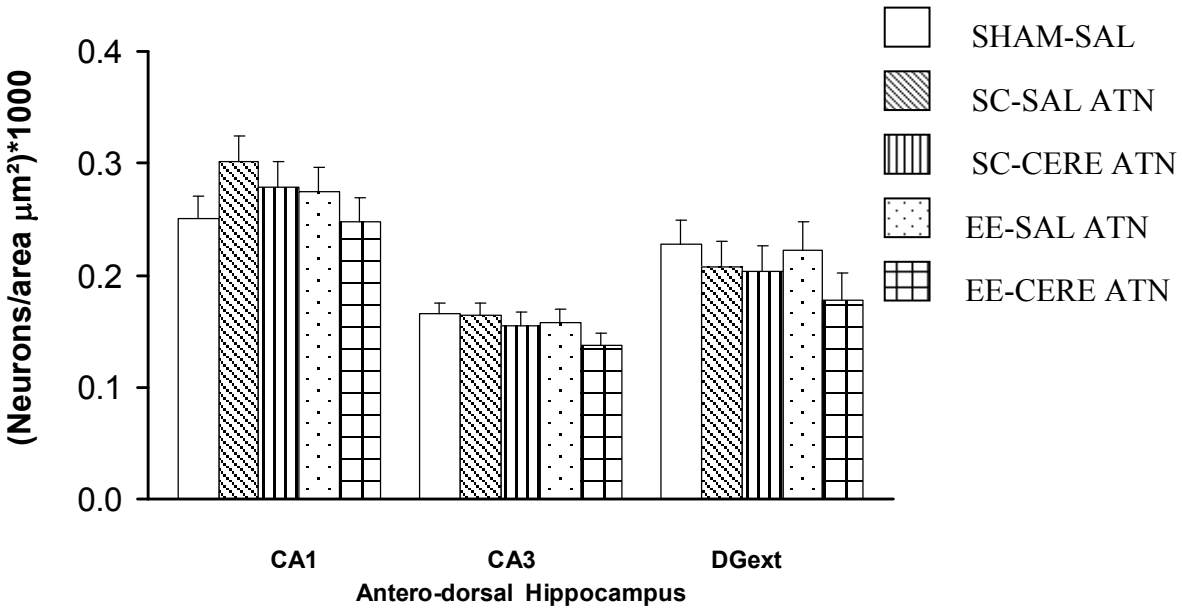


Fig 42. Enrichment and Cerebrolysin and Fos-counts in the antero-dorsal hippocampus. Fos-counts (number of neurons/area µm²) in the hippocampus. Data shown as mean (+ SEM) number of neurons/area µm² counts multiplied by a 1000. Results are given for the CA1 and CA3 fields and the external blade of dentate gyrus (DG ext) of the antero-dorsal hippocampus. ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE= administered Cerebrolysin; SAL= administered saline.

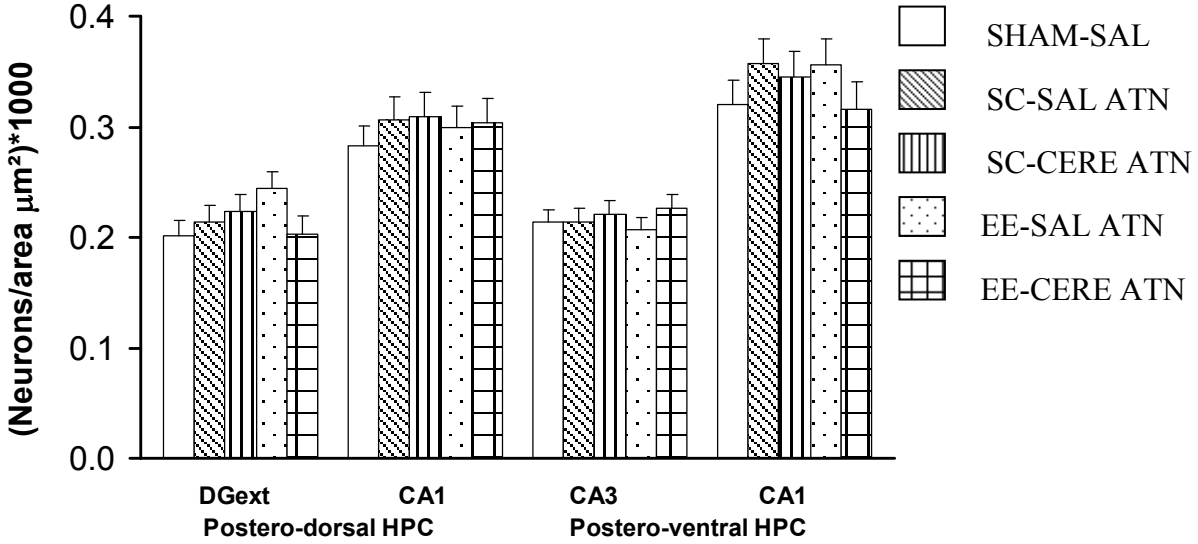


Fig 43. Enrichment and Cerebrolysin and Fos-counts in the posterior hippocampus. Fos- counts (number of neurons/area µm²) in the posterior hippocampus. Data shown as mean (+ SEM) number of neurons/area µm² counts multiplied by a 1000. Results are given for the ventral and dorsal CA1 and CA3 fields and the external blade of the dentate gyrus (DG ext) of the posterior hippocampus. ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment.; CERE= administered Cerebrolysin; SAL= administered saline.

Cortical Control Areas

In the primary motor cortex (MOp), lesions to the ATN did not result in any changes in the level of activation, but exposure to enrichment induced hypoactivation. A significant group effect was detected ($F_{(4,29)} = 3.63$; $p < 0.05$), with planned comparisons identifying that the level of activation in the EE-CERE was significantly different from that in the SHAM-SAL (p's < 0.001) (see Fig 44). While the The EE-SAL also showed lower levels of activation in comparison to shams the difference did not reach the specified level of significance ($p = 0.03$). Analysis of just the lesion groups revealed a significant effect for Housing ($F_{(1,22)} = 4.42$; $p < 0.05$), confirming that enrichment resulted in induction of hypoactivation in the primary motor cortex. No other effects or interactions were observed.

Similarly, in the primary somatosensory area (Ssp) a significant group effect was detected ($F_{(4,29)} = 3.08$; $p < 0.05$), with only the EE-CERE group reaching the specified level of significant ($p < 0.001$) difference from SHAM-SAL; (EE-SAL vs SHAM-SAL difference $p = 0.02$) (see Fig 44). Examination of the lesion groups also only revealed a significant effect for Housing only ($F_{(1,22)} = 6.88$; $p < 0.05$). No other effects or interactions were observed.

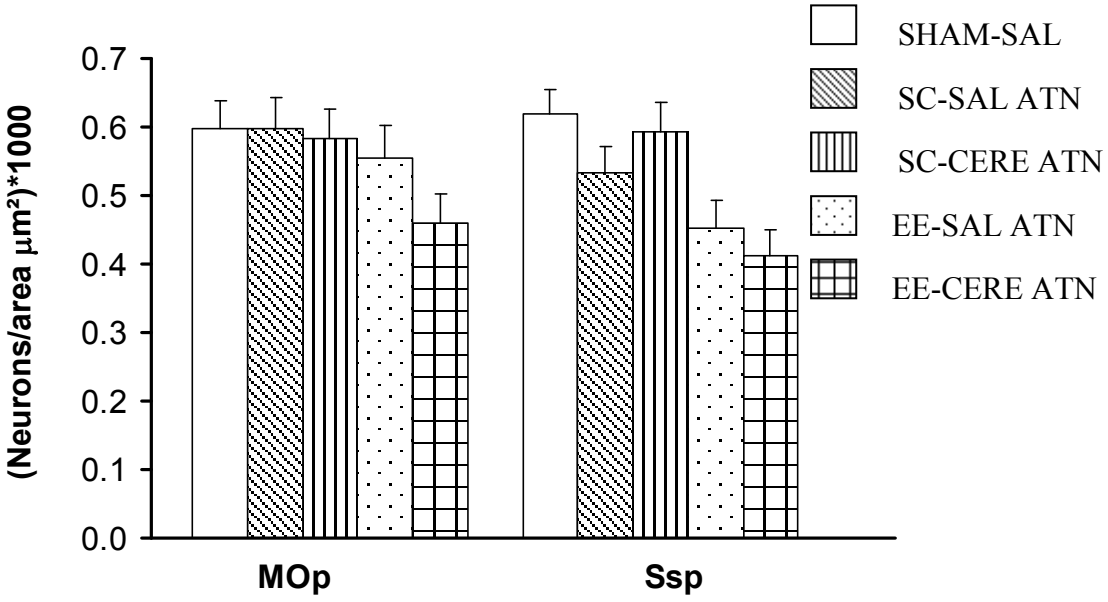


Fig 44. Enrichment and Cerebrolysin and Fos-counts in the cortical control areas. Fos-counts (number of neurons/area µm²) in the control areas. Data shown as mean (+SEM) number of neurons/area µm² counts multiplied by a 1000. MOp = primary motor cortex; Ssp = primary somatosensory cortex; ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE= administered Cerebrolysin; SAL= administered saline.

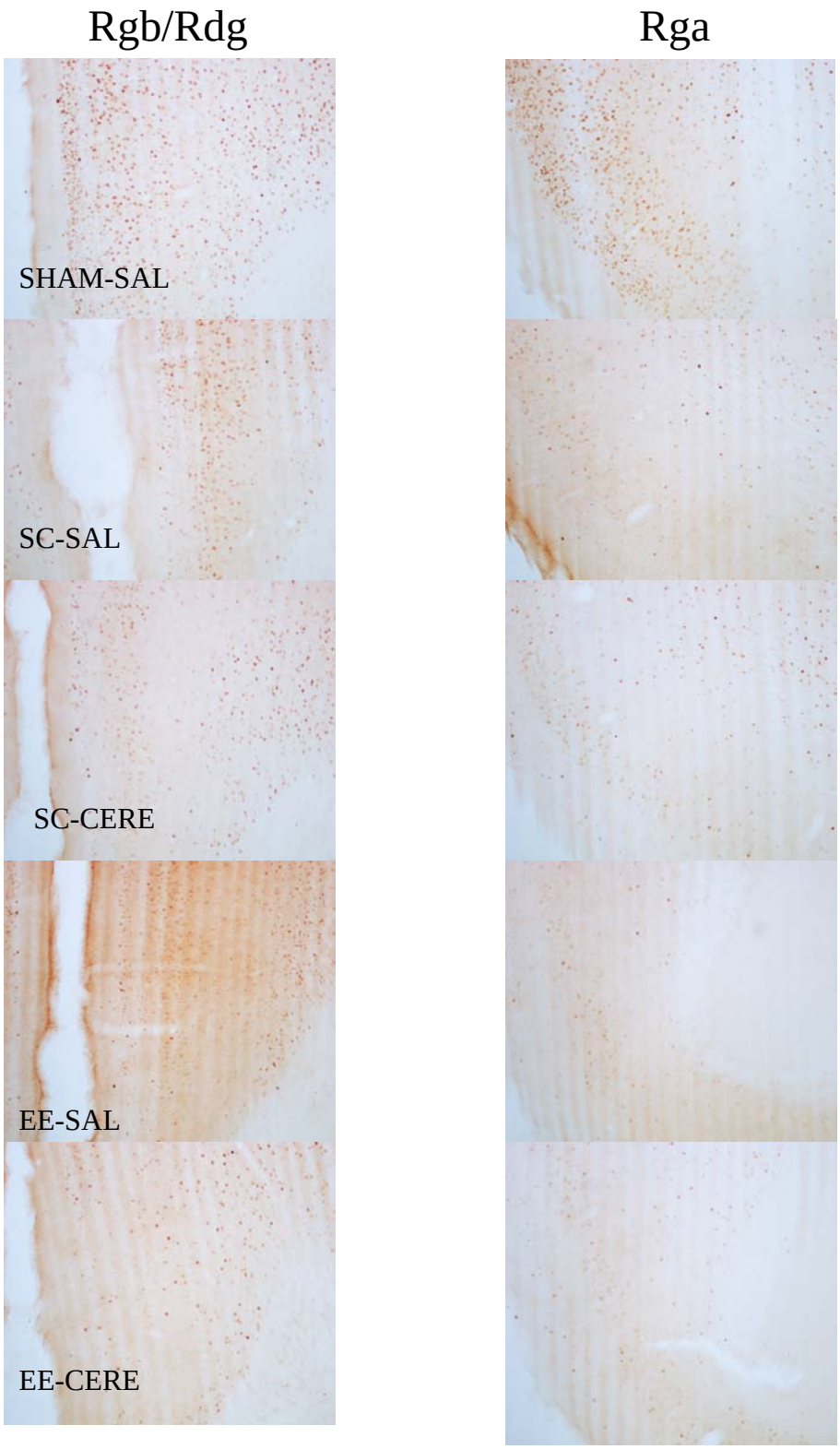


Fig 45A. Photomicrographs depicting Fos levels in retrosplenial cortex areas rostral granular retrosplenial cortical area (Rgb), dysgranular retrosplenial cortical area (Rdg), and caudal granular retrosplenial cortical area (Rga) in all groups. SC = housed in standard group; conditions; EE = enriched environment; CERE = administered Cerebrolysin; SAL = administered saline.

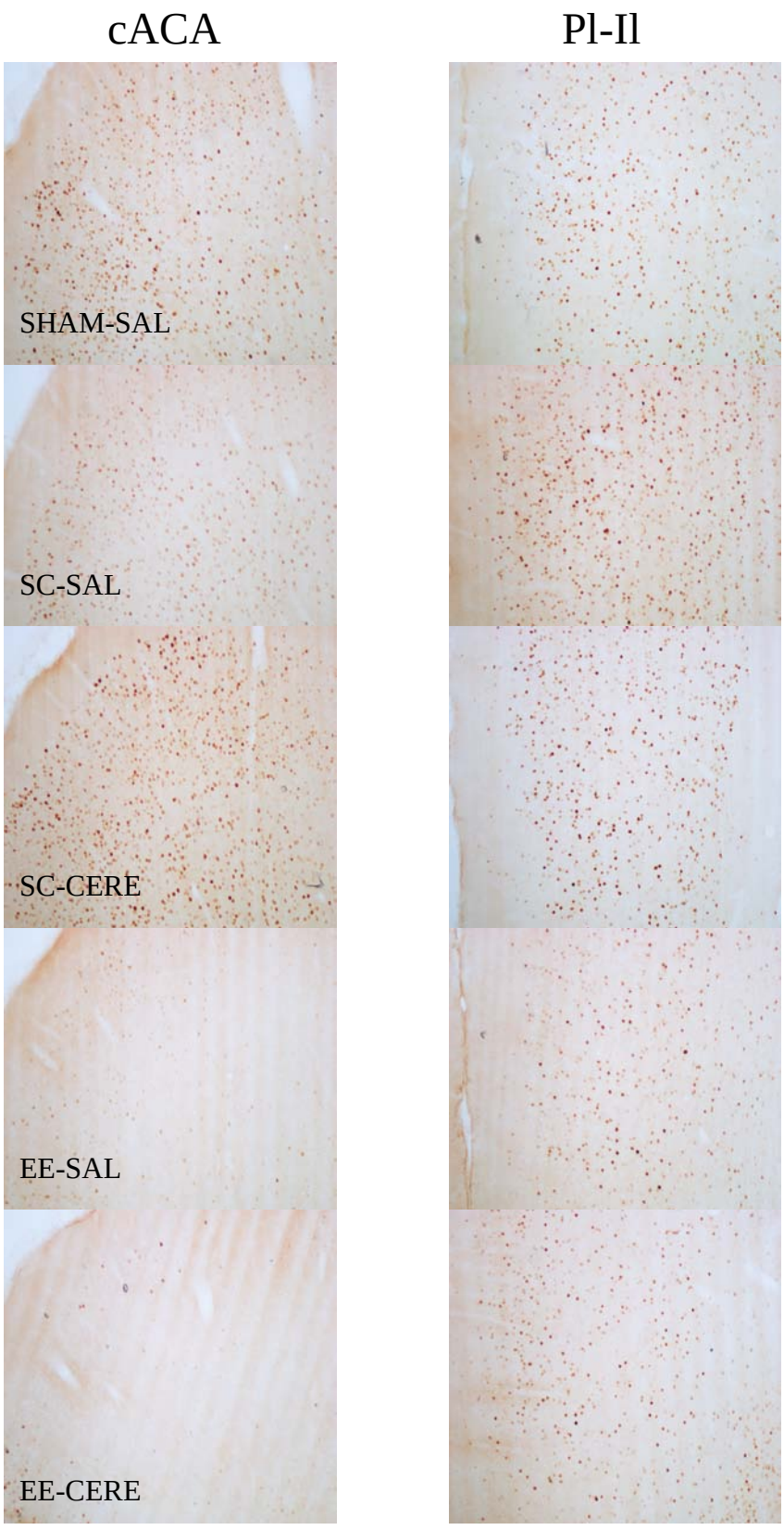


Fig 45B. Photomicrographs depciting Fos levels in cortical areas caudal anterior cingulate (cACA) and prelimbic-infralimbic cortices (Pl-Il) in all groups. SC = housed in standard group conditions; EE = enriched environment; CERE = administered Cerebrolysin; SAL = administered saline.

9.3.5 Individual performance, lesion size, spatial working memory and Fos- activation

Figure 46 shows individual performance of all rats with ATN lesions groups across the 10 sessions, as well as separately for the first and last three sessions of the spatial working memory task conducted immediately after the 30 day period of enrichment exposure and/or Cerebrolysin injections. The correlation coefficients are provided in Table 10. As evident from the scattergrams, there was limited overlap in scores for the ATN rats that experienced post-operative treatment compared to those in the SC-SAL group, especially by the end of training. No consistent pattern of relationship between performance on the cross-maze task and size of the ATN lesion emerged. Only one significant correlation emerged between the average performance on the cross-maze (across 10 sessions) and the size of lesion of the EE-SAL group, which is counter-intuitive (larger lesion, better performance) and may be a chance finding.

To evaluate whether the level of Fos activation was correlated with the level of performance demonstrated by lesion groups correlations were performed between average performance across 10 spatial working memory sessions and levels of activation in brain areas where group differences in activation were detected (see Fig 47). Across all regions examined only two significant correlations emerged. In the SC-CERE group better performance on the 40-s delay trials on the cross-maze was significantly associated with a lower level of activation in the anterior cingulate area (see Table 11). While in the SC-SAL group better levels of performance were associated with higher levels of activation in the superficial cell layer of the Rga but only for the three sessions of pre-Fos training.

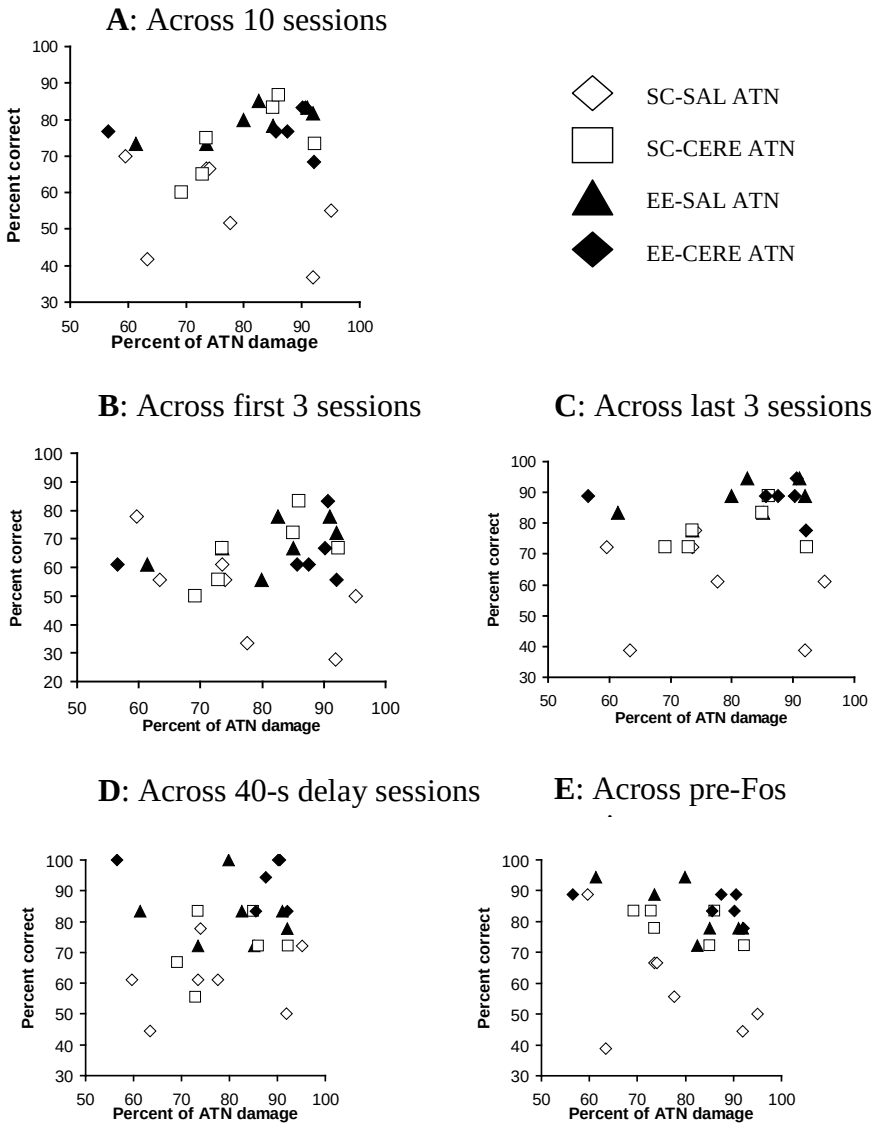


Fig 46. Enrichment and Cerebrolysin and percent of damage sustained to the ATN versus Spatial Working Memory performance. Scatterplots showing the correlation between the extent of bilateral ATN damage sustained and mean percent correct responses on the spatial working memory task in the cross-maze following treatment intervention **A**: across all ten sessions; **B**: across the first three sessions and **C**: across the last three sessions **D**: across 40-second sample-test delay sessions; **E**: across pre-Fos sessions. ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE = administered Cerebrolysin; SAL= administered saline.

Mean percent correct responses on the cross-maze	**SC-SAL average lesion size** *df* = 5	**SC-CERE average lesion size** *df* = 4	**EE-SAL average lesion size** *df* = 5	**EE-CERE average lesion size** *df* = 4
Across all 10 sessions	-0.41	0.68	**0.78**	0.05
First 3 sessions	-0.70	0.70	0.58	0.21
Last 3 sessions	-0.24	0.38	0.58	-0.14
Delayed sessions	0.68	0.33	-0.07	-0.34
Fos-sessions	-0.50	-0.64	-0.73	-0.45

Table 10. Enrichment and Cerebrolysin and correlations between Spatial Working Memory performance and lesion size. Correlation coefficients observed between mean percent correct responses on the cross-maze task across all 10 sessions and separately for the first and last three sessions, as well as for sessions with a 40-second sample-test delay and pre-Fos sessions versus average ATN lesion size sustained by each group. Bold indicates significance at $p < 0.05$.

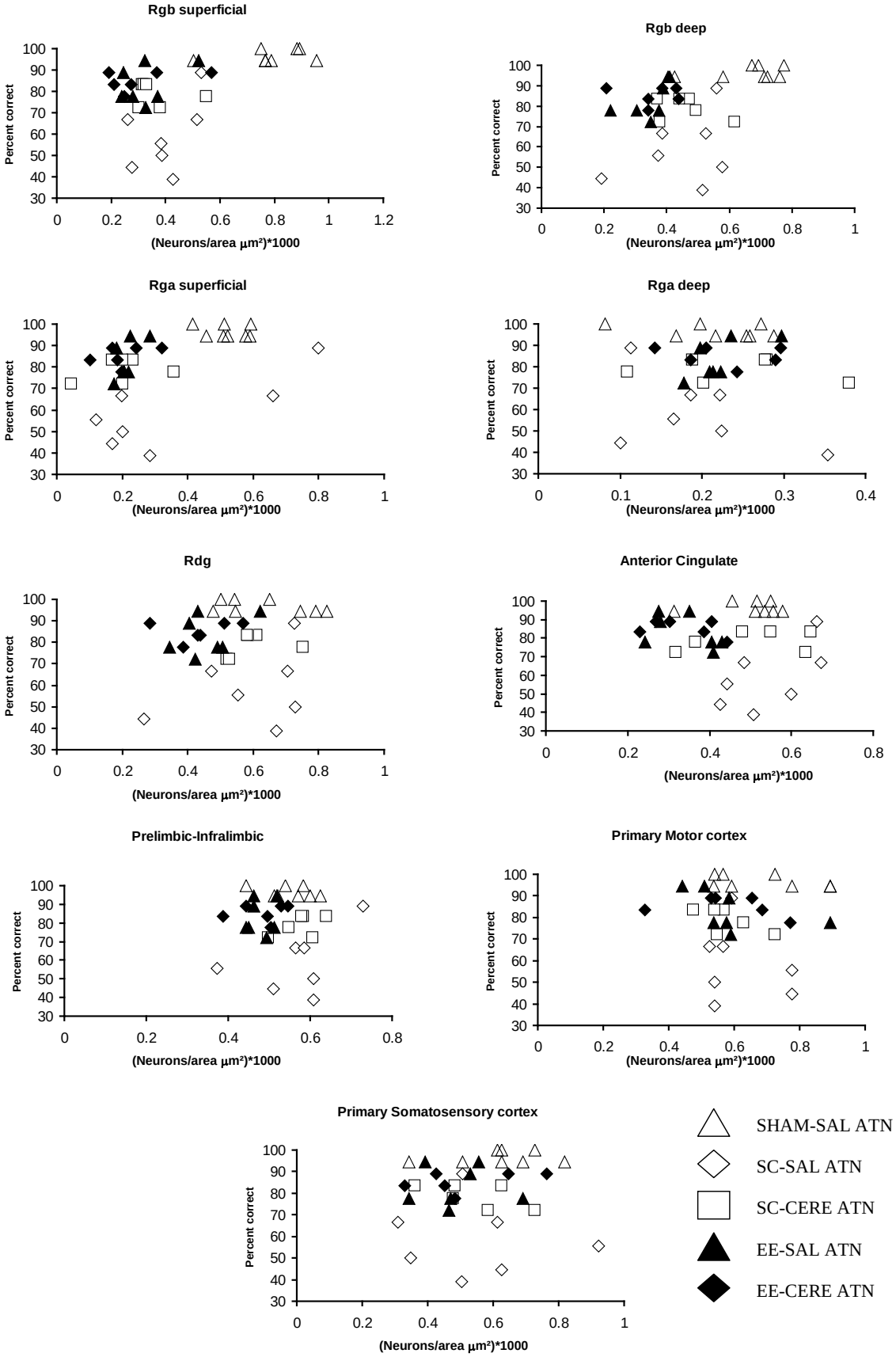


Fig 47. Enrichment and Cerebrolysin and Fos activation versus performance on the Spatial Working Memory task. Scatterplots showing the correlation between the extent of Fos activation and mean percent correct responses on the spatial working memory task in the cross-maze across the 3 days of the pre-Fos behavioural activation. Rgb = rostral granular retrosplenial cortex; Rga = caudal granular retrosplenial cortex; Rdg = dysgranular retrosplenial cortex. ATN= neurotoxic lesion of the anterior thalamic nuclei; SC = housed in standard group conditions; EE = enriched environment; CERE = administered Cerebrolysin; SAL= administered saline.

	SC-SAL			SC-CERE			EE-SAL			EE-CERE		
	10 sessions	delayed	Pre-Fos	10 sessions	delayed	Pre-Fos	10 sessions	delayed	Pre-Fos	10 sessions	delayed	Pre-Fos
cACA	0.70	0.22	0.61	-0.60	**-0.87**	0.33	0.52	0.10	-0.49	-0.34	0.14	-0.42
Pl-Il	0.44	0.05	0.48	-0.24	-0.17	0.51	0.10	0.39	0.10	0.13	0.63	0.19
RGB super	0.45	-0.21	0.48	0.04	0.49	-0.18	-0.45	0.12	0.41	0.07	0.40	0.45
RGB deep	0.55	0.22	0.35	-0.39	-0.13	-0.39	-0.63	0.15	0.64	0.22	-0.06	-0.09
RDG	0.52	0.15	0.34	-0.14	0.17	0.33	-0.50	-0.09	0.37	0.34	0.44	0.26
RGA super	0.64	0.35	**0.76**	-0.08	0.23	0.31	-0.52	0.25	0.63	-0.15	0.52	0.43
RGA deep	-0.19	-0.24	-0.50	-0.44	-0.56	-0.16	-0.19	0.74	0.71	0.08	0.01	-0.22
Ssp	-0.30	-0.09	-0.11	0.56	0.45	-0.60	-0.47	-0.53	0.00	0.16	0.64	0.49
Mop	-0.51	-0.28	-0.22	0.07	0.45	-0.64	-0.01	-0.67	-0.50	-0.29	0.23	-0.34

Table 11. Enrichment and Cerebrolysin and correlations between Spatial Working Memory performance and Fos activation. Correlation matrix representing correlation coefficients between mean performance on the 10 sessions of the cross-maze task (10 sessions); 40-s sample-choice delay sessions (delayed) and three pre-Fos sessions versus level of Fos activation in brain regions examined presented separately for each lesion group. **Bold** indicates significance at $p < 0.05$. SC = housed in standard group conditions. EE = enriched environment. CERE= administered Cerebrolysin and SAL= administered saline; cACA = caudal anterior cingulate cortex; Pl-Il = prelimbic-infralimbic cortex; RGB super and deep = rostral granular retrosplenial cortical area superficial and deep layers; RGA super and deep = caudal granular retrosplenial cortical area superficial or deep cell leayers; RDG= dysgranular retrosplenial cortical area; Ssp = primary somatosensory cortex; MOp = primary motor cortex.

9.4 Discussion

The current study provides a third successful replication of the therapeutic effects of enrichment following anterior thalamic lesions. As was previously demonstrated (see Chapter 6), a 30-day continuous exposure of ATN lesioned rats to enriched environment soon after surgery resulted in marked amelioration of the ATN induced deficit on the non-matching to sample spatial working memory task in the cross-maze. The novel contribution of the current study was that a long-term (30 day) administration of the neurotrophic type substance Cerebrolysin started 48-hours post-surgery also promoted recovery of function after anterior thalamic lesions and was generally as effective as enrichment alone. This finding adds significantly to the body of evidence on the effectiveness on Cerebrolysin in promoting behavioral recovery after hippocampal system lesions (Francis-Turner & Valouskova, 1996; 1999), and provides the first preliminary evidence of the possible therapeutic advantages of the drug in cases of diencephalic amnesia. Combination of the two therapeutic approaches, enrichment and Cerebrolysin, during the acquisition of the standard cross-maze task (sample-test delay 5-10 seconds) did not result in any additional behavioral improvements for the ATN animals above what could be observed after only enrichment or only Cerebrolysin exposure. However, when a 40-s delay between the sample and test runs was introduced the combined treatment emerged, with the Enriched-Cerebrolysin rats demonstrating improved performance in comparison to other treatment groups.

As has previously been demonstrated (see Chapters 6 & 7) standard-housed ATN rats showed marked impairments in spatial working memory, emphasising the robustness of working memory deficits after ATN lesions (Aggleton, et al., 1995a; Warburton & Aggleton, 1999; Warburton, et al., 1999; Warburton, et al., 2001; Ward-Robinson, et al., 2002). The impairments demonstrated by the ATN rats on the spatial alternation task were largely due the inability of the animals to employ an allocentric/direction type strategy in order to solve the maze, and a tendency to rely on an egocentric strategy. Exposure to enrichment clearly ameliorated the spatial working memory deficits in ATN rats. Treated ATN rats not only demonstrated improved performance on the egocentric-type trials but also showed an enhanced ability to acquire an allocentric/direction type strategy. Exposure to Cerebrolysin improved the rats' ability to solve the same-start/egocentric type trials, and had

positive effect on the rats' ability to utilise the allocentric/directional cues, although to a lesser degree than enrichment alone. The addition of a 40-s delay had little effect on the performance of the sham rats which continued to perform well, or the standard housed ATN rats which continued to find the task challenging. The Cerebrolysin only rats were mildly negatively affected on both trial types. Most interestingly, the combination of both treatment modes resulted in improved performance shown by Enrichment-Cerebrolysin group on both trial types of the delayed task with this group reaching the levels of the control animals.

Using Fos as a marker this study mapped how anterior thalamic lesions alter neuronal activation, and how exposure to therapeutic intervention may influence the changes in the pattern of activation during behaviour testing on the standard spatial working memory task in the cross-maze (5 to 10 second sample-test delay). Marked hypoactivation was observed in the retrosplenial cortex following anterior thalamic lesions. In the standard caged, saline only ATN animals the hypoactivation was particularly pronounced in the rostral granular retrosplenial cortex (Rgb) in both the superficial and deep cell layers, and to lesser extent in the superficial cell layers of the caudal granular retrosplenial cortex (Rga). No ATN-lesion induced changes were observed in the dysgranular retrosplenial region (Rdg). Previously, Jenkins and colleagues (2004) reported that the ATN-lesion induced hypoactivity is most dramatic in the dense aggregation of darkly stained small cells in layer II and upper layer III of the Rgb and Rga. While these superficial laminae typically contain a high proportion of Fos-positive cells in intact rats, this marker has been reported to be almost completely absent following anterior thalamic lesions (Jenkins, et al., 2004; Poirier & Aggleton, 2009). The findings of the present research further confirm this conclusion, although the level of hypoactivity observed in the superficial cell layers of the Rga was not as dramatic as previously reported. Recently, Poirier and Aggleton (2009) argued that the ATN induced hypoactivation in the retrosplenial region becomes more widespread with a passage of time. The authors supported their conclusion by demonstrating that changes in the superficial cell layers of the Rgb emerge early after surgery (1-2 weeks), while hypoactivation in the deep cell layers of Rgb takes longer to develop and only becomes evident at 8 weeks survival time post-surgery. The dysgranular region (Rdg) appears unaffected at early survival times but hypoactivation in this area becomes evident at 1 year post-surgery survival (Poirier &

Aggleton, 2009). In the current study rats were sacrificed 4 months post-surgery. At this time point one would expect that changes in superficial as well as deep cell layers of Rgb would become evident, but changes in Rdg may not yet emerge. Indeed, in the current experiment both superficial and deep layers of Rgb showed marked reduction in Fos staining in the standard housed, saline only ATN-lesioned rats, but these rats did not show a change in marker in the Rdg. Although, it may be possible that the current data are task-dependent (as no behavioural controls were used), it should be noted that Aggleton group found a substantial c-*fos* reductions in Rgb superficial cell layers even in their home-cage ATN-lesioned controls (Jenkins, et al., 2004).

No lesion induced changes were observed in the hippocampus. Previously, Jenkins, et al., (2002a) reported mixed results with regard to hippocampal activation. In their experiment the hippocampal (anterior and posterior) Fos levels in the ATN animals did not differ from these in normal animals that had remained in a familiar room (Sham-familiar vs ATN lesion-novel), but were lower in comparison to the Shams that were exposed to a novel room. The authors suggested that this finding indicates that the ATN lesions blocked the rise in c-*fos* expression that normally follows exposure to a novel environment. It is possible that the same process operated in the current study, as despite previous observation that exposure to enrichment results in hippocampal activation (Kempermann, et al., 1997) no such changes were observed in the enriched ATN animals and testing was conducted in a room with which they were highly familiar.

In the current study, no changes were also noted in the primary motor and somatosensory areas of the standard ATN saline rats. This was contrary to Jenkins and colleagues (2002a) observation of elevated c-*fos* activation in the motor area of the ATN lesioned rats. However, the motor area hyperactivation is not a consistent effect as it was not observed across different lesion method conditions (NMDA versus electrolytic) or immunohistochemical agents (c-*fos* vs *zif-268*) (Jenkins, et al., 2004).

The novel contribution of the current study was the observation that exposure to enrichment after ATN lesions was associated with general induction of yet further hypoactivation in the cortical areas. The hypoactivation was not region specific, with reduced cell counts observed most notably in the enriched rats in the Rgd, anterior

cingulate cortex, prelimbic-infralimbic cortex, primary motor and somatosensory areas and to a lesser extent in the deep laminae of Rgb. No enrichment associated changes were observed in the hippocampus. There was also no indication that administration of Cerebrolysin was associated with any further changes in activation in the lesioned animals other than some indication of reduction in activation in the superficial Rga region.

The Fos activation findings support the observation that anterior thalamic lesions alter the pattern of activation in the retrosplenial cortex area. This hypoactivation is particularly striking in the cell layers II and upper III. As thalamic inputs terminate in these regions Jenkins and colleagues (2004) argued that the hypoactivity observed is a consequence of disconnection of the retrosplenial cortex from the ATN inputs. The authors further suggested that severity of the amnesic syndrome observed in Korsakoff's patients as well as in Alzheimer's disease patients is due to the presence of the ATN induced pathology in the retrosplenial cortex (see Chapter 3). The current results highlight the question of the functional significance of retrosplenial hypoactivity after ATN lesions. Here, we have demonstrated that clear behavioural gains can be obtained in ATN lesion animals exposed to enrichment, with evidence that enrichment was effective in reducing the classic allocentric-type spatial memory deficits seen in ATN animals. This change in behaviour was however, associated with induction of more pronounced hypoactivity in the cortical areas. This finding is novel and unexpected and may mean that either the retrosplenial cortex is not critical for the spatial working memory task specifically or other mechanisms rather than *c-fos* are involved, and / or changes in the relative balance of *c-fos* activity across different brain regions may explain the level of behavioral performance in a spatial working memory task (see Chapter 10 for more in depth discussion).

The current study presented the first evidence that administration of a neurotrophic drug Cerebrolysin can result in behavioural improvements in ATN lesioned rats on the task of spatial working memory. It also demonstrated that the combination of Cerebrolysin with exposure to enrichment can possibly further enhance performance of ATN lesioned rats when spatial working memory is examined using a delay. The study provided confirmation of previous findings that ATN lesions produce hypoactivity in the retrosplenial cortex region. However,

behavioural recovery was not associated with the changes in the levels of activation and, if anything, exposure to enrichment resulted in further reduction in Fos expression in the cortical regions.

Chapter 10

General Discussion and Conclusions

This chapter provides a general overview of the scientific contributions of the current research, and identifies possible caveats and future research directions. More in depth discussion of individual experimental findings is provided in each experimental chapter (Chapters 6, 7, 8, 9).

10.1 Contributions of the current research to the field of recovery of function

The current research investigated the possibility of recovery of function in diencephalic amnesia. To our knowledge no attempt has yet been made to ameliorate behavioral deficits observed after lesions to anterior thalamus, which is strongly implicated in contributing to the severity of the amnestic deficits observed in patients with diencephalic amnesia. The research described in this thesis has provided the first evidence that deficits associated with the injury to the ATN may be amenable to therapeutic intervention. This evidence was consistent across three experiments using a cross-maze task to examine spatial working memory.

The long-lasting memory deficits that follow bilateral ATN lesions, together with evidence of similar deficits after disconnection lesions of the ATN and the hippocampal system, point to the potential importance of the thalamic region as a core part of a distributed system supporting episodic memory (Aggleton & Brown, 2006; Byatt & Dalrymple-Alford, 1996; Henry, et al., 2004; Warburton, et al., 2000; 2001). The contribution of the diencephalic structures to the severity of the amnesic syndrome suggests that there is considerable merit in determining whether memory deficits produced by experimental ATN lesions are amenable to treatment. Environmental enrichment has long been used as a therapeutic intervention in

experimental animal studies. While most of the studies have concentrated on the effects of enriched environmental conditions in various models of TBI (Hoffman, et al., 2008; Kline, et al., 2007), stroke (Briones, et al., 2004; Puurunen, et al., 2001) and degenerative diseases (Arendash, et al., 2004; Lazarov, et al., 2005), post-operative enrichment has also been demonstrated to promote recovery of function after acute brain injury, such as lesions to the hippocampus (Einon, et al., 1980; Galani, et al., 1997; Kelche & Will, 1982; Pacteau, et al., 1989) and other components of the extended hippocampal system (Bindu, et al., 2005; Dhanushkodi, et al., 2007). However, variable outcomes have been observed and lesion and task specificity has often been documented (Dalrymple-Alford & Kelche, 1987; Galani, et al., 1997; Kelche et al., 1987; Will et al., 1981). It is therefore not necessarily the case that enrichment effects would be found after thalamic lesions.

This thesis has shown that exposure to enrichment, either soon after surgery or 40 days later, can be effective in promoting behavioral recovery in animals with ATN lesions. The current studies employed careful, highly selective lesion techniques and only included lesions that produced at least 50% damage to the ATN complex with minimal damage to any of the surrounding thalamic structures. As pointed out in the Introduction, previous ATN lesion studies that have employed selective lesion procedures have demonstrated that damage to the ATN is associated with severe and apparently permanent deficits on the tasks of spatial memory. Performance in the cross-maze or T-maze task has been repeatedly used as a clear and robust indicator of the type of spatial working impairment observed after ATN lesions (see Chapter 3). Currently, across three different experiments, we have been able to consistently demonstrate that deficits in spatial working memory in the cross-maze were markedly ameliorated by exposure to post-operative enrichment. The replications of enrichment induced recovery across studies emphasized the robustness of the results obtained, indicating that the enrichment-induced benefits were unlikely to be due to non-specific factors. Moreover, enrichment also induced behavioral recovery when it was introduced 40 days post surgery, after clear ATN-induced impairments had already been demonstrated. Enrichment also continued to exert a beneficial effect on spatial working memory long after exposure to enrichment was discontinued. This is a particularly encouraging observation, as it offers hope that therapeutic interventions may be successful even in well-established cases of diencephalic amnesia.

One of the most contentious issues in the field of brain damage is the meaning of the term "recovery". In their review, Stein, Finger and Hart (1983) suggested that CNS insults may force the subjects to alter their behavioral strategies such that a problem can be solved in a new or different way. The researchers cited some early findings which demonstrated that rats with motor-parietal lesions were able to "recover" their ability to negotiate a runway when a conventional "end or goal" analysis was used, but careful observation of the behavior revealed that lesioned rats' ballistic and temporal movements were different (Gentile, Green, Nieburgs, Schmeltzer & Stein, 1978). Stein and Hoffman (2003) suggested that the recovery in lesioned animals may be a reflection of compensation or an ability of the animals to develop strategies to solve learning problems. Will and colleagues (2004) also supported the same idea, arguing that recovery seen after enrichment is most likely a reflection of compensation, with complex environment promoting subjects' ability to learn different strategies for dealing with general problems, such as maze learning. For example, they have suggested that enriched hippocampal rats may have developed a non-hippocampal mediated strategy in the spontaneous alternation task which allowed the animals to solve it successfully (Will, et al., 1983). Stein and colleagues (1983) advocated that careful analysis of the strategy selection will need to occur in order to establish whether recovery has occurred.

In the current study, improved performance of the ATN rats on the cross-maze was not only determined by their increased ability to solve egocentric-type trials, an ability supposedly independent of ATN function, but also allocentric/directional type trials. In the cross-maze task employed, the enriched ATN rats performed above chance when the test run used the opposite start direction to that used in the sample run whereas standard-housed ATN rats were severely impaired on these trials. As the ATN is considered to be essential for normal spatial working memory function, the improvements demonstrated by the ATN-enriched rats on these trials suggest that exposure to enrichment did not simply result in compensation but induced changes in animals' ability to utilize spatial cues in the environment to guide responses. Considering the evidence the intact rats rely on more than one strategy when solving a cross-maze, such as direction, place and response (Skinner, et al., 2003) it might be the case that an integration of several strategies rather than the use of just the visual cues permitted the ATN enriched rats to

successfully solve the task. When Stringer and colleagues (2005) examined how the hippocampal rats solve the T-maze they noted that the rats were impaired in use of both place and directional strategies, but not in their use of response strategies. These researchers suggested that there might be some common underlying mechanism for place learning and direction learning, arguing that direction learning is another form of place learning. They hypothesized that direction could be equivalent of a crude place (i.e., the rats may simply be learning to approach a door), or direction and place learning could be a form of conditional discrimination (i.e., if at Position A, turn right; if at Position B, turn left). Hence improved performance of the enriched rats on the cross-maze task may not solely reflect improved allocentric memory, but an improved ability of the rats to integrate different spatial strategies, such as learning to go in a particular direction on the basis of a sense of direction that guides path integration, and go to a particular place on the basis of distal visual cues.

The current research also provided first evidence of the possibility of pharmacologically induced functional recovery after ATN lesions. In recent years, numerous pre-clinical studies have been conducted looking at possible agents and substances that can promote recovery after brain injury (see Liepert, 2008 for review). The discovery of endogenous, brain-derived trophic factors that affect neuronal growth and viability has supported proposals that degenerative disorders (Ramaswamy, Soderstrom, & Kordower, 2009; Zuccato & Cattaneo, 2009) and possibly acute brain injury (Marklund, Bakshi, Castelbuono, Conte, & McIntosh, 2006) might be treated with specific factors which selectively affect neural systems that become dysfunctional. The main difficulty with using naturally occurring nerve growth factors is the need for direct intracerebral application. A substance with trophic-like properties, low in toxicity and yet able to cross the blood brain barrier, would be a promising line of treatment. The neuroprotective drug Cerebrolysin is able to cross the blood brain barrier and is able to reduce necrotic and apoptotic changes in the damaged brain tissue as well as enhance glucose metabolism; it also has low toxicity (Gschanes, et al., 1997; Windisch, et al., 1998). Cerebrolysin has been previously demonstrated to be effective in producing recovery of spatial reference memory after hippocampal (Karoleva, et al., 1994) and fimbria-fornix (Valuskova & Francis-Turner, 1999) lesions. This evidence made it a viable candidate for examining the possibility of promoting recovery after diencephalic damage.

Post-surgery administration of Cerebrolysin for 30 days promoted behavioral recovery in ATN lesioned rats on the spatial working memory task, and the magnitude of recovery was comparable to that produced by enrichment alone. This represents the first attempt in using Cerebrolysin to promote recovery in the diencephalic amnesia model and further extends the potential therapeutic effectiveness of the drug. The mechanism of action of Cerebrolysin is not clear, but it has been shown to enhance glucose utilization in the disturbed brain regions (Kofler, et al., 1990), as well as promote neurogenesis in the dentate gyrus, potentially by inhibition of the spontaneous apoptosis of progenitors increasing the number of BrdU-positive newborn cells (Tatebayashi, et al., 2003). It is possible to speculate that these neural changes underlie the behavioral effects of Cerebrolsyin observed after ATN lesions. Furthermore, there is evidence that Cerebrolysin can produce marked increase of synaptophysin-immunoreactive presynaptic terminals in entorhinal cortex, the dentate gyrus, and hippocampal subfields CA1, CA2, and CA3 (Reinprecht, et al., 1999; Windholz, et al., 2000). This increased synapse density in the hippocampus may possibly underlie the improved memory functioning observed in treated ATN rats. Although the evidence from the current experiment concerning Cerebrolysin effectiveness is preliminary, it clearly points to the fact that there is value in exploring further the potential of different pharmacological agents, such as Cerebrolysin, to treat diencephalic amnesia.

Another unique contribution of the current study to the field of recovery of function was an attempt to combine two different modes of treatment to promote recovery after ATN lesions. Unfortunately, only a very limited number of animal studies have tried to apply a similar strategy (Johansson, et al., 1997; Puurunen, et al., 2001), which is regrettable, as it has been suggested that in the human domain that a combination of pharmacology and psychosocial strategies represents the best approach to promote optimum recovery (Nolan, 2005). In the current thesis, the combination of enrichment and Cerebrolysin resulted in cumulative improvement for lesioned rats on the spatial working memory task when a 40-second delay between a sample and test phase was introduced. However, no evidence of cumulative benefit was seen on the standard (approximately 5 to 10-second delay) version of the task. While the current evidence is limited, as only 3 sessions of delayed trials were analyzed, it provides some indication that when the

retention demand of the task increases, that the combination of treatment modalities leads to better behavioral outcomes.

10.2 Contributions of the current research towards understanding of the role of ATN in memory

In addition to providing first evidence on the possibility of recovery of function after ATN lesions the current research also contributed to further expanding our understanding of the role that the ATN play in memory processes. Both clinical and animal evidence have previously pointed to the fact that structures within the medial thalamus contribute to memory. Evidence from human cases strongly implicates the mammillothalamic tract and/or disruption of the ATN in the amnesic syndrome associated with thalamic amnesia (Harding, et al., 2000; Van der Werf, et al., 2000; 2003). Aggleton and Brown (1999) suggested that damage to the extended hippocampal system, which is comprised of hippocampus, fornix, mammillary bodies and the ATN are a common feature of diencephalic amnesia. Animal models using primarily rodents have confirmed that ATN are implicated in episodic-like memory processes (Aggleton & Brown, 1999; Mair, et al., 1998; Wolff, et al., 2006). The Aggleton group has demonstrated that it is the acquisition of allocentric spatial memory, that is, memory which is dependent on the use of environmental cues, which is particularly sensitive to hippocampal and ATN lesions (Aggelton & Pearce, 2001). They primarily based their conclusion on data that ATN animals are grossly impaired in performing spatial alternation task in a T-maze and related spatial memory tasks (Aggleton, et al., 1996, 1995a,b; Gaffan, et al., 2001; Warburton & Aggleton, 1999; Warburton, et al., 1999; 2000; 2001; Wilton, et al., 2001).

The present research has been able to provide further supporting evidence for the involvement of ATN in episodic memory. Across all experiments, the ATN lesioned rats not receiving any therapeutic intervention demonstrated marked deficits on the spatial-working memory task in the cross-maze. In support of the previous literature (Warburton, et al., 1999), pre-training on this task did not reduce the magnitude of the deficit. The current study showed that these deficits persist long-term even when the extensive and repeated post-surgery testing is used. Furthermore, consistent with the results provided by the Aggelton laboratory, the ATN rats in the current thesis demonstrated more pronounced deficits on the allocentric/direction-type

trials in the cross-maze. In previous T-maze studies the “opposite start position” trials were used only as probes (Aggleton, et al., 1996; 1995a). On these probe trials the ATN rats were observed to demonstrate more marked deficits that on the standard “same start position” trails, a pattern that was seen indicative of allocentric-type memory impairment. However, the failure of ATN animals in solving the “opposite start position” trials when these were used as probes might have been a reflection of reaction to novelty and change in procedure. In the current study, the animals were originally trained pre-surgery on both types of trials in the cross-maze and both types of trials (egocentric and allocentric type) were used to later examine the performance of rats post-lesion. Despite the repeated exposure to the “opposite start position” trials the ATN rats continued to perform at a chance level on these trials highlighting further the contribution of ATN to allocentric-type memory. The cross-maze set-up tends to encourage the use of multiple strategies (for e.g. direction as well as allocentric) (see Chapter 3 for detailed discussion) and cues even in sham rats (Futter & Aggleton, 2006), which makes it difficult to conclude whether poor performance of the ATN rats on the “opposite start position” trials is reflective of a pure allocentric deficit. What is clear is that when animals are given an opportunity to rely on egocentric cues (“same start position” trials) better preserved performance is observed. Here, across experiments, some of the ATN animals were able to achieve high accuracy over sessions on the egocentric-type trails (nearly 80%), which supports the conclusions made by Aggleton and colleagues (1996) that ATN is not involved in egocentric learning. Of interest too is the observation that lesions that damage the total 100% of the target ATN nuclei were not necessary to produce severe impairments on the spatial memory task; lesions over 50% damage still induced marked behavioral impairments. However, much smaller lesions of 20%-40% damage were not sufficient to result in significant behavioral deficits (see Chapter 8). This observation highlights the notion that the most robust behavior impairments are obtained when lesions to the ATN involve all of its subcomponents (Aggleton, et al., 1996; Byatt & Dalrymple-Alford, 1996). The beneficial effect of treatment on spatial-working memory performance seemed to be independent of the lesion size and rats with either nearly total or relatively small (just over 50%) degree of damage benefited equally from being exposed to enrichment and/or injected with Cerebrolysin.

There is now considerable evidence avaliable that the ATN are also involved in spatial reference memory (Warburton, et al., 1999; 2000; 2001; Wolff, et al., 2008). In the present study, we examined ATN rats performance on a novel reference memory task that required spatial pattern separation for three different types of problems (close, medium, wide) based on fixed locations in a 12-arm maze. While the ATN lesioned rats found the adjacent arm discrimination more difficult than other discriminations, they also demonstrated deficits in solving the easier, wide and medium separations. There is theoretically little demand for pattern separation in the intermediate and wide problems, as there is little or no overlap of relevant spatial information in those tasks. Hence the existence of a general impairment in ATN rats on these discriminations suggests that the ATN are involved in more general aspects of spatial memory, such as consolidation, rather than spatial pattern separation. The 12-arm radial maze task used was modeled on that described by McDonald and White (1995) although these researchers used an 8-arm radial maze with only two conditions, widely separated arms (180 degrees) and two adjacent/close arms. McDonald and White (1995) found that rats with fornix lesions were impaired at discriminating between adjacent arm locations only, suggesting that hippocampal system involvement may only be necessary for place learning when cues identifying arm locations are ambiguous.

The finding that ATN rats found all spatial discriminations difficult to solve suggests that lesions to fornix and ATN may not necessarily always result in similar impairments. In fact, there have been some reports that ATN, fornix, and hippocampal lesions may produce different patterns of deficits, with mixed differences found in terms of conditional memory tasks that test the acquisition of various contextual or visually guided arbitrary associations (Sziklas & Petrides, 2002; 2004; 2007). Sometimes, rats with ATN lesions show more severe spatial memory and conditional learning deficits than that shown by rats with fornix injury (Sziklas, et al., 1998; Sziklas and Petrides, 1999; Warburton and Aggleton, 1999). Specifically, impaired acquisition after both ATN and hippocampal lesions, but intact performance after fornix lesions, has been found when a rat must choose an object based on its location (Sziklas et al., 1998; Sziklas & Petrides, 1999). By contrast, hippocampal lesions impair acquisition of a conditional task in which rats select a location, based on a visual cue, but in this instance rats with ATN or fornix lesions appear to be able to use either egocentric cues or place cues and achieve normal rates of

acquisition (Sziklas et al., 1998; Sziklas & Petrides, 1999; 2002; 2004; 2007). From this perspective, the deficit on the radial-arm maze discrimination problems after ATN lesions in the current study is thus valuable in providing a second example of a dissociation between the effects of fornix and ATN lesions, but this time outside the context of conditional associative learning, (but also see section 10.4). Alternatively, while the majority of the available evidence suggests that the ATN lesion effects are derived from their prominent connections with the hippocampal system, it is also possible that there is a role for the relatively weak connections between the Intralaminar nuclei (ILn) and the retrohippocampal region, especially the perirhinal cortex (Van der Werf, et al., 2002). The ILn lesions (Gibb, et al., 2006) have no effect on standard hippocampal-dependent spatial memory tasks, yet have a profound effect on associative memory when a spatial component provides one of the arbitrary cues. Hence to some extent the impairments demonstrated by the ATN rats on the spatial pattern separation task may reflect hippocampal as well as prefrontal system contributions, resulting in more marked impairments being observed than when only fornix lesions are performed.

There is a general agreement in the literature that the ATN are not involved in memory for reward value (Mitchell & Dalrymple-Alford, 2005), which is seen to be subsumed under the function of the mediodorsal nuclei. Current results further support the notion that ATN are not required for optimal reward memory function as lesions to the ATN did not significantly affect rats' ability to discriminate between solutions of different concentrations.

Overall, the behavioral results obtained across experiments conducted in this thesis clearly confirm the ATN's involvement in episodic-like memory and reinforce the view that both the ATN and the hippocampus are critical components of an integrated hippocampal-diencephalic system.

10.3 Neurobiological Contributions

The current study provided additional support for some of the neurobiological changes that occur in the brain in response to the ATN lesion. In his recent review of the extended hippocampal system model, Aggleton (2008) suggested that the severity of the amnesic deficits observed after damage to the subcomponents of the system can be explained by the presence of

dysfunction in another region, namely the retrosplenial cortex. Aggleton (2008) highlighted that retrosplenial dysfunction is a common feature of both temporal and diencephalic amnesias and that the loss of retrosplenial cortex function may not only exacerbate effects of the temporal and diencephalic lesions but may also help explain why the two types of amnesia share so many features. Several studies have now come from the Aggleton laboratory that demonstrated the presence of marked neuronal hypoactivity in the retrosplenial cortex region after unilateral and bilateral lesions to the ATN (Jenkins, et al., 2004; Poirier & Aggleton, 2009) as well as after lesions to the hippocampus (Albasser, et al., 2007) and transection of the mammillothalamic tract (Jenkins, et al., 2004). Aggleton (2008) suggested that the retrosplenial hypoactivity after ATN lesions is not merely caused by global disconnection as the anterior thalamic lesion leaves intact other inputs into retrosplenial cortex.

The present study detected evidence of marked retrosplenial hypoactivity following ATN lesions. At four months survival time, the ATN induced hypoactivity was observed in the superficial and deep cell layers of the rostral granular retrosplenial cortex (Rgb), as well as to a lesser extent in the superficial cell layers of the caudal granular retrosplenial cortex (Rga). Research by Poirier and Aggleton (2009) demonstrated that Rgb, and in particular the superficial cell layers of Rgb, become affected early on post-ATN injury (1-2 weeks), while changes in the deep cell layers of Rgb take longer to emerge (8 weeks). The presence of superficial and deep cell layer Rgb hypoactivity detected in the current thesis fits the time line of retrosplenial changes described by Poirier and Aggleton (2009). In the present thesis no changes in the dysgranular retrosplenial region (Rgd) were detected. In their research Poirier and Aggleton (2009) demonstrated that hypoactivation in the Rdg may take a long time to develop (1 year), and hence the current survival time of 4 months would have been too short for the Rdg changes to become evident.

Previous attempts to elucidate the mechanisms responsible for reinstitution of function led to the suggestion that injury to the brain has “distal” as well as proximal effects, supporting the idea of diathesis (Stein, 1994). The key element of the diathesis theory is that the injury to the brain may affect far-removed structures closely related morphologically or functionally to the region destroyed. The behavioral recovery is thus seen to be tied to the rate and extent to which

the suppression of functioning in these distal regions dissipates. In the current thesis, we originally hypothesized that retrosplenial cortex may be the structure which is contributing to the observed behavioral recovery after ATN lesions, given the evidence that ATN lesions appear to suppress the activity of this region.

Contrary to expectations, exposure to the therapeutic modalities of enrichment and/or Cerebrolysin did not result in improvements in the levels of Fos activation in the retrosplenial cortex. The behavioral gains observed following exposure to enrichment and Cerebrolysin were not correlated with improved neural activation in the retrosplenial or any other regions assessed, such as prefrontal cortex and hippocampus. In fact, exposure to enrichment resulted in further hypoactiviation in the retrosplenial cortex region.

In the literature on enrichment other instances of the dissociation between functional and structural changes have been observed which indicates that a complex pattern of interaction between behavior and neurobiology exists. For example, the exposure to enrichment in AD enriched transgenic mice resulted in shorter swim distances to reach the hidden platform in the water maze and fewer working memory errors on a radial water maze task (Jankowsky et al., 2005) but produced no changes in the amount of neuritic plaque burden in the hippocampus or the levels of amyloid-β present. Similarly, exposure to enriched environment of Huntington's disease (HD) mice did not rescue the HD-induced reduction in dendritic spines in striatal medium spiny neurons and pyramidal neurons of cingulate cortex or ameliorated the shrinkage of striatum and cingulate cortex (Spires, et al., 2004), while still producing a positive behavioral effects. In attempt to explain this disparity the authors suggested that the reserve developed in the enriched HD mice could be based at least partially on the changes in the hippocampal neurogenesis that underlie some of the beneficial effects of enrichment whereas the differential molecular and morphological effects induced by environmental enrichment may indicate that the spine pathology does not contribute as much to symptomatology as the molecular changes (Spires et al., 2004). Alternatively, it is possible to speculate that some behavioral effects are mediated by anatomical changes and others by molecular changes.

The currently observed dissociation between the level of retrosplenial cortex activation and behavioral changes in enriched rats pose a challenge to Aggleton's model of covert pathology as it suggests that the retrosplenial cortex may not be crucial for spatial memory performance. Indeed, there is evidence that lesions to the retrosplenial cortex do not always result in robust spatial memory deficits and lack of or relatively minor impairment has been observed on T-maze alternation (Harker & Wishaw, 2004; Pothuizen, et al., 2008) and Morris Water maze tasks even after extensive retrosplenial cortex lesions (Harker & Whishaw, 2002; Sutherland, et al., 1988; Vann & Aggleton, 2002, 2004; Van Groen, et al., 2004; Warburton, et al., 1998). Another hypothesis could be that the retrosplenial cortex is not crucial for the performance of the spatial working memory task in the cross-maze, but may still play a role when other types of spatial memory functions are evaluated or other behavioral paradigms are used, which is a question requiring further investigation. Alternatively, it might be the case that enrichment is having an effect on other ATN-lesion induced changes in the retrosplenial cortex, rather than Fos, which in turn translated into behavioral improvements seen. For example, Garden and colleagues (in press cited in Poirier and Aggleton 2009) also noted that ATN lesions induce loss of long-term depression (LTD) in the superficial Rgb tissues but not in the deep cell layer of Rgb. ATN lesions also have been shown to result in reductions in adrenergic, cholinergic, serotonergic and GABAergic receptors (Van Groen et al. 1993) in retrosplenial cortex. Enrichment could be potentially counteracting one or a number of these changes or its effects may also be more evident when other IEG's are examined such as *zif268*.

Another option could be that enrichment effect is operating on other components of the memory circuitry and not the retrosplenial cortex. One such possible candidate could be the prefrontal cortex. After reviewing literature on the involvement of prefrontal cortex in memory Kesner and Rogers (2004) proposed that it is responsible for the application of rules that guide behavior, while the hippocampus is more responsible for event-type memory. They have suggested that these rule and event based systems both work in concert and independently. The researchers also conducted a series of experiments which examined spatial memory on the delayed non-matching-to-sample task in the radial maze and were able to establish that in rats with dorsal hippocampal lesions the spared medial prefrontal cortex allowed for compensation for the deficits when the sample-trial delay was short (10 seconds). However, the dorsal

hippocampus became a necessary structure for spatial working memory following a longer delay (5 min), whereas the medial prefrontal cortex failed to show compensation for this time window. In a similar vein, Mogensen and colleagues (Mogensen, Moustgaard, Khan, Wörtwein & Nielsen, 2005) observed that rats with fimbria-fornix lesions performed better on the acquisition of a spatial delayed alternation task in a T-maze in comparison to rats with combined fimbria–fornix and prefrontal cortex lesions, which was seen as in indication that the prefrontal cortex contributes to mediation of post-traumatic functional recovery.

While it is possible that prefrontal cortex may be one of the areas which contributed to the behavioral change observed in treated ATN rats on the spatial working memory task, the presence of prefrontal cortex hypoactivitation in the enrichment groups is also puzzling. There is considerable evidence that spatial memory performance is associated with increases in Fos activity in the prefrontal cortex (de Pisapia Slomski & Braver, 2007; Jansma, Ramsey, Slagter & Kahn, 200; McCarthy, et al., 1994). Enrichment induced hypoactivation in the prefrontal areas suggests that other mechanisms may be operating and indicates that behavioral performance may not necessarily directly translate into increased levels of activation. In fact, hypoactivity may actually reflect more efficient processing of information and hence better task performance. For example, lower levels of stress and decreased attention to those stimulus dimensions that do not predict successful spatial performance have been shown to result in lessened IEG activity (Redhead, Roberts, Good, & Pearce, 1997; but see Whishaw & Mittleman, 1986). Shires and Aggleton (2008) and Poirier and colleagues (Poirier, Amin & Aggleton, 2008) have recently proposed that levels of spatial memory performance need not simply be expressed in increased levels of IEG in any particular region that underlies memory function, but rather by dynamic patterns of IEG expression across brain regions. One possible way to capture these dynamic changes may be via application of structural equation modeling to estimate the casual relationships between patterns of Fos activation across brain regions and behavioral performance.

Overall, the current findings lend support to the observation put forward by Aggleton's laboratory that lesions to the ATN result in IEG hypoactivity in the retrosplenial cortex. However, the functional significance of this effect remains debatable, as in the current thesis behavioral gains noted after exposure to enrichment were associated with further hypoactivation

in retrosplenial as well as prefrontal areas. Several explanations have been put forward which require further investigation, including that enrichment possibly promotes changes in other IEG's or other neural markers in the retrosplenial cortex, that it induces dynamic patterns of change in IEG expression across brain regions, or that it promotes changes in other brain regions.

10.4 Relevance of current research to human populations

As was pointed out in Chapter 4, the main aim of laboratory research is not to provide treatments directly applicable to the human domain, but to demonstrate the fundamental processes of brain plasticity which can than be used to guide the intervention in clinical populations. With that premise in mind there are several valuable insights that could be gained from the current research to inform better treatment protocol for patients with diencephalic amnesia.

Most importantly, the demonstration that enrichment and Cerebrolysin could promote recovery of function after ATN lesions, at least on the task of spatial working memory, suggests that individuals with diencephalic amnesia can also potentially benefit from therapeutic intervention. This evidence challenges the current belief that the cognitive changes experienced by the sufferers are permanent and irreversible (Kopelman, 2002). The efficacy of Cerebrolysin in enhancing recovery of function suggests that there is value in further exploring the therapeutic potential of this drug. Cerebrolysin has already been used with Alzheimer's dementia (Ruther, et al., 2002) and stroke patients (Ladurner, et al., 2005) and has proven safety in humans. Extending the drug application to sufferers of diencephalic amnesia and potentially Korsakoff's patients is now indicated. This is a particularly exciting prospect, as thus far research on pharmacological treatment of Korsakoff's syndrome has been limited and yielded controversial results (Angunawela & Barker, 2001; Cochrane, et al., 2005).

In the field of brain injury recovery, it is generally proposed that therapeutic intervention should begin immediately after injury and as soon as the patient can tolerate environmental stimulation. Many clinicians believe that unless recovery occurs within a relatively short space of time post-injury that there will be no recovery at all. Unfortunately, immediate therapeutic intervention may not always be possible due to delays in accessing treatment and also because in conditions such as Korsakoff's syndrome and thalamic stroke a period of post-injury recovery is

mandatory. The success of the current study in demonstrating that therapeutic intervention could be beneficial in promoting recovery when introduced some time following acute brain injury, and when arguably post-injury neuronal changes have for the most part stabilized, offers hope to diencephalic amnesia sufferers. It also suggests that reducing environmental stimulation by confining patients to structured hospital environments may not be the optimal therapeutic strategy. While it is true that a long course of medical and psychosocial therapy may be expensive and time consuming it nonetheless may be necessary for any recovery to be observed in patients. Such therapy must be conducted in a supportive and "enriched" environment, with close cooperation and coordination between treating professionals.

The research presented in this thesis suggests that a combination of therapeutic approaches may possibly yield the most beneficial effects in diencephalic amnesia. The current thesis has provided preliminary evidence that environmental and pharmacological combination was more effective in promoting recovery on a 40-sec delay version of the spatial working memory task. In the research laboratory we tend to examine single agents to determine the specific mechanisms associated with functional recovery. As different pharmacological agents are identified and different rehabilitative techniques are developed it may be necessary to combine them to produce maximum effect. Similar evidence that the combination of drug and behavioral treatment is most beneficial is beginning to emerge in the Alzheimer's disease literature, where the combination of donepezil and errorless practice was reported to produce stronger cognitive outcomes (Rothi, et al., 2009). Additionally, the more is learned about the continuum of brain changes experienced by sufferers the better we will be able to coincide treatment with different therapeutic windows of efficacy. It is therefore possible that environmental enrichment coupled with pharmacological agents will hold the best hope for functional recovery for diencephalic amnesia patients.

10.5 Unresolved issues, limitations and future directions

Behavioral limitations- It is important to note that the conclusions with regard to therapeutic effects of enrichment and Cerebrolysin in the current study were primarily based on the evidence of improved performance on one task of spatial working memory. The previous literature on enrichment suggests that task specific effects may exist with performance across tasks being

differentially affected (Bindu, et al., 2005; Dalrymple-Alford & Kelche, 1987; Galani, et al., 1997; Kelche, et al., 1987; Will, et al., 1981). In the present study, task-specific effects were also observed. Enrichment did not have any effect on the lesion-induced deficits in acquiring fixed spatial discriminations in the radial-arm maze. The presence of enrichment-induced recovery on the spatial working memory task, but not on the spatial reference memory task, limits the generality of the conclusions from the current study in terms of spatial memory. It raises the question of whether reference memory in general is resistant to the effects of an enriched environment in rats with ATN lesions. More importantly, the cross-maze procedure provided unambiguous evidence of improved working memory, but it was not clear that the overall improvement reflected allocentric spatial memory specifically.

A follow-up study conducted in our laboratory, with the participation of the present researcher, has addressed this question (Wolff, Loukavenko, Will & Dalrymple-Alford, 2008). In that study ATN rats were trained to swim from a constant single start point to a submerged platform held in a fixed position in a Morris water maze, which encouraged the establishment of spatial representations that emphasized escape-specific associations, either with simple direction or a relatively fixed set of extra-maze cues and a relatively fixed swim trajectory (Eichenbaum, et al., 1990). As multiple visual cues were available during training, a strong test of whether these representations could be used in a flexible manner was provided by the comparison of performance on subsequent probe trials that used novel start positions and those trials that used the previous constant (“regular”) start position. Rats with ATN lesions that were housed in standard conditions showed a substantial deficit when probe trials with new starts were used that explicitly challenged the flexibility of the spatial representations that they had acquired. The performance of the enriched ATN group and of the ATN standard group was comparable on these ‘‘regular’’ trials during probe testing. There was however, dramatically superior performance shown by the ATN enriched group on the novel-start probe trials. These findings provided convincing evidence for recovery of both allocentric spatial reference memory in general and its flexible use in novel task demands.

It remains uncertain why the ATN enriched rats did not show improvements in the acquisition of simultaneous spatial discriminations in the radial-arm maze task, particularly in the

instance when the radial-maze arms were adjacent and the spatial cues to guide performance would engage spatial pattern separation processes that were presumably utilized by the enriched ATN rats in the task used in the water maze study. It is likely that subtle procedural differences in the cognitive demands of various tasks may have contributed to these differences. The radial-arm maze enclosed the rat inside the central hub from which the rat was required to make a choice, whereas both the use of more distant start positions and more open environments afforded by the elevated cross-maze used for the alternation task and the Morris maze would more readily encourage the use of distal environmental cues and path integration strategies. In addition, vestibular stimulation by passive rotation of the rats during the radial-arm maze testing, which is known to impair the use of spatial information (Kirwan, et al., 2005), may have adversely disrupted the ATN enriched rats. The possibility that enrichment induced effects may be vulnerable to interference, such as rotation, suggests that recovery of function may be fragile and that distraction can negatively affect the rats' performance. An interesting extension of the current study with an aim to examine possible disruptive effects of rotation would be to use a cross-maze positioned on a turntable and rotate the apparatus for "opposite start position" trials instead of carrying the rat across to the opposite start area. Additionally, it would be interesting to examine whether manipulation of salience of the extra-maze cues or use of probe trials with less or more cues will affect the enriched rats' performance on the cross-maze may provide insights into how the enriched rats use the extra-maze cues to guide their responses. Assessing the performance of enriched ATN animals on additional tests of episodic-like memory is also warranted. For example, using a test paradigm developed by Eacott and colleagues (2005) which assesses episodic memory by evaluating the rats' ability to learn that object locations are stable within context but are different between contexts, may provide insights into how enrichment affects the rats ability to acquire "when" and "what" aspects of episodic representation. Using tasks on which ATN-induced deficits have already been demonstrated such as a spatial memory task in a 12-arm-radial maze (Moran & Dalrymple-Alford, 2003) or a temporal order memory task (Wolff, et al., 2006) may further help extend the generality of enrichment effects currently observed.

Other behavioral factors which may potentially explain the improved performance of the enriched ATN rats concern the enrichment experience itself. Various studies in the past have tried

to delineate which components of enrichment are crucial for promoting recovery, such as exercise (Gomez-Pinilla, et al., 1998; Kleim, et al., 1996, 1997), social aspect (Einon, et al., 1980; Finger & Stein, 1982) or physical complexity (Will, et al., 1986). It was also suggested that the interaction of factors rather than any single element of enrichment explains behavioral effects (van Praag, et al., 2000). However, increasing evidence indicates that social and inanimate stimulation can have different effects on selective brain regions. Inanimate stimulation has been noted to produce structural and biochemical changes mainly in the cortical regions and hippocampus (Van Praag, et al., 2000; Wurbel, 2001), and irrespective of the social component has been shown to accelerate habituation to novelty (Schrijver, et al., 2002). Lack of social stimulation irrespective of inanimate background has been associated with changes in pre-frontal, cortico-striatal monoamine pathways (Robbins, Jones & Wilkinson, 1996; Wurbel, 2001). As the current study utilized both social and inanimate stimulation it is difficult to conclude which aspect of the environment was most crucial to recovery observed. Ideally future studies will examine the impact of different aspects of the environment on the behavior of ATN rats (social vs. isolate, or enriched vs. barren). Across different experimental studies in the literature the enrichment protocol is always the most variable part, with different research groups utilizing different lengths of exposure, cage size and physical complexity set-ups. These variations in experimental design may account for inconsistent results obtained in the field of enrichment. Developing a standardized enrichment protocol which clearly specifies the duration of exposure, the type of cages and objects used may be important in promoting consistency between studies and across laboratories when behavioral effects of enrichment are examined. A standardized protocol has now been introduced at University of Canterbury laboratory (B. Harland & J. Dalrymple-Alford, personal communication, 27 October, 2009).

In the current study we have made the first attempt to use pharmacological intervention to observe the possibility of recovery of function after ATN lesions. The evidence obtained with regard to the effectiveness of Cerebrolysin in reducing the behavioral deficits is encouraging. It was particularly interesting to see that the combination of both enrichment and Cerebrolysin was beneficial on the 40-sec delay spatial working memory task. However, more convincing evidence may be needed before a case is made for making broad conclusions about the impact of cumulative treatment effects. Only 3 sessions of delayed trials were currently analyzed and more

extensive testing with a delay paradigm may be required. Challenging the animals during the delay by introducing distraction or interference or by extending the delay interval may also be useful in ascertaining whether the treated rats are able to demonstrate response flexibility and withstand distraction or whether they are only able to demonstrate better performance while holding the needed spatial information "on-line." As noted above for the enrichment effects, investigation of Cerebrolysin effects on other tasks of memory including reference memory in the Morris Water maze and temporal order memory will be needed to establish the generality of the drug effect. In the current thesis we have chosen Cerebrolysin over a vast array of other available drugs based on its postulated neuroprotective properties as well as low toxicity and ease of administration. The effectiveness of other pharmacological agents particularly those belonging to the acetylcholinesterase inhibitor group, which have already been shown have some efficacy in Alzheimer's dementia (Kurz, Farlow & Leferve, 2009), may be also worthwhile to explore following ATN lesions. Evidence exists that the stimulation of acetylcholine release in the hippocampus is decreased following thalamic injury (Savage, et al., 2003; 2007) and a limited number of studies with small sample sizes found some beneficial effects of acetylcholinesterase inhibitors in Korsakoff's patients (Iga, et al, 2001; Sahin, Gurvit, Bilqic, Hanagasi, & Emre, 2002).

In the field of recovery of function in clinical populations, one of the most important determinants of outcome is considered to be the context in which the recovery occurs. Individual characteristics of the organism, and in particular sex of the individual, are now seen as one of the factors that may effect the selection of appropriate course of treatment and the prediction of outcome. (Stein, et al., 1993). In laboratory settings the sex of the rats may be a variable that may potentially influence the treatment outcome. In the current thesis, female rats were used across all studies (male rats being used in other experiments at the time). The vast majority of experiments that examined enrichment-induced recovery after acute brain injury have used male rats and the ability of male rats to benefit from enrichment is well-established (Einon, et al., 1980; Galani, et al., 1997; Kelche & Will, 1982; Pacteau, et al., 1989; Wolgin & Teittelbaum, 1978). However, it is possible that to some extent sex differences may be influencing recovery after ATN lesions. A number of studies have now been able to demonstrate that female hormones estrogen and progesterone are associated with better recovery after brain injury and experimental stroke (see

Stein, Wright & Kellermann, 2008 for review). However, in the context of neurodegenerative disorders, it was female 3xTg-AD mice that demonstrated worse behavioral performance on the water maze in comparison to males (Clinton, et al., 2007). In environmental enrichment studies some researchers suggested that females may respond better to environmental stimulation in comparison to males. For example, Pereira and colleagues (2008) found that ischemic female rats benefited more from enrichment, demonstrating better performance on the working memory protocol of the water maze in comparison to males. Frick & Fernandez (2003) reported that in female mice the enrichment induced improvements in the water maze were associated with increased hippocampal levels of synaptophysin, whereas behavioral outcomes were related to reduced synaptophysin levels in males. Bennett and colleagues (2006) suggested that it is possible that different neuronal mechanism may underlie the behavioral changes in males and females. Hence, an appropriate extension of the current study would be to examine the enrichment effects in ATN lesioned male rats. The current data on the ability of the female ATN rats to demonstrate recovery is valuable as it adds to the understanding of how sexual dimorphism may potentially mediate recovery (Nithianantharajah & Hannan, 2009).

Neurobiological limitations - The current study attempted to provide some preliminary evidence pertaining to the neurobiological changes that accompany behavioral recovery following ATN rats' exposure to enrichment and/or Cerebrolysin. The results were encouraging, as we have been able to confirm the presence of marked hypoactivity in the retrosplenial cortex following ATN lesions. However, enrichment induced behavioral improvements were associated with further hypoactivity in the retrosplenial region. Clearly, the neurobiology responsible for the behavior change that occurs following therapeutic intervention requires further investigation. The main limitation of the current neurohistochemical investigation was an absence of matched pseudo-trained controls. The issue of behavioral controls in IEG experiments has recently been discussed by Shires and Aggleton (2008). IEG activity is non-specific and Fos activation can be triggered by random events, including general sensory stimulation and motor activity. The researchers argued that commonly used caged-controls may not be appropriate when assessing memory and learning related IEG changes. The experiences of the caged-controlled animals might be different from those engaged in a memory task as the caged-controls are not interacting with the environment. Consequently, the caged controls demonstrate reduced levels of IEG

activation in the somatosensory, motor and frontal areas which can cloud the interpretation of IEG differences in the experimental groups. In their study Shires and Aggleton (2008) tried to determine the differences in IEG activity in intact rats by exposing one group to an active learning task in the Morris Water Maze and the other only to the procedural aspects of the task, as well as then matching the groups on swim time and swim distance. The researchers found that procedural controls showed lower levels of activation of Fos in the prefrontal areas, but did not differ in the level of activation in the hippocampus even though water maze learning is considered to be hippocampus dependent. This unexpected finding highlights the point that the best behavioral controls for IEG experiments should be yoked to produce a clear picture of what changes in IEGs are lesion and/or treatment dependent. In the current experiment, the observation that enrichment led to behavioral improvements but yet produced reduced activation in the retrosplenial and prefrontal regions implies that the hypoactivation observed was unlikely to be due to non-specific aspects of the task. A more extensive study utilizing yoked matched controls, which would be forced to the left or right arm on each run from yoked start areas in the cross-maze task may allow for more unequivocal conclusions to be drawn with regard to the changes in levels of Fos activation following ATN lesions and enrichment.

Future research directions may also focus on investigating other markers for neurobiological changes that may occur following enrichment and/or Cerebrolysin administration. For example, Cerebrolysin has been shown to enhance the growth of neurons in tissue cultures (Lindner, et al., 1975) and treatment with Cerebrolysin resulted in marked increase in presynaptic terminals in the dentate gyrus, and hippocampal subfields CA1, CA2, and CA3 (Reinprecht, et al., 1999; Windholz, et al., 2000). Moreover, in apo-E deficient mice that were treated with Cerebrolsyin an inverse correlation between the performance of the mice on the Morris Water Maze and the dendritic and synaptic content was observed (Masliah, et al., 1999) This evidence suggests that the behavioral changes observed in ATN rats following Cerebrolysin administration might be related to its ability to modify synaptic structure and function, which warrants the use of synaptophysin assays following Cerebrolysin treatment. Additionally, Cerebrolysin is known to modulate the expression of the blood brain barrier (BBB)-GLUT1 glucose transporter gene by increasing the mRNA transcript stability (Boado, 1996). As it has been shown that that this transporter is regulated by neurotrophic factors the effect of

Cerebrolysin on the BBB-GLUT1 model might support this as another possible target for Cerebrolysin treatment effects (Boado, 1996).

Exposure to enrichment has been associated with a multitude of cellular and molecular changes with each one specifically, or probably in combination, being potentially responsible for the enrichment induced behavioral improvements observed (see Nithianantharajah & Hannan, 2009). Some neurobiological processes seem to hold more promise than others when investigating the recovery of function following diencephalic amnesia. Many researchers have demonstrated that enrichment leads to structural changes in dendrites. Increased dendritic branching has been observed in the occipital cortex (Volkmar & Greenough, 1972), temporal cortex (Greenhough, Volkmar & Juraska, 1973) and hippocampus (Rampon, et al., 2000). Increased dendritic spine density may indicate enhanced synaptic contacts. Within the hippocampus increased dendritic complexity and enhanced spine counts have been reported for dentate granule neurons and pyramidal cells in area CA1, CA3 and dentate gyrus in animals housed in enriched environment (Juraska, et al., 1985; Rampon, et al., 2000). Examining changes in dendritic morphology in the hippocampus and possibly other brain areas such as prefrontal and rhinal cortices may present one avenue for establishing the mechanisms underlying recovery following ATN lesions, and this work in now underway in our laboratory. Another line of research has focused on enrichment induced neurogenesis. Kempermann and colleagues (1997) have been able to demonstrate that enrichment increases neurogenesis in the hippocampus and Young and colleagues (1999) also noted that it is associated with reduced apoptosis in the region. Various links have been suggested between hippocampal neurogenesis and behavior, including the proposal that newly born neurons are directly involved in the transient storage of hippocampus-dependent memories, and may also increase the storage capacity of the hippocampus, or alternatively, that the birth of new neurons contributes to the refinement of the hippocampal circuitry which promotes adaptation to functional demands (see Ehninger & Kempermann, 2008 for review). These proposed links between adult neurogenesis and hippocampal-dependent cognition implies that enrichment induced enhancement of hippocampal neurogenesis may be associated with behavioral changes observed in enriched ATN rats.

On a physiological level enrichment has been shown to increase measures of synaptic plasticity in the hippocampus, in particular long-term potentiation (LTP) (Duffy, Craddock, Abel & Nguyen, 2001; Foster & Dumas, 2001), as well as enhance LTP and decrease long-term depression (LTD) in the anterior cingulate cortex (Shum, et al., 2007). Recordings of excitatory postsynaptic potentials in the hippocampus and cingulate cortex in enriched ATN rats may help to establish whether the behavioral gains are a result of improved synaptic plasticity. At a molecular level, changes in the neurotransmitter levels have been associated with exposure to enrichment including changes in glutamenergic, GABAergic, cholinergic, serotonergic and dopaminergic systems (Nichols, et al., 2007, Segovia, et al., 2008). As noted in Chapter 4, Galani and colleagues (2007) were able to observe that rats that underwent serotonergic depletion and were exposed to enrichment demonstrated improved sensory-motor and memory function. Exposure to enrichment also resulted in significant increase of the serotonin turnover observed several weeks after surgery in the ventral and dorsal hippocampus of the lesioned animals, which led the researchers to suggest that enrichment enhanced the function of unaffected serotonin neurons. Moreover the serotonin levels in the ventral hippocampus and the norepinephrine levels in the dorsal hippocampus were altered by exposure to enrichment and were negatively correlated with reduced anxiety response on the elevated plus maze demonstrated by enriched rats. Segovia and colleagues (2008) also reported that enriched rats subjected to handling stress demonstrated lower dopamine and acetylcholine levels in the prefrontal cortex. Changes in individual neurotransmitter levels or the dynamic pattern of change in the balance of neurotransmitters may also be underlying improved performance of the enriched ATN rats.

One of the most extensively replicated findings in relation to enrichment is that exposure to enrichment has been associated with increased levels of brain derived factor (BDNF) and nerve growth factor (NGF) (Ickes, Pham, Sanders, Albeck, Mohammed & Granholm, 2000; O'Callaghan, Griffin & Kelly, 2009; Torasdotter, Metsis, Henriksson, Winblad & Mohammed, 1998) with the neurotrophic changes being most extensively studied in the neocortex and hippocampus. BDNF is considered to be important in a number of neurodegenerative disorders such as Alzheimer's dementia, Huntington's and Parkinson's diseases (Zuccato & Cattaneo, 2009) and levels of BDNF and NGF have been linked to cholinergic innervation of hippocampus and spatial memory (Pham, et al., 1999). It might be worthwhile to explore whether BDNF

changes underlie the memory impairment in diencephalic amnesia and whether the levels of BDNF can be rescued by enrichment.

It seems most likely that enrichment as well as Cerebrolysin-induced behavioral outcomes observed after ATN lesions are mediated by variety of cellular (neurogenesis, synaptogenesis and synaptic plasticity) and molecular (gene expression, including those encoding neurotrophins, neurotransmitter receptors, transcription factors, cytoskeletal proteins and regulators of neurogenesis) factors, rather than by a change in any one particular factor. Beginning to understand and explore the mechanisms that underlie behavioral recovery following ATN lesions may provide insights into the mechanistic basis of brain recovery in general and also lead to novel therapeutic approaches which can boost brain function in sufferers of diencephalic amnesia.

10.6 Summary

In summary, the current thesis provided first evidence that severe episodic-like memory impairments following anterior thalamic lesions can be ameliorated by exposure to enriched environment, administration of a pharmacological agent Cerebrolysin or both. The enrichment effects on spatial working memory were particularly robust and were observed even when the introduction of enrichment was delayed post-surgery. The effects were also maintained in the long-term. Despite the success in revealing that substantial behavioral improvements can be obtained by exposing animals to enrichment and Cerebrolysin, the anterior thalamic lesioned rats did not demonstrate a full reinstitution of function, although there was some evidence using delay trails that combination of both treatment modalities resulted in an optimal behavioral outcome. Task-specific effects of enrichment were also observed, with enriched ATN animals continuing to demonstrate impairments on a task of spatial discrimination. However, subsequent work (Wolff, et al., 2008) has generalized the positive effects of enrichment, demonstrating an enrichment enhanced ability of ATN animals to flexibly use spatial representations to guide responses on a different task of spatial reference memory. The research discussed in the current thesis represents a first step towards investigating the possibility of recovery of function after thalamic lesions and additional research focusing on using a wider array of episodic memory tasks as well as other pharmacological agents will be necessary. However, present findings pose

an intriguing prospect of possibility of recovery of function after diencephalic amnesia and may add to knowledge concerning the adaptive response of hippocampal-dependent processes to thalamic injury and the neural mechanisms subserving memory.

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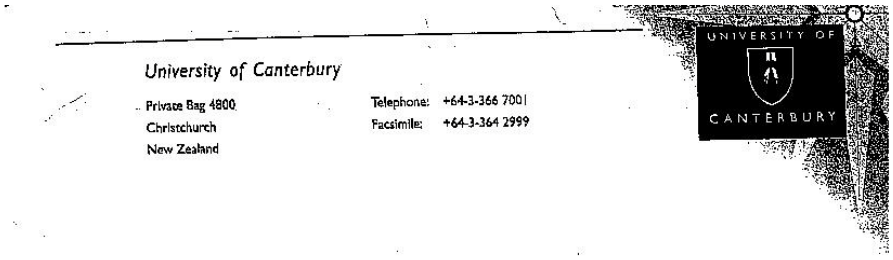
APPENDIX A

Animal Ethics Approval



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16 July 2003

Elena Loukavenko
Department of Psychology
UNIVERSITY OF CANTERBURY

Dear Elena

I am pleased to inform you that the Animal Ethics Committee has approved your application entitled: 2003: **11R - Memory Systems and Recovery of Brain Function.**

Yours sincerely

G. S. Gillon