The Role of the Amygdala and Other Forebrain Structures in the Immediate Fear Arousal Produced by Footshock Exposure

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ABBREVIATIONS

ASR	acoustic startle response (reflex)
ANOVA	analysis of variance
AP5	DL-1-amino-5-phosphonopentanoic acid
AP	anterior - posterior
BLA	basolateral nucleus of the amygdala
BNST	bed nucleus of the stria terminalis
CA1	field CA1 of hippocampus
CA2	field CA2 of hippocampus
CA3	field CA3 of hippocampus
CA4	field CA4 of hippocampus
CCK	cholecystokinin
CNQX	6-cyano-7nitroquinoxaline-2, and 3-dione
CNS	central nervous system
CRH	corticotrophin- releasing hormones
CS	conditioned stimulus
DV	dorsal - ventral
GABA	gamma – aminobututyric acid
GAD	glutamic acid decarboxylase
ML	medial - lateral
NE	norepinepherine
NMDA	N-methyl-D-aspartate
PET	Human Positron Emission Tomography

PnC	caudal pontine reticular nucleus
PTSD	post traumatic stress disorder
SCR	stimulus conditioned response
SEM	standard error of measurement
SP	substance P
UCS or UC	unconditioned stimulus
VTA	ventral tegmental area

ABSTRACT

When a human or animal is threatened or confronted with a stimuli signalling danger, internal defence mechanisms are activated that evoke feelings of fear and anxiety. These emotional responses promote the behaviour patterns necessary for an organism's survival. Animal research seeks to understand how these emotions affect behaviour both physiologically and neurologically in order to develop effective treatment for those suffering from severe anxiety disorders. The aim of this thesis was to examine the role of the amygdala, and dorsal and ventral hippocampus in relation to immediate fear arousal brought on by footshock. This was assessed by examining whether muscimol would interfere with the acoustic startle response before or after footshock presentation, and then comparing these reactions to a control group that received saline infusions. The results of this research are extremely important because they identify various brain structures involved in the fear-arousing effects of footshock as measured by the shock sensitization of acoustic startle. Laboratory rats received muscimol (0.1ug and 0.01ug) infusions into the basolateral amygdala, dorsal and ventral hippocampus. These three brain regions have been identified as playing a prominent role in fear neurocircuitry. The results demonstrated that the GABA A receptor agonist muscimol in doses of 0.1ug and 0.01ug reliably blocked shock sensitization of the acoustic startle response. The muscimol doses did not alter the shock reactivity amplitudes therefore indicating a normal perception of the fear arousing properties of footshock. Therefore, the present study's results suggest that a decrease of GABA activity in the amygdala, dorsal and

ventral hippocampus may be essential for the neuronal basis of fear acquisition and expression of unconditioned and conditioned stimuli.

1.0 INTRODUCTION

1.1 The Emotion of Fear

Animals survive in nature through their effective innate defensive reactions. When an animal is confronted with a sudden or new stimulus it reacts immediately it either runs away (flees), freezes, or adopts an aggressive response (fight). These responses vary from animal to animal but they are automatic and without the thought that typifies humans (Bolles, 1970). Evolutionary theory proposes that an organism's survival is dependent upon its ability to learn quickly and with little training (Öhman & Mineka, 2001). The organism must learn to fear a potential stimulus that could threaten its survival. Learning is an adaptation that occurs when an organism must cope with the various environmental changes that occur throughout its lifetime. Permanent changes in the brain occur when an animal learns to fear a stimulus. Learned fear is different to sensitized fear in that learned fear requires a permanent change in the brain's neurocircuitry, which is achieved through past encounters within aversive contexts (Öhman & Mineka, 2001).

The fear model states that fear is activated automatically by specific fear arousing stimuli. This automaticity of the defence response is important for an organism's survival and ability to adapt to changing environments (Öhman & Mineka, 2001). A slight hesitation may be the difference between an organism living and procreating, or dying and ending the transmission of its genes to new generations. When confronted with danger an automatic rapid response is needed instead of a complex cognitive analysis of

the situation. People who suffer from severe phobias often recognise that the fear they experience is excessive and unreasonable. It is proposed that fear responding that is evoked by certain fearful or aversive stimuli are not influenced or changed by cognition; rather once the response is activated it runs its course of action (Öhman & Mineka, 2001). The fear defence system is controlled by specific neuro-circuitry in the brain. Recognition that the fear mechanism has an ancient history suggests that those areas most likely to be involved would be located in the more primitive structures of the brain, such as the limbic system (Öhman & Mineka, 2001).

1.2 How Fear is Measured in Animals

Fear is expressed through various behavioural modalities, such as verbally, cognitively, motorically, and physiologically. Experiments using selective association, selective sensitization, and latent inhibition only measure one or two of the fear response systems (Koch, 1999). It is thought that what closely relates to fear in an animal is the measurement of their defensive responding, such as increased heart rate, facial muscle activity, increased blood pressure, freezing, or the fear potentiated startle reflex response. Davis and his colleagues (1992) used the potentiation of the startle reflex to measure fear in their animals. Startle potentiation has been observed to occur many times when an animal has been fear conditioned to a fear potentiating stimulus. Fear potentiation is the startle reflex of the whole body of the animal. In humans eyeblink amplitudes have been readily used and observed (Koch, 1999). These various expressions of fear are the activation of defensive behavioural systems that rise in reaction to threatening stimuli.

These expressions of fear have all been identified as the most important components for inferring fear (Koch, 1999).

1.3 The Acoustic Startle Response

The startle response can be obtained in many animals and humans; it causes the subject to react to a visual, acoustic, or tactile stimulus resulting in rapid movements of facial and body muscles (Landis & Hunt, 1939). The reaction begins with eyelid closure, then facial muscles contract moving to the neck and skeletal muscles (Koch, 1999). Other physiological reactions include an increase in heart rate, while other behaviours are slowed down and are less likely to occur. The startle response has been identified to perform a protective function from injury and predators; it also increases the likelihood of survival and prepares the organism for the fight/flight response (Koch, 1999). Despite startle's simple nature it can be manipulated by a variety of variables. Startle has a non-zero baseline which means it can be magnified and reduced (Koch, 1999). Therefore, many researchers have used startle as a behavioural tool to understand sensorimotor response plasticity (Koch, 1999). The greatest amount of research on the acoustic startle response (ASR) has taken place with mammals, namely rats, mice, cats, and humans (Koch, 1999). ASR research has found that the same stimulus parameters used on animals generate the exact same response patterns in humans (Koch, 1999). Results from many animal studies have been generalised to humans. Due to the parallel similarity found among rats and humans, it has been proposed that the neuronal mechanisms involved in the ASR may help explain human sensorimotor integration (Koch, 1999).

The ASR varies in stimulus intensity and inter-stimulus interval which affects the startle magnitude and latency. The ASR varies among participants and can be influenced by genetic differences such as diurnal rhythm, the sensory environment, background noise, illumination pre-pulses, and various drugs (Koch, 1999). The ASR can be magnified or enhanced by conditioned and unconditioned aversive stimuli. The ASR can be modified through the variation of experimental conditions and by manipulating the perceptual and emotional state of the organism (Davis, 1996; Davis, Walker, & Yee, 1997). Drugs have been widely used to change the magnitude of the ASR and to assess effects on sensorimotor reactivity in both animals and humans (Davis, 1980; Davis, Falls, Campeau, & Kim, 1993). For example, anxiogenic drugs such as yohimbine reduce the inhibitory neurotransmission of the CNS and heighten the ASR (Fendt & Schnitzer, 1994). In contrast, drugs that decrease the excitability of the CNS, such as ethanol or diazepam inhibit the ASR (Berg & Davis, 1984; Grillon, Sinha, & O'Malley, 1994). The infusion of these different drugs into specific brain structures has brought about a number of investigations of the various pathways that mediate and moderate the ASR. Such manipulations are thought to either enhance or inhibit the transfer of information between sensory receptors and motor effector systems (Koch, 1999).

Because startle is assumed to have a protective function against predators and survival, it is proposed that in a threatening situation or aversive event, the ASR is enhanced as a result. It is readily observed that when experimental rats are presented with an aversive event the ASR is consistently enhanced (Koch, 1999). Research utilising rats has demonstrated that when an animal is presented with a cue predicting an aversive

event (fear potentiated startle), the presentation of bright illumination, electric footshock or a loud noise results in the enhancement of startle (Davis, 1989). Other research has found that the ASR has been observed to increase after lesions of the septum, olfactory bulbectomy, and stimulation of the amygdala, VTA and the lateral periaqueductal area. The infusion of drugs into particular brain structures can also enhance or reduce the magnitude of the ASR (Davis, 1980).

1.4 Sensitization

Sensitization is achieved in a short time frame when an enhanced response to fear stimuli occurs after the fear state has already been activated. Öhman and his colleagues (2001) discovered that fear-relevant stimuli elicit an increase in stimulus conditioned response (SCR) in contrast to fear-irrelevant stimuli, and the threat of electric shock augments the response. Sensitization refers to the enhancement of a response after the presentation of a strong stimulus. The majority of ASR sensitization research is based on a dishabituation design. This posits that the magnitude of the ASR pre-shock habituates due to repeated presentation of startling stimuli and is compared to the ASR after the footshocks. However, Davis (1989) shows in his studies that aversive shocks increase the ASR magnitude way above the initial level (pre-shock, before habituation occurs). The term sensitization refers to this notion, enhancement of the ASR before habituation occurs. Sensitization is a non-associative form of learning due to the fact that the subject does not associate a particular event with an aversive stimulus. Researchers suggest that

the underlying mechanisms of sensitization in the acoustic startle response are similar to that of the fear-potentiated startle reflex.

Some researchers have put forward the idea that animals are conditioned to various background noise during ASR experiments (Fendt & Fanselow, 1999; Richardson, 2000). Richardson and Elsayed (1998) found that shock sensitization of the ASR is mediated by context conditioning. They used two manipulation experimental designs that reduced the conditioning to the test context, and proposed that the manipulations would have little effect if shock sensitization is mediated by unconditioned fear (Richardson & Elsayed, 1998). Their results suggest that the shock sensitization paradigm is in fact mediated by contexual conditioning rather than a state of fear that is unconditionally elicited by shock. Animals shocked in a different context than the testing environment, or given extensive pre-exposure to the test context previous to shock did not exhibit sensitization of the startle response (Richardson & Elsayed, 1998).

1.5 Pavlovian Fear Conditioning

Seligman's theory of fear acquisition proposes that intense fear can be established through Pavlovian Conditioning for stimuli that have threatened an organism's survival in the past in comparison to stimuli that have never been a threat (fear irrelevant stimuli), or fear stimuli that have only recently emerged in our lifetime (fear relevant stimuli, eg. guns, cars, electric outlets, (Öhman & Mineka, 2001). Prepared associations are easy to acquire and require only one trial of learning, but they are more resistant to extinction in

comparison to non-prepared associations (Öhman & Mineka, 2001). Seligman's theory has been supported by many animal studies regarding many types of learning including fear conditioning. The early premise of learning theorists such as Seligman, Thorndike, and Pavlov was that all conditioned stimuli and unconditioned stimuli combinations are learnt with relative ease (Öhman & Mineka, 2001).

The fear potentiated startle paradigm was developed in 1951 and has been further investigated by Davis and his colleagues (1993). It involves training animals to associate a neutral stimulus (light or tone) with an aversive stimulus (mild electric footshock, Koch, 1999). After the subject receives a number of these pairings the conditioned stimulus (CS) elicits a state of fear and is measured by the potentiation of the ASR. The state of fear is the conditioned response to the CS (light), and the potentiation is how the fear is measured in the animal (Koch, 1999). The presentation of the CS also produces other fear related reactions such as freezing, an increase in blood pressure, and bradycardia (Davis, 1992; Le Doux, 1996). Pavlovian conditioning is the most frequently used by many behavioural researchers. Pavlovian Conditioning involves 1 – 2 training sessions of 10 – 20 pairings of a 3.7 second light with a 0.5 second electric footshock of mild intensity (0.6mA), presented 3.2 seconds after the onset of the light. Fear conditioning tests are typically performed 4 – 24 hours after conditioning (Koch, 1999). The initial fear test consists of tone or noise alone trials in the absence of the light to get animals consistently responding to the noise (ASR). Then the light is presented with the tone or noise trials later in the testing procedure. The difference in ASR magnitudes between the noise alone and light plus noise trials is the measure of fear that the animal

exhibits (Koch, 1999). Research has found that rats tend to show very low levels of the fear potentiated ASR when they have received very high levels (eg. 1.6mA) of electric footshock. This may be due to the high intensity of the footshock triggering an active instead of a passive defence mechanism that reduces the fear potentiated ASR (Walker & Davis, 1997).

Contextual fear is another effect shown to occur in Pavlovian conditioning. In contextual fear the subject also begins to fear the context in which they receive the light and shock pairings. Contextual fear is also a conditioned effect and is acquired quite quickly (Fendt et al., 1999). The fear potentiated startle reflex can be blocked or interfered with by certain drugs. Some examples of such drugs are the benzodiazepine agonists, dopamine antagonists, and opioid agonists (Fendt et al., 1999). The properties and actions of these drugs have been shown to modify the animal's state of fear. In particular, the anxiolytic effects of these drugs have been demonstrated in human studies (Davis, 1979; Davis et al., 1993; Fendt et al., 1999). Therefore, the fear potentiated startle reflex is a phenomenon that occurs across many species and has been observed in humans (Grillon & Davis, 1997). These findings lend support to the view that the expression of fear in humans and animals are similar in their underlying casual mechanisms.

1.6 The Role of the Amygdala in Fear

Ample research has demonstrated that the amygdala plays a regulatory role in an animal's emotional response. The limbic system was thought to be primarily responsible for controlling the neural basis of emotion. However, this assumption soon diminished as specific nuclei and pathways of the limbic system emerged as playing key roles in memory and cognitive, as well as emotional functions (LeDoux, 1993). The limbic areas most important for emotional processes are less involved in cognitive processes. LeDoux (1996) has identified the amygdala as the central structure responsible for the neural fear circuitry which has more efferent than afferent connections within the cortex. Therefore, the amygdala sends out more information to the cortex than it actually receives. Animal research has found that in very stressful events the prefrontal cortex; where high levels of thinking takes place, shuts down and behaviour is then controlled by subcortical defence mechanisms. Human Positron Emission Tomogrophy (PET) studies have shown similar findings in that working memory in the prefrontal cortex areas are deactivated during extremely stressful situations (LeDoux, 1993).

A large number of studies have implicated the amygdala as the region of the mammalian brain responsible for fear processing. When certain emotional stimuli are presented to an animal, neurons in the amygdala become active. The efferent fibers of the central nucleus of the amygdala project through the stria terminalis and the ventral amygdalofugal pathway to areas in the diencephalons and brain stem, which have been identified as important in the expression of fear-related behaviour (LeDoux, 1987; Smith & De Vito, 1984). Electrical stimulation of the amygdala has been observed to enhance

startle amplitudes and also produce physiological changes such as elevated blood pressure that are apparent in fear episodes (Rosen & Davis, 1988; Davis, 1997). The pathway identified as controlling behavioural fear originates from fibres within the amygdala, which extend to the ventral periaqueductal gray in the midbrain (Fanselow, 1994; Le Doux, Iwata, Chicchetti, & Reis, 1988). In contrast, the pathway important in producing physiological changes is identified as originating within the ventral amygdalofugal pathway and extends to the lateral hypothalamus. Therefore, the amygdala has been the primary focal point in fear research.

Research has established that the amygdala is an integral component in the expression of emotions for both animals and humans and that it contributes less to cognitive processing (Fendt & Fanselow, 1999; LeDoux, 1993). Both the amygdala and the hippocampus are involved in storing and retrieving memories about emotional experiences and events (LeDoux, 1993). LeDoux (1993) states that the differences between the amygdala and the hippocampus is based on the finding that the amygdala stores and retrieves emotional experience, while the hippocampus consolidates and retrieves non-emotional experiences. Research carried out in order to understand the underlying mechanisms involved in forming, establishing, and retrieving emotional experiences and memories have used the fear conditioning paradigm. Questions approached by this research has looked at how innate emotional responses are activated and controlled by environmental events, how learning of novel environments occur, and how information about aversive events are stored and used for future encounters of threatening environments (LeDoux, 1993).

1.7 Neurobiology of the Amygdala

The amygdala has been identified as being central to the neural circuitry employed in mediating and modulating fear behaviours for both humans and animals. It is located in front of the hippocampus and adjacent to the temporal lobe. The amygdala is made up of thirteen nuclei, containing numerous subnuclei (Pitkänen, 2000). The nuclei that have been identified as playing an integral role in fear include the basolateral complex (which consists of the lateral, basal, and accessory basal nuclei) and the central nucleus (which is divided into the capsular, lateral, and medial divisions). These nuclei are crucial to both sensory input and motor output for fear learning and memory systems, as well as behavioural responses to fear (Rosen, 2004). The nuclei of the basolateral complex is a rich sensory area that receives sensory input from the thalamus and cortex (Rosen, 2004). Within this complex, auditory and visual information is received via the lateral nucleus. The basolateral complex also receives various inputs from the hippocampus and the prefrontal cortex. The direction of information flow in the basolateral complex is generally lateral to medial, with extensive reciprocity and communication within the amygdala (Pitkänen, Savender, & LeDoux, 1997). It has been posited that the basolateral complex's most prominent role is to interpret sensory information (Davis & Whalen, 2001); Rosen & Schulkin, 1998, which influences other amygdala nuclei and brain areas that produce a fear response. The lateral nucleus projects to the basal and accessory basal nuclei of the complex, and has direct efferent pathways to the central nucleus.

The central nucleus of the amygdala has vast projections to nuclei found in the midbrain and brainstem, resulting in quick automatic behavioural and endocrine fear responses to possible threats of danger (Davis, 1992; Hostege, 1995). The prefrontal cortex innervates the central nucleus which controls the expression of learned behaviour (Quirk, Likhtik, Pelletier, & Pare, 2003). The central nucleus receives instinctual information from the brainstem and reciprocally projects the information to other brain areas. The central nucleus has various important roles in fear, it produces fear behaviour and receives instinctual information that in turn influences the central nucleus's activity within the amygdala. Another important output pathway that has been identified is the basal nucleus efferent to the nucleus accumbens. Nauta and Domesick (1984) suggested that this pathway links motivation and motor control with organised active behaviour (Amorapanth, LeDoux, & Nader, 2000; Gray, 1999; Swanson, 2000; Swanson & Petrovich, 1998; Yim & Mogenson, 1982). Therefore, the central nucleus projections to the lower brain areas are responsible for reactive automatic behaviours, and endocrine fear responses such as freezing, while the efferents of the basal nucleus of the amygdala are involved in fear avoidant responses that are hypothesised to traverse the accumbens, striatum, and thalamus (Amorapanth et al., 2000). Recently, the medial nucleus of the amygdala has been shown to play a regulatory role in endocrine responses via efferent projections to the hypothalamus (Dayas, Buller, & Day, 1999).

The amygdala has been shown to have an association with the olfactory system. The amygdala receives olfactory information through a number of pathways (Pitkanen, 2000; Shipley, McLean, & Ennis, 1995). The amygdala's medial nucleus receives

extensive input from the main and accessory olfactory systems, however, the lateral, basal, and central nuclei do not receive any direct input from the olfactory system. Conversely, the accessory basal nucleus receives significant input from the olfactory system, and projects directly to the lateral nucleus of the amygdala (McDonald, 1998; Savander, Go, LeDoux, & Pitkanen, 1996), therefore suggesting that the basolateral complex and the central nucleus fear circuit of the amygdala are able to participate in the fear expression of the olfactory system, especially olfactory fear conditioning (Otto, Cousens, & Herzog, 2000). Finally, the bed nucleus of the stria terminalis is not strictly part of the amygdala, but is thought to be an extension of the amygdala (Alheid, de Olmos, & Beltramino, 1995). The bed nucleus of the stria terminalis has many reciprocal connections to the amygdala and is thought to play a role in unconditioned fear behaviour.

1.8 The Fear Circuitry of the Acoustic Startle Response

The neuronal basis of the fear potentiated ASR has been intensely researched by many groups which have provided a relatively complete picture of the fear circuitry involved. Research involving lesions and drug infusions of the amygdala has identified the amygdala as an important structure in the sensitizing effects of electric footshock (Fendt et al., 1994; Davis, 1992; Sananes & Davis, 1992; Schanbacher, Koch, Pilz, & Schnitzler, 1996). The startling stimulus, such as a loud noise is aversive and produces a state of fear or anxiety in the subject. The amygdala has been proposed to control and activate the startle reflex. The circuitry identified in sensitization of the ASR by

footshock is thought to begin at the PnC, which projects from the medial central amygdaloid nucleus via the caudal division of the ventral amygdalofungal pathway to the PnC (Koch, 1999; Davis et al., 1993; Campeau & Davis, 1995).

When the central nucleus or basolateral amygdala is lesioned, fear in the ASR is blocked (Davis et al., 1993). It has been suggested that the association between neutral and aversive stimuli takes place in the lateral/basolateral nuclei of the amygdala (Campeau & Davis, 1995). The perirhinal cortex relays the visual or auditory CS information to the amygdala. Infusion of NMDA antagonists such as DL-2-amino-5phosphonopentanoic acid (AP-5) into the basolateral amygdala has been shown to block fear acquisition, proposing the view that NMDA receptors are involved in the process of association between neutral and aversive stimuli in the amygdala (Campeau, Miserendino, & Davis, 1992; Gewirtz & Davis, 1997). It has been proposed that the fear potentiated ASR circuit receives its fearful input from the PnC (Davis et al., 2003). Research carried out by Davis and his colleagues (1993) found that when the amygdala is lesioned, the fear-potentiated startle response is blocked, suggesting that the pathway from the amygdala and PNC is also damaged. It was also discovered that the amygdala – PNC pathway is crucial for the reinstatement of fear after Pavlovian conditioning (Fendt et al., 1999).

Research has revealed that the amygdaloid pathway may distribute glutamate and/or corticotrophin- releasing hormones (CRH). Results demonstrated that infusions of AP5 and x-helical CRH (a CRH antagonist) into the PnC resulted in blocking the fear

potentiated ASR (Fendt, Koch, & Schnitzler, 1996; 1997). Infusions of the glutamate receptor antagonists 6-cyano-7nitroquinoxaline-2, and 3-dione (CNQX) into the amygdala have also demonstrated blocked expression of conditioned fear; indicating that the intra-amygdaloid projections are activated by the CS, using glutamate receptor actions (Kim & Davis, 1993). The dopaminergic projection from the VTA to the amygdala has also been identified as playing a role in the expression of the fear potentiated ASR (Borowski & Kokkinidis, 1996; Lamont & Kokkinidis, 1998).

Infusions of CRH into the lateral ventricle, PnC, or infusion of cholecystokinin (CCK), Substance P (SP) into the PnC or amygdala have been shown to have enhancing effects on sentisitization. This indicates that these peptides are involved in sensitization. In contrast, research has shown sensitization can be blocked by infusion of SP antagonists into the PnC, or $\chi 2$ – adrenergic agonist ST – 91 into the amygdala. It has been observed that the enhancement of ASR by electric foot shock, bright illumination, or neuropeptide agonists outlasts the duration of the aversive stimulus. In contrast, the ASR in the fear potentiated startle is only enhanced momentarily when the aversive stimulus (CS) is presented suggesting that two different neuronal or neurochemical factors contribute to the potentiation of the ASR phenomenon. It has recently been put forward that the fear-potentiated ASR reflects a rapid conditioned response to a fear provoking stimulus but does not provide an ideal model for various states of anxiety in humans (Koch, 1999). Anxiety is not brought upon by certain specific stimuli, rather it is a state of discomfort and apprehension. Distinctions can be made between conditioned (fear) and unconditioned (anxiety) forms of ASR enhancement. For example, the anxiety disorder

post-traumatic stress disorder (PTSD) is generally caused by an explicit traumatic experience that is similar to that of fear conditioning. The brain structures that mediate conditioned and unconditioned forms of aversive fear learning have considerable overlap, but also have some differences in the brain mechanisms involved (Koch, 1999).

The hippocampus has also been identified as playing a role in the enhancement of the ASR by CRH, but not in the fear potentiation of the ASR. The amygdala is necessary for both the sensitization of the acoustic startle reflex and fear potentiation of the ASR by bright illumination, not in enhancement of ASR by CRH. Areas predicted to be involved in enhancement of the ASR are the direct projections of the bed nucleus of the stria terminalis that projects to the PnC, but also the indirect route of the bed nucleus of the stria terminalis to the PnC via the amygdala. Other nuclei between the central amydala and PnC have also been implicated in contributing to sensitization. These are the periacqueductal gray, the laterodorsal tegmental nucleus, and the mesencephalic nuclei (Koch, 1999; Davis et al., 1993).

1.9 The Role of the Hippocampus in Fear

Considerable research has concentrated on how the fear conditioning circuitry of the amygdala fits in with other larger brain regions. The brain is thought to have two different learning systems to perform the function of rapidly learning novel information and acquiring information about one's environment. Therefore, much research has concentrated on the functions of the hippocampus and posterior neo-cortex (McClelland,

McNaughton, & O'Reilly, 1995; Norman & O'Reilly, 2003; O'Reilly & McClelland, 1994; O'Reilly & Rudy, 2001). It was Scoville and Milner's (1957) investigation into bilateral resection of the medial temporal lobe that was shown to cause anterograde amnesia in humans. This resulted in a sudden interest in the neuropsychology of memory (Milner, Squire, & Kandel, 1998). The focus of many studies was the hippocampus and the most famous patient was H.M. who had substantial bilateral damage to the hippocampus (Corkin, Amaral, Gonzalez, Johnson, & Hyman, 1997; Scoville & Milner, 1957).

Many animal studies were carried out in order to determine the role of the hippocampus in amnesia. These studies were carried out until the 1960s and revealed that animals with large hippocampal lesions were still able to learn a variety of new tasks. The abnormalities that were highlighted were not in new task acquisition, but instead a tendency to persist in the learned responses once they were no longer appropriate (Bannerman, Rawlins, McHugh, Deacon, Yee, Bast, Zhang, Pothuizen, & Feldon, 2004). There was also evidence of preservative responding during extinction (Jarrard & Isaacson, 1965), difficulty in reversal learning (Kimble & Kimble, 1965), and excessive responding in operant schedules (Clark & Isaacson, 1965). These observations and results led to the formulation of hippocampal theories and investigation into the role that the hippocampus may play in behavioural inhibition (Bannerman et al., 2004).

Researchers have agreed that the major function of the hippocampus is to encode episodic and spatial memories (Squire, 1992; Vargha-Khadem, Gadian, Watkins, Connelly, Van Paesschen, & Mishkin, 1997). It has also been suggested that the hippocampus plays an important function in learning and forming memories. Human amnesia is commonly associated with damage to the medial temporal lobe, and it is postulated that the hippocampus is critically involved in severe declarative memory deficits. Spatial memory deficits in rodents are thought to model some aspects of episodic memory deficits in human amnesics. Research on rodents with hippocampal lesions have demonstrated memory deficits in maze tasks, which suggests that the hippocampus in rodents is important for spatial learning and memory (Bannerman, Grubb, Deacon, Yee, Felden, & Rawlins, 2003).

Recently, it has been revealed that the hippocampus is not only involved in forming memories, but also plays a general role in information processing and the successive regulation of behaviour. Bannerman and his colleagues (2004) recently described and defined the specific regions of the hippocampus. The dorsal hippocampus was identified as 50% of the subregion of the hippocampus volume, beginning at the septal pole (posterior hippocampus) and being primarily involved in spatial learning and memory (Bannerman, Grubb, Deacon, Yee, Feldon, & Rawlins, 2002). The ventral hippocampus subregion was defined as 50% of the hippocampus volume beginning at the anterior hippocampus, and being primarily concerned with anxiety-related behaviours. The ventral hippocampus has also been recently implicated in emotional processes that

are different to the amygdala, but is suggested to be associated with fear rather than anxiety (Bannerman et al., 2004).

1.10 The Various Functions of the Dorsal Hippocampus

Research has shown that the major input to the dorsal hippocampus is visuo-spatial information from primary sensory cortical areas projected via the association cortex, and the peririhinal and entorhinal areas (Amaral & Witter, 1995; Burwell & Amaral, 1998a; Burwell & Amaralb, 1998b; Dolorfo & Amaral, 1998a; Dolorfo & Amaral, 1998b). Other sensory input such as the olfactory input tends to be evenly distributed across the dorsoventral hippocampus (Moser & Moser, 1998). Research investigating different subregions of the hippocampus has revealed that lesions of these various areas have quite varying and distinct behavioural effects. Early behavioural experiments (Hughes, 1965; Nadel, 1968; Stevens & Cowey, 1973; Sinnamon, Feniere, & Kootz, 1978) and anatomical studies of the hippocampus (Siegel & Tassoni, 1971, Swanson & Cowan, 1977; Witter, 1986) have demonstrated a difference in the roles played between the dorsal and ventral hippocampus.

Moser and his colleagues (Moser, Moser, & Andersen, 1993) demonstrated that lesions to the dorsal hippocampus severely disrupted spatial learning in a Morris watermaze, resembling complete hippocampal lesions, while lesions to the ventral hippocampus showed no such effect. These results were later replicated again by Moser and his colleagues using ibotenic acid lesions instead of aspiration lesions (Moser, Moser,

Forrest, Andersen, & Morris, 1995). Results demonstrated that spatial learning was in tact, but animals still displayed impairment in the watermaze task, with only 26% of the dorsal hippocampus still remaining, and 60% of the ventral hippocampus still in tact. The use of ibotenic acid lesions resulted in having discrete and specific lesions to the intended areas as well as having the effect of preserving nerve fibers and cerebrovasculature passing via the lesioned area (Jarrard, 1989).

Moser's (Moser et al., 1993; Moser et al., 1995) results add to the view that different subregions of the hippocampus tend to be involved in various functions of the brain. A number of studies have also examined the various effects of dorsal and ventral hippocampal cytotoxic lesions on spatial learning using a variety of learning paradigms and have revealed similar results to that of Moser (Moser et al., 1993; Moser et al., 1995; Bannerman, et al., 2002; Bannerman, Yee, Good, Heupel, Iversen, & Rawlins, 1999; Kjelstrup, Tuvnes, Steffenach, Murison, Moser, & Moser, 2002; McHugh, Deacon, Rawlins, & Bannerman, 2004). The results of these studies have demonstrated impairment in spatial reference memory acquisition in the watermaze task and T-Maze tasks, (Bannerman, et al., 2002; Hock & Bunsey, 1998), and performance on the radial maze (Pothuizen, Zhang, Jongen-Relo, Feldon, & Yee, 2004) after receiving dorsal hippocampal lesioning, therefore demonstrating similar results as complete hippocampal lesions. In contrast, lesioning of the ventral hippocampus did not impair performance of the above tasks.

The difference found between the dorsal and ventral hippocampus in spatial learning may be less conclusive than it is conveyed, instead the ventral hippocampus may contribute to spatial learning under certain conditions (De Hoz, Knox, & Morris, 2003; Ferbinteanu & McDonald, 2000; Ferbinteanu, Ray, & McDonald, 2003). De Hoz and colleagues (2003) have demonstrated that rats with excitotoxic dorsal hippocampal lesions were able to acquire a spatial reference memory task after training in a Morris watermaze. Animals with complete hippocampal lesions did not show this improvement in performance, the results of the experiment suggest that under certain conditions the ventral hippocampus is able to contribute to spatial learning. These results support the view that there are anatomical differences along the septotemporal axis of the hippocampus suggesting extensive connectivity between the dorsal and ventral hippocampus, as well as having some functional interindependence between the two subregions (Amaral & Witter, 1989; Amaral & Witter, 1995).

1.11 The Various Functions of the Ventral Hippocampus

The ventral hippocampus subregion is quite different from its dorsal counterpart in its anatomical connections. The ventral hippocampus projects to the prefrontal cortex, while the dorsal hippocampus does not (Barbas & Blatt, 1995; Goldman-Rakic, Selemon, & Schwartz, 1984; Jay & Witter, 1991; Verwer, Meijer, Van Uum, & Witter, 1997). The ventral hippocampus is connected to the bed nucleus of the stria terminalis (BNST) and the amygdala (Henke, 1990; Krettek & Price, 1977; Petrovich, Canteras, & Swanson, 2001; Pitkänen, Pikkarainen, Nurminen, & Ylinen, 2000; Swanson & Cowan, 1977; Van

Groen & Wyss 1990), and also to other subcortical structures associated with the hypothalamic-pituitary-adrenal (HPA) axis (Amaral & Witter, 1995; Siegel & Tassoni, 1971; Swanson & Cowan, 1977; Witter, 1986). Many of the amygdala nuclei have reciprocal projections with the hippocampus, mainly affiliated with the basal and lateral nuclei (Pitkänen et al., 2000). The evidence of strong connectivity between the ventral hippocampus, hypothalamus, and amygdala suggests a possible role for the ventral hippocampus in fear and/or anxiety, therefore accounting for some evidence demonstrating the relationship between ventral hippocampal lesions and inhibited emotional responses. The most extensively studied paradigm is contextually conditioned freezing.

Freezing in rats is a fear response that immobilises the animal after fear conditioning. The rat associates an unconditioned aversive stimulus, such as footshock, with a conditioned stimulus and expresses their fear in the situation where escape is not possible (Fanselow, 1984). Many studies have demonstrated that hippocampal lesions both disrupt the acquisition and expression of contextual fear conditioning (Good & Honey, 1997; McNish, Gewirtz, & Davis, 1997). Some research has shown over recent years that the ventral hippocampus shows similar results to complete hippocampal damage in reducing the fear response of freezing after receiving a mild form of footshock (Richmond, Yee, Pouzet, Veenman, Rawlins, Feldon, & Bannerman, 1999; Kjelstrup et al., 2002). The reduction of freezing was demonstrated for both conditioning to a tone and a context where shock had already been delivered previously during the training stage. A similar decrease in freezing was also shown after ventral subiculum lesions

(Maren, 1999). In contrast, Richardson and his colleagues (Richmond et al., 1999) demonstrated that dorsal hippocapal lesions did not have any type of effect on freezing (Maren, Aharonov, & Fanselow, 1997).

The results of the various experiments suggest that the ventral hippocampus tends to be involved in conditioned freezing unlike its dorsal neighbour. However, these conclusions are still under debate and must be treated with some caution (Anagnostaras, Gale, & Fanselow, 2001; Anagnostaras, Gale, Fanselow, 2002, Bast, Zhang, & Feldon, 2001; Bast, Zhang, & Feldon, 2003; Kjelstrup et al., 2002). For instance, conditioned freezing may not be the best paradigm used for investigating the relationship between the dorsal and ventral hippocampus in anxiety-related behaviours, it is vitally important to explore other paradigms. Alternative approaches to the conditioned freezing paradigm is the use of pharmaceuticals to temporarily manipulate areas in the brain with intracerebral drug infusions. Many drug infusion experiments into the ventral and dorsal hippocampus have revealed that they both play a role in memory processing in conditioned fear responses (Anagnostaras et al., 2001; Anagnostaras et al., 2002, Bast et al., 2001; Bast et al., 2003; Sanders, Wiltgen, & Fanselow, 2003).

1.12 Role of ventral hippocampus and amygala in anxiety-related processes

Experiments investigating the effects of amygdaloid and ventral hippocampal lesions have revealed that they both contribute differently to the processing of fearful or anxiety provoking stimuli (Iwata, LeDoux, Meeley, Arneric, & Reis, 1986; Phillips & LeDoux, 1992; Richmond et al., 1999). This view is not a novel account (Gray & McNaughton, 2000) as research has shown that fear and anxiety are related to one another as it has been suggested that they should be thought of as a unitary construct (Davis & Shi, 1999; Gray & McNaughton, 2000; Ramos, Berton, Mormede, & Chaouloff, 1997). Davis and his colleagues state that fear is a phasic response to conditioned aversive cues, while anxiety is a tonic and unconditioned response to an aversive cue or situation (Davis & Shi, 1999). Gray and McNaughton (2000) have a similar view stating that fear is an avoidant or escape response (phasic) where the subject must escape a possible dangerous situation in order to survive. Anxiety arises from conflict or uncertainity and is a result of diffuse aversive stimuli: for example, a subject wonders whether it should enter or approach a dangerous situation. It is also proposed that fear is a pre-cursor of anxiety and that anxiety serves as an inhibitory fear response.

Research using the fear potentiated startle response have shown some supporting evidence for the separate roles that the hippocampus and amygdala play in fear and anxiety related behaviours. As the ASR is elicited by a sudden auditory stimulus that results in the skeletal muscles rapidly contracting, it has been assumed to be an unconditioned response to fearful stimuli. The potentiated startle reflex is mediated by the brain stem circuit (Davis, Gendelman, Tischler, & Gendelman, 1982; Yeomans &

Frankland , 1996) and modulated by the forebrain areas (Koch, 1999). The bed nucleus of the stria terminalis (BNST) and the amygdala are closely connected to the ventral hippocampus (Pitkänen et al., 2000; Swanson & Cowan, 1977) which access and control the startle circuit (Davis & Shi, 1999; Koch, 1999). Emotional responses have been shown to influence startle reactivity (Koch, 1999; Lang, 1995). In fact the enhancement of the startle reflex is a diagnostic criterion for human anxiety disorders and is also employed in animal models of fear and anxiety (Davis & Shi, 1999; Rodgers, 1997). The startle reflex is a protective response to an aversive or threatening stimulus and is able to be enhanced by negative emotional states, or attenuated by positive emotional states (Lang, 1995). In the fear potentiated conditioning paradigm rats are first taught to be fearful of the light (CS) that predicts the following footshock (UCS). The conditioned fear to the light is then measured by the startle response of the animal when the light is presented in the experimental chamber absent of the footshock. It has been shown by many studies that the fear-potentiated startle response can be blocked by lesions of the amygdala (Davis, 1992, Davis et al., 1982; Davis & Shi, 1999). However, lesions of the hippocampus have not been able to show reliable affects of the startle magnitude (Bast et al., 2003; Kemble & Ison, 1971; Leaton, 1981; Lee & Davis, 1997a; Lee & Davis, 1997b; Pouzet, Feldon, Veenman, Yee, Richmond, Nicholas, Rawlins, & Weiner, 1999). Microinfusions of a variety of drugs such as NMDA agonists or antagonists, tetrodotoxin, muscimol, and picrotoxin into the dorsal and ventral hippocampus have tended to attenuate the startle response (Bast et al., 2001; Bast et al., 2003; Wan, Caine, & Swerdlow, 1996; Zhang, Bast, & Feldon, 2000; Zhang, Bast, & Feldon, 2002). In contrast, the NMDA receptor antagonist MK-801 tended to show a potentiated basal

startle response when infused into the dorsal hippocampal region (Zhang, Bast, & Feldon, 2000). Therefore, no specific hippocampal role has been identified in the fear potentiated startle response.

A very different picture is beginning to emerge when the startle response is elicited by an anxious state, induced by intracerebroventricular (i.c.v) microinfusions of CRH. CRH infusions tend to elicit a response that resembles that of anxiety or stress (Dunn & Berridge, 1990). This is displayed through physiological, endocrinological, and behavioural changes which include an increase in the startle amplitude. Increasing evidence has shown that the hippocampus and its connectivity with the BNST represent a neural circuit that is intrinsic to the enhancement of the CRH startle. Research by Lee and Davis (1997a; 1997b) has been able to demonstrate that the primary action of CRH is most likely to be the BNST. However, cytotoxic lesions of the BNST completely block the CRH-enhanced startle response, but they did not show any effect on the acquisition of classical fear-potentiaed startle response to an explicit visual CS. In contrast, cytotoxic lesions of the central and basolateral nuclei of the amygdala have been shown to block the fear potentiated startle reflex, but have no effect on the CRH-enhanced startle. Therefore, this dissociation between the BNST and the amygdala lesions of the fear potentiated startle paradigm demonstrate and further lend support to the view that there are various components involved in the emotional states of fear and anxiety, with diverse underlying neural substrates. The research carried out by Lee and Davis (1997a; 1997b) is still unclear, however it does suggest that the ventral hippocampus may have projections to the BNST, which may be an important neural pathway that influences the

various states of anxiety (Amaral & Witter, 1995; Canteras & Swanson, 1992; Cullinan, Herman, & Watson, 1993; Swanson & Cowan, 1977) and that is different to the neural fear circuit of the amygdala.

1.13 The Present Study Summary

When a human or animal is threatened or confronted with danger, internal defence mechanisms are activated that evoke feelings of fear and anxiety. These feelings promote the behaviour patterns necessary for an organism's survival. Animal research seeks to understand how these emotions affect behaviour both physiologically and neurologically in order to develop effective treatment for those suffering from severe anxiety disorders (Fendt & Fanselow, 1999). Our research will attempt to understand the neural basis of fear and anxiety in rodents. This was carried out by modelling fear in laboratory rats using the fear-potentiated startle paradigm. The amygdala has been identified as playing a key role in fear learning in a variety of experiments and research. It has been demonstrated that damage or interference to this area causes a disruption in neurotransmission and conditioning effects. The purpose of this thesis is to examine the role of the amygdala, and dorsal and ventral hippocampus in relation to immediate fear arousal brought on by footshock. This was assessed by examining whether muscimol will interfere with the ASR before or after footshock presentation, and then comparing these reactions to a control group that receive saline infusions. The results of this research are extremely important because they identify various brain structures involved in the fear-arousing effects of footshock. We expected that the agonistic action of gamma –

aminobututyric acid A receptors (GABA A) produced by muscimol in the amygdala would block the shock-associated fear. However, the key question was whether or not other forebrain structures, like the dorsal and ventral hippocampus that are connected to the amygdala and also contribute to fear arousal.

2.0 METHOD

2.1 Subjects

Approximately 70 naïve male albino rats of the Wistar strain were used for the experiments. There were nine groups of rats with 7-8 subjects per group. 48 received varying doses of 0.1ug and 0.01ug of the drug muscimol (experimental group) and the other 22 (control group) received saline. The rats were bred in the University of Canterbury's Psychology laboratory and were held in a climatically controlled colony environment, in grouped-housing with free access to food and water. The subjects weighed approximately 300-350g when used.

2.2 Apparatus

The acoustic startle reflex amplitudes and footshock delivery were measured in four identical cages (16.5cm x 8cm x 9cm), which were located inside sound attenuating melamine chambers (60cm x 34cm x 56cm). The sides and lid of each startle cage (Med Associates, Fairfield, VT) were manufactured from stainless steel horizontal rods 0.25cm in diameter and were situated 1.5cm apart. The floor of the startle cage consisted of stainless steel rods, with a 0.45cm diameter. A metal frame 10cm away from each cage housed a 6.0cm speaker. The startle cages were mounted on a Med Associates load cell-based startle platform (25cm x 11.5cm x 4.5cm). Movement amplitude were rectified, digitised, and recorded by Med Associates software which controlled the white noise and scrambled shock stimuli. The acoustic stimulus was produced by a programmable audio

generator which consisted of a 100-ms white noise burst with a rise-decay time of 10ms. The ambient noise level in the chambers were 36dB as measured by a Bruel & Kjaer (Model 2235; Denmark) sound level meter (A Scale). The 600-μA footshock was delivered through the floor grid by constant current stimulators connected to commutators located on top of each sound attenuating chamber with stimulation leads attached to each startle cage.

2.3 Surgery

Surgery was performed in accordance with protocols approved by the Animal Ethics Committee at the University of Canterbury. Subjects first received atropine sulphate (0.12mg/kg) prior to anaesthesia in order to dry-up mucous secretions. Twenty minutes later they were then anaesthetised with sodium pentobarbitone (90mg/kg) and put in a Stoelting stereotaxic instrument (WoodDale, IL). Subjects received a mepivacaine (local anaesthetic) scalp injection (20mg/ml) before surgery and an anti-inflammatory local injection of ketofen (10mg/ml) prior to suturing (as required by the ethical guidelines). The horizontal plane of each subject's skull was levelled using the landmarks Bregma (anterior) and Lamda (posterior). Stereotaxic co-ordinates were calculated by using the stereotaxic atlas of the rat brain by Paxinos and Watson (1998). Stainless Steel guide cannulas (C313G, Plastics One, Roanoke, VA) were implanted bilaterally with an outer diameter of 0.71mm. The co-ordinates for bilateral implants into the amygdala were AP - 2.8, ML ± 4.8 and DV – 8.2, ventral hippocampus AP -5.6 ML ± 4.6 DV – 6.4 and dorsal hippocampus AP – 5.6 ML ±3.6 DV – 3.0. The implants were

fixed to the skull using dental cement and 4 stainless steel jeweller's screws (Lomat, Quebec, Canada). Subjects were left to recover for approximately 7 days before commencement of testing.

2.4 Procedure

2.4.1 Baseline Acoustic Startle (Pre-drug)

The procedure of the proposed experiment was approved by the Animal Ethics Committee of the University of Canterbury. Seven days post-surgery each subject's acoustic startle threshold was measured. The rat were first placed into the startle apparatus and given a 5-minute period of acclimatisation. They then received 2 sessions of 30 white noise bursts with a fixed interval of 20 seconds between each noise burst. The decibel level of the white noise bursts were alternated between 91, 95 and 99dB in intensity. For each subject, the noise level that produced an average startle amplitude between 100 and 400 units was selected and used for all subsequent testing.

2.4.2 Drug Infusion and Acoustic Startle Testing

The day after baselining the rats were returned to the startle apparatus and given another session of 20 noise bursts with a 30second interval between noise bursts at the pre-selected decibel level. The subject was then taken out of the box and infused with the drug muscimol (0.1μg/μlor 0.01μg/μl) or saline. The dummy cannulas were then

removed (C313DC, Plastics One) and 28-gauge (0.36mm) stainless steel infusion needles (C313I, Plastics One) were then inserted into each cannula. Polyethylene tubing (PE20, Plastics One) was pre-loaded with either muscimol or saline and attached to each cannula implant of the subject. The polyethylene tubing was attached to a 2-µl Hamilton syringe and infused over a 1 minute period at a volume of 0.5 µl per side with infusion pumps (Model 310, Stoelting). After infusion stopped, the needles were left for a further 2 minutes, and then removed and replaced with dummy cannulas. The subject was then returned to the startle apparatus and again put through a post-drug test. The test consisted of a 1 minute acclimatisation period, followed by a session of 20 noise bursts with a fixed interval of 30 seconds, then a session of 10 unsignalled footshocks with 10 second intervals, and lastly a session of 10 noise bursts with a fixed interval of 30 seconds.

2.5 Perfusion and Histology

70 subjects with bi-cannula implants into the amygdala, dorsal hippocampus and ventral hippocampus were culled and then perfused intracardially with saline, followed by a 10% formalin solution. The brains were removed quickly and stored in the formalin solution for one-day, and then transferred to a sucrose solution and refrigerated. The subjects' brains were then sliced (50µm) after 2-3weeks storage using a cryostat and were then mounted onto gel coated slides. The slides were then stained with cresyl violet and later evaluated using a microscope and the stereotaxic atlas of the rat brain by Paxinos and Watson (1998) so that the guide cannula placements could be verified.

3.0 RESULTS

3.1 Amygdala

3.1.1 Histology

Two rats in the amygdala group were excluded from the study after developing loose acrylic headcaps. The guide cannula locations in the basolateral amygdala for the remaining rats in the group (Saline N = 7, Muscimol 0.1ug N = 8, Muscimol 0.01ug N = 8) are depicted in Figure 1. In all 16 of the cannulated experimental rats, the centres of the infusion sites (the tips of the infusion cannulae) were located in the targeted areas within or around the border of the BLA. Guide cannula placements were implanted and aimed 1mm above the BLA (co-ordinates AP – 2.8mm from bregma, ML ± 4.8mm from the sagital suture, DV- 8.2mm from the skull surface). Visible tissue damage was restricted to the immediate surrounding area of the guide and infusion cannulae.

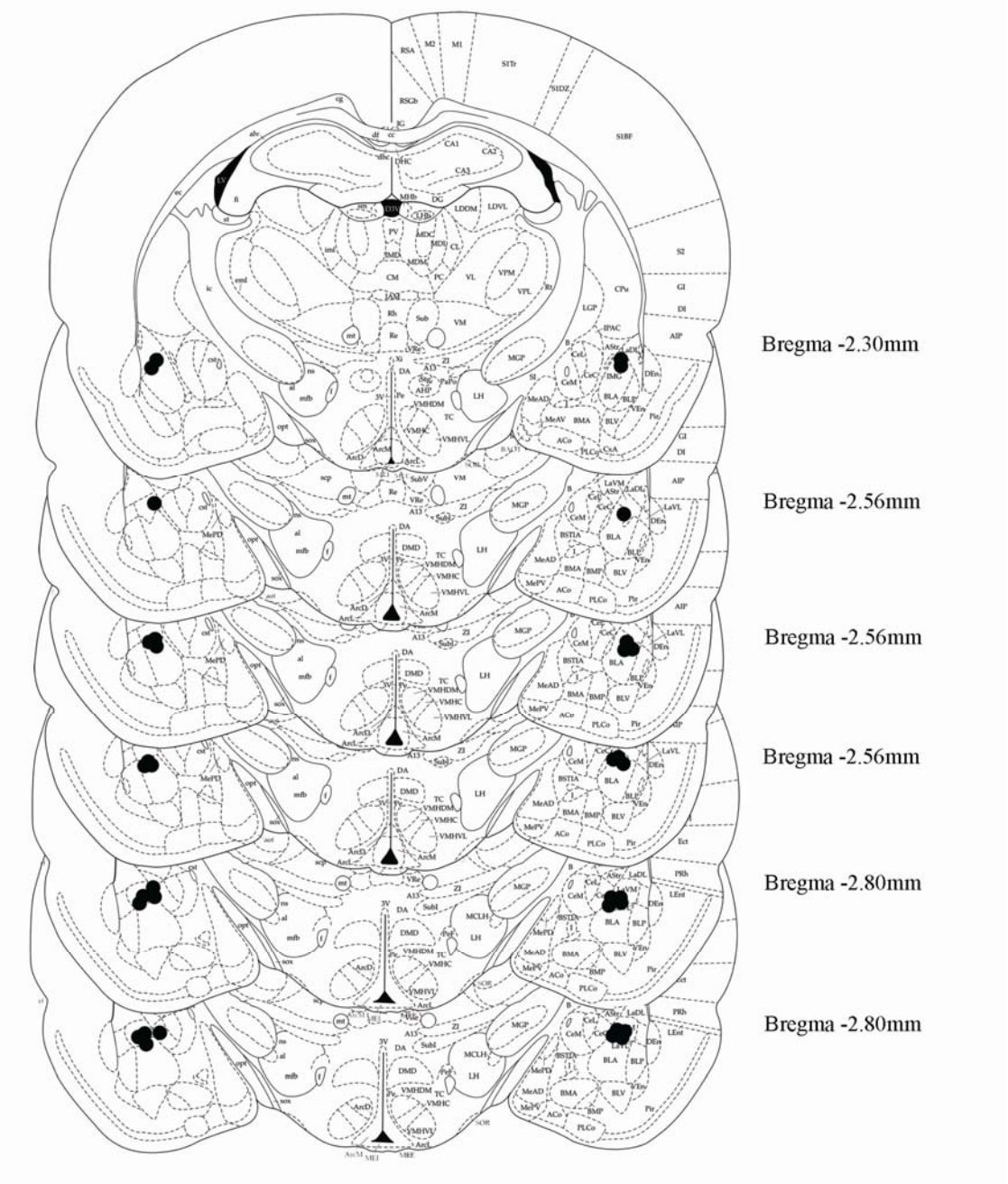

Figure 1. A schematic representation of the guide cannula locations for the Saline (N = 7), Muscimol 0.1ug (N = 8), and Muscimol 0.01ug (N = 8) infusion groups. Guide cannula placements were implanted and aimed 1mm above the Basolateral Amygdala (co-ordinates AP – 2.8mm from bregma, ML ± 4.8mm from the sagital suture, DV- 8.2mm from the skull surface). The representative sections (-2.30mm - -2.80mm from bregma) were taken from The Rat Brain in Stereotaxic Coordinates, by Paxinos and Watson (1998).

3.12 Acoustic Startle and Selected Decibel Levels

The acoustic startle levels of the individual animals did not differ significantly across the saline and amygdala groups during the baseline screening session, $F(1,21) = 0.72$, $p = 0.41$, ns. Therefore, any differences seen in the startle levels of the animals post sensitization were not a result of variable baseline levels. The decibel levels selected for each subject also did not differ significantly between the saline and muscimol groups, $F = (1,21) = 0.29$, $p = 0.60$, ns. The noise burst decibel level varied between 91 – 99 dB, with an average of 94.30 dB.

3.13 Effects of Muscimol Drug Infusion into the BLA for Shock Sensitization

In this experiment subjects firstly received a baseline session of 20 white noise bursts, followed quickly by the infusion of muscimol 0.1ug, 0.01ug, or saline, and were then placed back into the experimental chamber for the sensitization experiment of 20 white noise bursts, 10 footshock, and lastly 20 white noise bursts. The behavioural data is shown in Figure 2. Subjects that received intracranial infusions of saline served as a control group (N=7) for the animals that received intracranial infusions of muscimol 0.1ug or 0.01ug prior to the sensitization session. A repeated measures ANOVA of the acoustic startle data for the muscimol drug group 0.1ug revealed main effects of startle session, $F(2,26) = 11.35$, $p < 0.0003$ and a significant drug treatment x startle session interaction $F(2,26) = 12.75$, $p < 0.0001$. A repeated measures ANOVA of the acoustic startle data for the muscimol drug group 0.01ug revealed main effects of startle session, $F(2,26) = 17.57$, $p < 0.00002$ and a significant drug treatment x startle session interaction

$F(2,26) = 7.86$, $p < 0.002$. Figure 2 depicts that intra BLA muscimol infusion did not significantly affect acoustic startle amplitudes baseline versus postdrug. A Bonferroni post-hoc analysis revealed that both doses of muscimol (0.1ug and 0.01ug) infused into the BLA blocked the shock sensitization of the acoustic startle response (see figure 2). Therefore, muscimol infusion into the BLA blocked the fear potentiating effects of footshock on the amplitude of the acoustic startle response. A one-way ANOVA of the difference scores (post shock – post drug acoustic startle scores) was carried out between the muscimol 0.1ug and 0.01ug (experimental) and saline (control) groups (see figure 3). A one way ANOVA of the difference scores revealed a significant main effect for drug treatment $F(1,13) = 15.02$, $p<0.0019$ (muscimol 0.1ug vs. saline), and $F(1,12) = 8.08$, $p<0.014$ (muscimol 0.01ug vs. saline) represented in Figure 3. The results revealed that infusions of muscimol 0.1ug and 0.01ug into the BLA significantly inhibited the shock-enhanced acoustic startle amplitudes of the subjects. A Bonferroni post-hoc analysis confirmed that the two varying doses of muscimol used in the current study significantly inhibited sensitization relative to the saline-treated control group. Therefore, the subjects in the two varying drug groups failed to demonstrate sensitization after receiving intra-BLA drug infusions of muscimol in comparison to the control group.

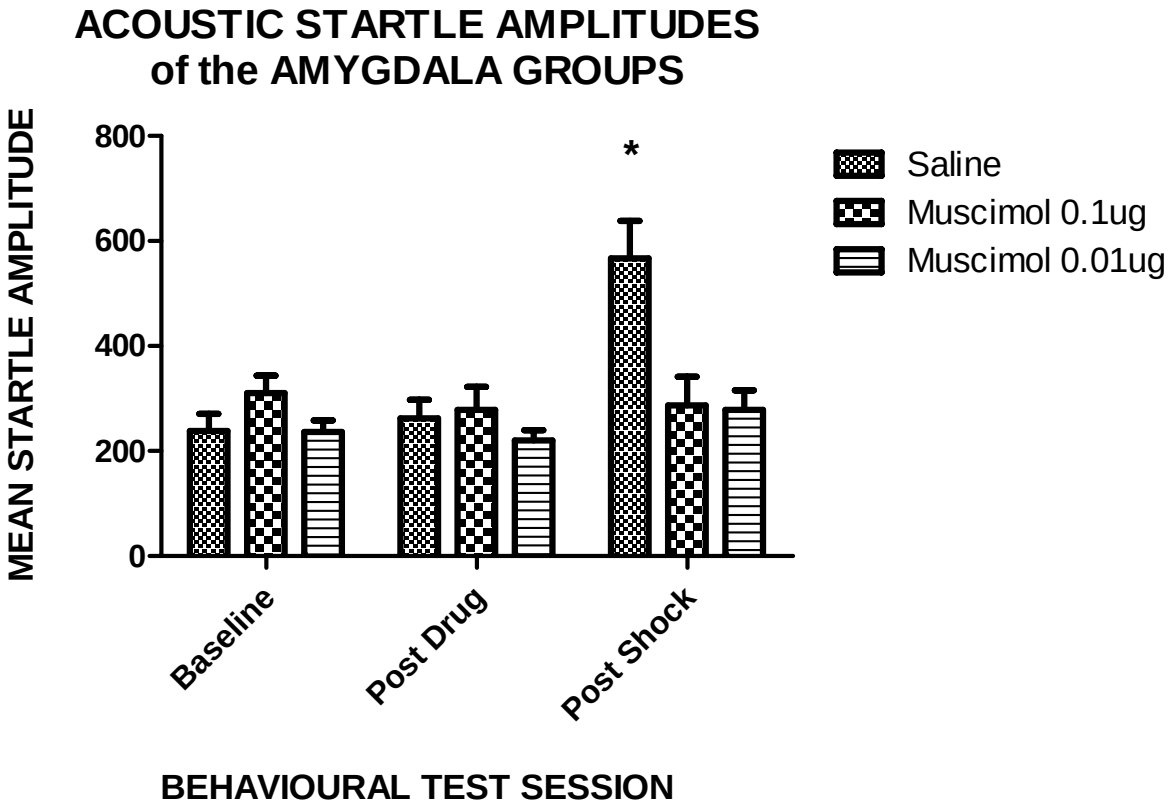

Figure 2. Mean (±S.E.M.) acoustic startle amplitude as a function of test session (baseline, postdrug infusion, and postshock) following intraBLA infusion of saline (N=7) or muscimol 0.1ug (N=8), 0.01ug (N=8) (*$p < 0.01$).

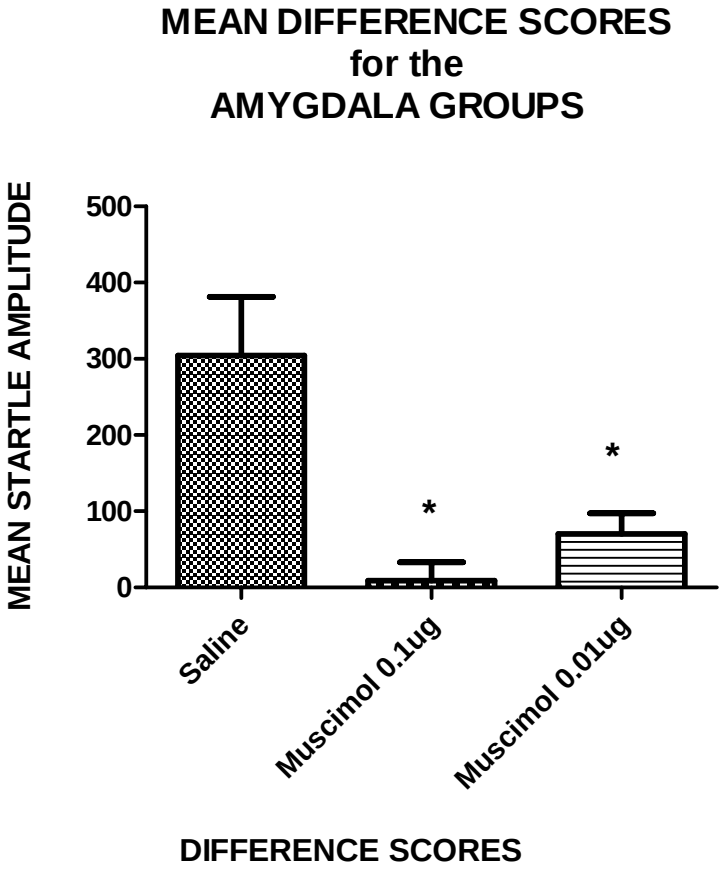

Figure 3. Mean difference score (post shock – post drug) following infusion of saline (N=7) or muscimol 0.1ug (N=8), 0.01ug (N=8) into the BLA.

3.14 Shock Reactivity

In order to demonstrate that drug infusions did not significantly attenuate the shock reactivity of the subjects a one-way ANOVA was used. There was no significant difference in shock reactivity between the muscimol drug group and the saline group, $F(1,21) = 3.10$, $p<0.09$, ns. Therefore, the anxiolytic actions of muscimol infused into the BLA cannot be effected by possible drug effects on sensorimotor responding as a suppression of movement amplitude was not induced by the muscimol infusion (see Figure 4).

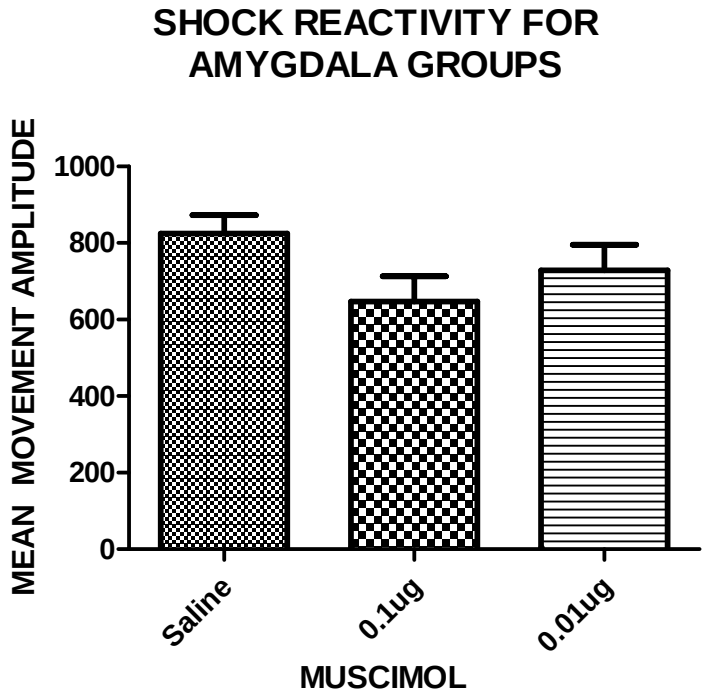

Figure 4. Mean movement amplitude recorded 100ms before shock and 100ms after shock onset following infusion of Saline (N = 7), Muscimol 0.1ug (N = 8), or Muscimol 0.01ug (N = 8) into the BLA. ANOVA did not reveal any significant difference between the groups.

3.2 Dorsal Hippocampus

3.2.1 Histology

One rat in the dorsal hippocampus group was excluded from the study after developing a loose acrylic headcap. The guide cannula locations in the dorsal hippocampus for the remaining rats in the group (saline N = 7, muscimol 0.1ug N = 8, muscimol 0.01ug N = 8) are depicted in Figure 5. In all 16 of the cannulated experimental rats, the centres of the infusion sites (the tips of the infusion cannulae) were located in the targeted areas within or around the border of the ventral hippocampus. Guide cannula placements were implanted and aimed 1mm above the ventral hippocampus (co-ordinates AP –5.6mm from bregma, ML ±3.6 mm from the sagital suture, DV-3.0 mm from the skull surface). Visible tissue damage was restricted to the immediate surrounding area of the guide and infusion cannulae.

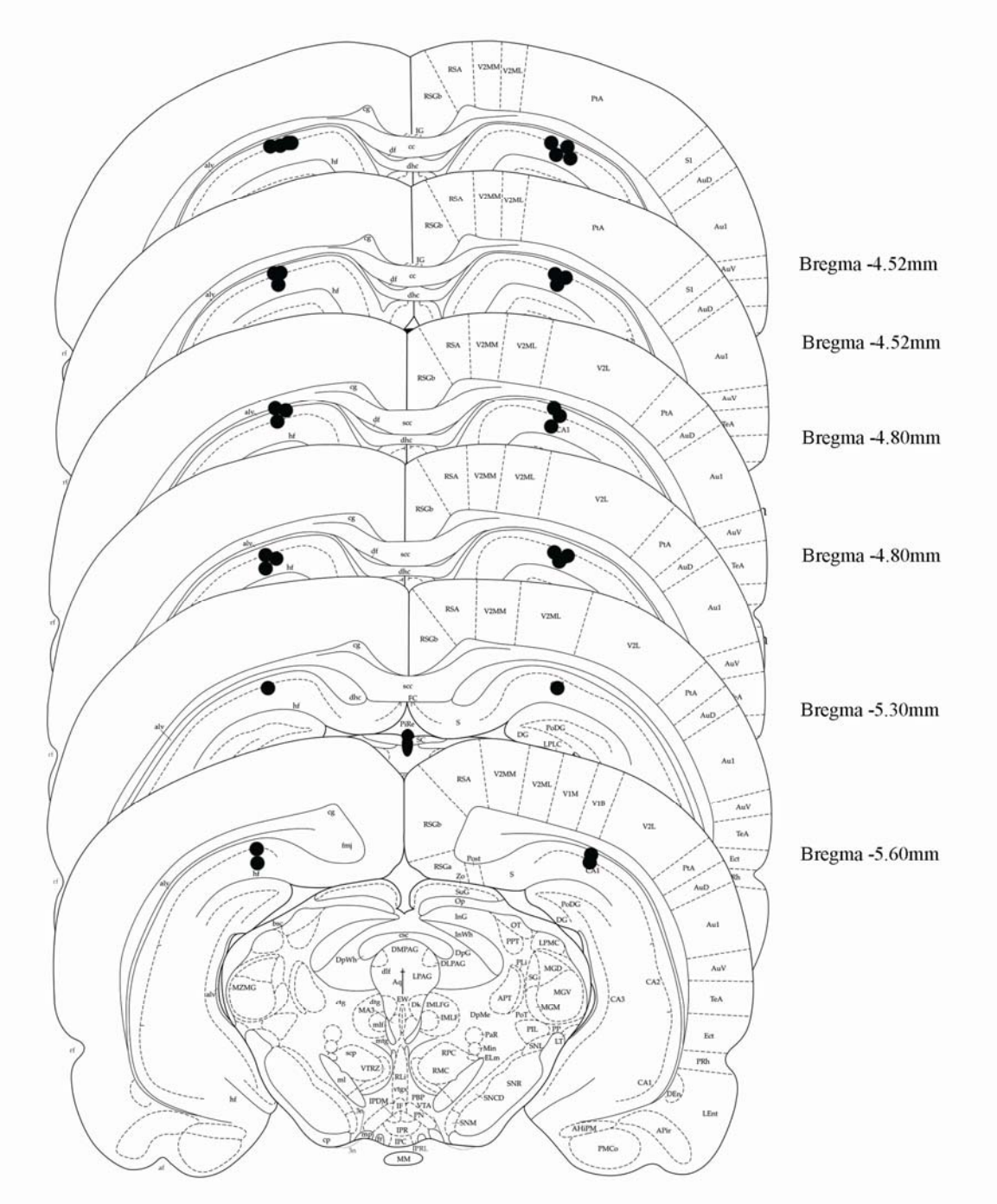

Figure 5. A schematic representation of the guide cannula locations for the Saline (N = 7), Muscimol 0.1ug (N = 8), and Muscimol 0.01ug (N = 8) infusion groups. Guide cannula placements were implanted and aimed 1mm above the Dorsal Hippocampus (co-ordinates AP – 5.6mm from bregma, ML ± 3.6mm from the sagital suture, DV- 3.0mm from the skull surface). The representative sections (-4.52mm - -5.60mm from bregma) were taken from The Rat Brain in Stereotaxic Coordinates, by Paxinos and Watson (1998).

3.2.2 Acoustic Startle and Selected Decibel Levels

The acoustic startle levels of the individual animals did not differ significantly across the saline and dorsal hippocampus groups during the baseline screening session, $F(1,21) = 0.35$, $p = 0.56$, ns. Therefore, any differences seen in the post sensitization startle levels of the animals were not a result of variable baseline levels. The decibel levels selected for each subject also did not differ significantly between the saline and muscimol groups, $F = (1,21) = 0.69$, $p = 0.41$, ns. The noise burst decibel level varied between 91 – 99 dB, with an average of 95.17 dB.

3.2.3 Effects of Muscimol Drug Infusion into the Dorsal Hippocampus for Shock Sensitization

In this experiment subjects firstly received a baseline session of 20 white noise bursts, followed quickly by the infusion of muscimol 0.1ug, 0.01ug, or saline, and were then placed back into the experimental chamber for the sensitization experiment of 20 white noise bursts, 10 footshock, and lastly 20 white noise bursts. The behavioural data are shown in Figure 6. Subjects that received intracranial infusions of saline served as a control group (N=7) for the animals that received intracranial infusions of muscimol 0.1ug or 0.01ug prior to the sensitization session. A repeated measures ANOVA of the acoustic startle data for the muscimol drug group 0.1ug revealed main effects of startle session, $F(2,26) = 28.28$, $p < 0.000001$ and a significant drug treatment x startle session interaction $F(2,26) = 6.88$, $p < 0.0094$ A repeated measures ANOVA of the acoustic startle data for the muscimol drug group 0.01ug revealed main effects of startle session,

$F(2,26) = 43.88$, $p < 0.00001$ and a significant drug treatment x startle session interaction $F(2,26) = 5.32$, $p < 0.01$. Figure 6 depicts that the intra dorsal hippocampus muscimol infusions did not significantly affect acoustic startle amplitudes (baseline versus postdrug). Bonferroni post-hoc analysis revealed that the 0.1ug muscimol dose infused into the dorsal hippocampus blocked the shock sensitization of the acoustic startle response (see figure 7). The 0.01ug dose of muscimol infused into the dorsal hippocampus showed a significant increase in startle amplitudes following drug infusions. Therefore, the muscimol infusion of 0.1ug into the dorsal hippocampus blocked the fear potentiating effects of footshock on the amplitude of the acoustic startle response. A one-way ANOVA of the difference scores (post shock – post drug acoustic startle scores) was carried out between the muscimol 0.1ug and 0.01ug (experimental) and saline (control) groups (see figure 7). ANOVA of the difference scores revealed a significant main effect for drug treatment $F(1,13) = 8.65$, $p<0.01$ (muscimol 0.1ug vs. Saline), and $F(1,13) = 7.50$, $p<0.02$ (muscimol 0.01ug vs. saline) represented in Figure 7. The results reveal that infusions of muscimol 0.1ug and 0.01ug into the dorsal hippocampus significantly inhibited the shock-enhanced acoustic startle amplitudes of the subjects. Post-hoc analysis using the Bonferroni test confirmed that the two varying doses of muscimol used in the current study significantly inhibited sensitization relative to the saline-treated control group. Therefore, the subjects in the two varying drug groups failed to demonstrate sensitization after receiving intra-dorsal hippocampus drug infusions of muscimol in comparison to the control group. These results are similar to those reported earlier following intra BLA and ventral hippocampus infusions of muscimol.

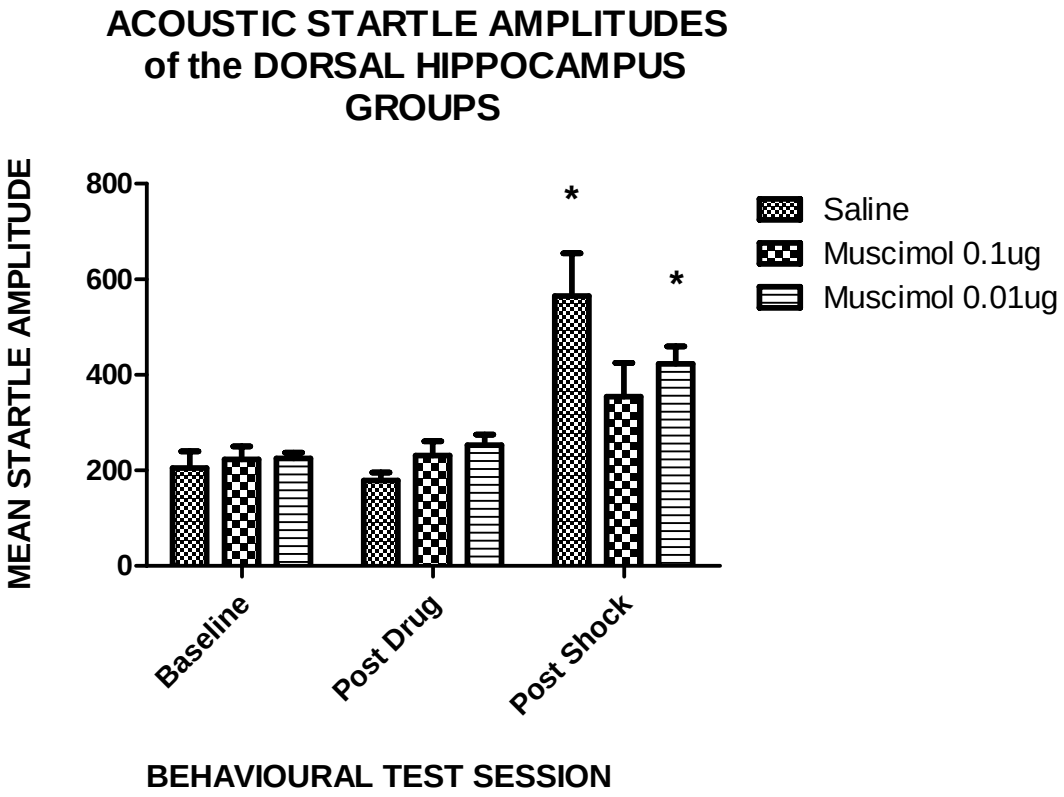

Figure 6. Mean (±S.E.M.) acoustic startle amplitude as a function of test session (baseline, postdrug infusion, and postshock) following intra dorsal hippocampus infusions of saline (N=7) or muscimol 0.1ug (N=8), 0.01ug (N=8) (*$p < 0.01$).

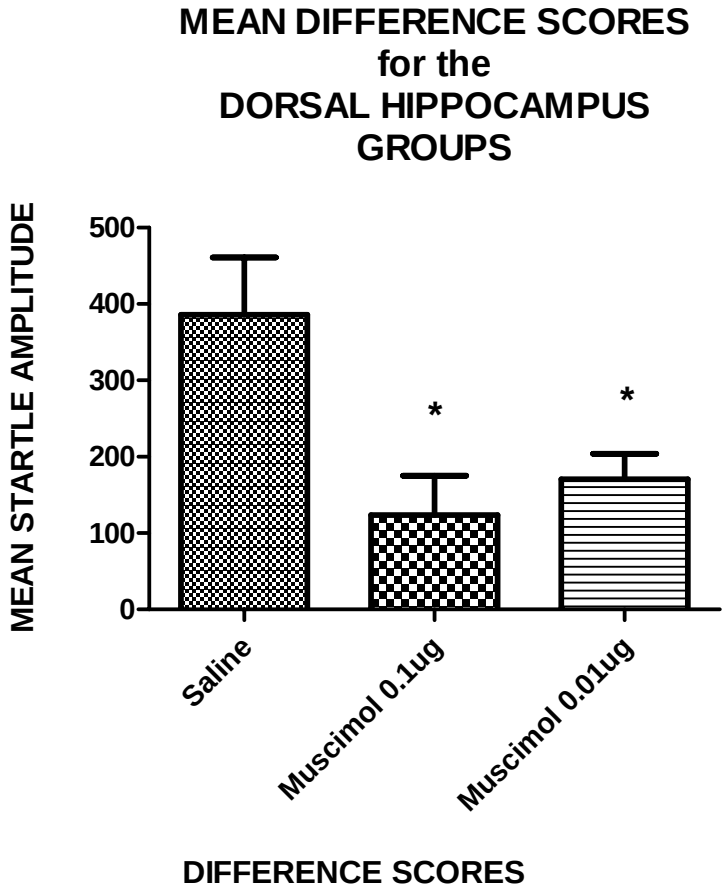

Figure 7. Mean difference score (post shock – post drug) following infusion of saline (N=7) or muscimol 0.1ug (N=8), 0.01ug (N=8) into the dorsal hippocampus.

3.2.4 Shock Reactivity

In order to demonstrate that drug infusions did not significantly attenuate the shock reactivity of the subjects a one-way ANOVA was used. There was no significant difference in shock reactivity between the muscimol drug group and the saline group, $F(1,21) = 0.97$, $p<0.34$, ns. Therefore, the anxiolytic actions of muscimol infused into the dorsal hippocampus cannot be affected by possible drug effects on sensorimotor responding as a suppression of movement amplitude was not induced by the muscimol infusion (see Figure 8).

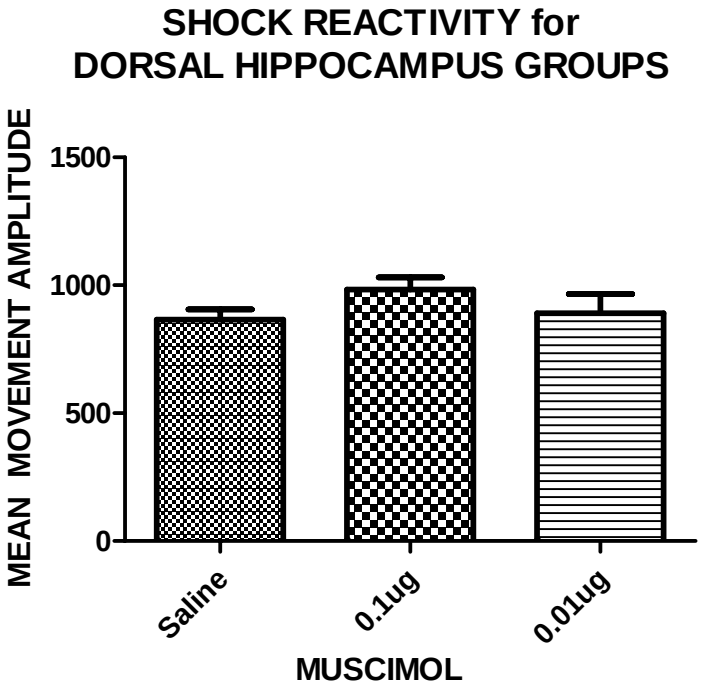

Figure 8. Mean movement amplitude recorded 100ms before shock and 100ms after shock onset following infusion of saline (N = 7), Muscimol 0.1ug (N = 8), or Muscimol 0.01ug (N = 8) into the dorsal hippocampus. ANOVA did not reveal any significant difference between the groups.

3.3 Ventral Hippocampus

3.3.1 Histology

Two rats in the ventral hippocampus group were excluded from the study after developing loose acrylic headcaps. The guide cannula locations in the ventral hippocampus for the remaining rats in the group (saline N = 8, muscimol 0.1ug N = 8, Muscimol 0.01ug N = 8) are depicted in Figure 9. In all 16 of the cannulated experimental rats, the centres of the infusion sites (the tips of the infusion cannulae) were located in the targeted areas within or around the border of the ventral hippocampus. Guide cannula placements were implanted and aimed 1mm above the ventral hippocampus (co-ordinates AP – 5.6mm from bregma, ML ± 4.6mm from the sagital suture, DV- 6.4mm from the skull surface). Visible tissue damage was restricted to the immediate surrounding area of the guide and infusion cannulae.

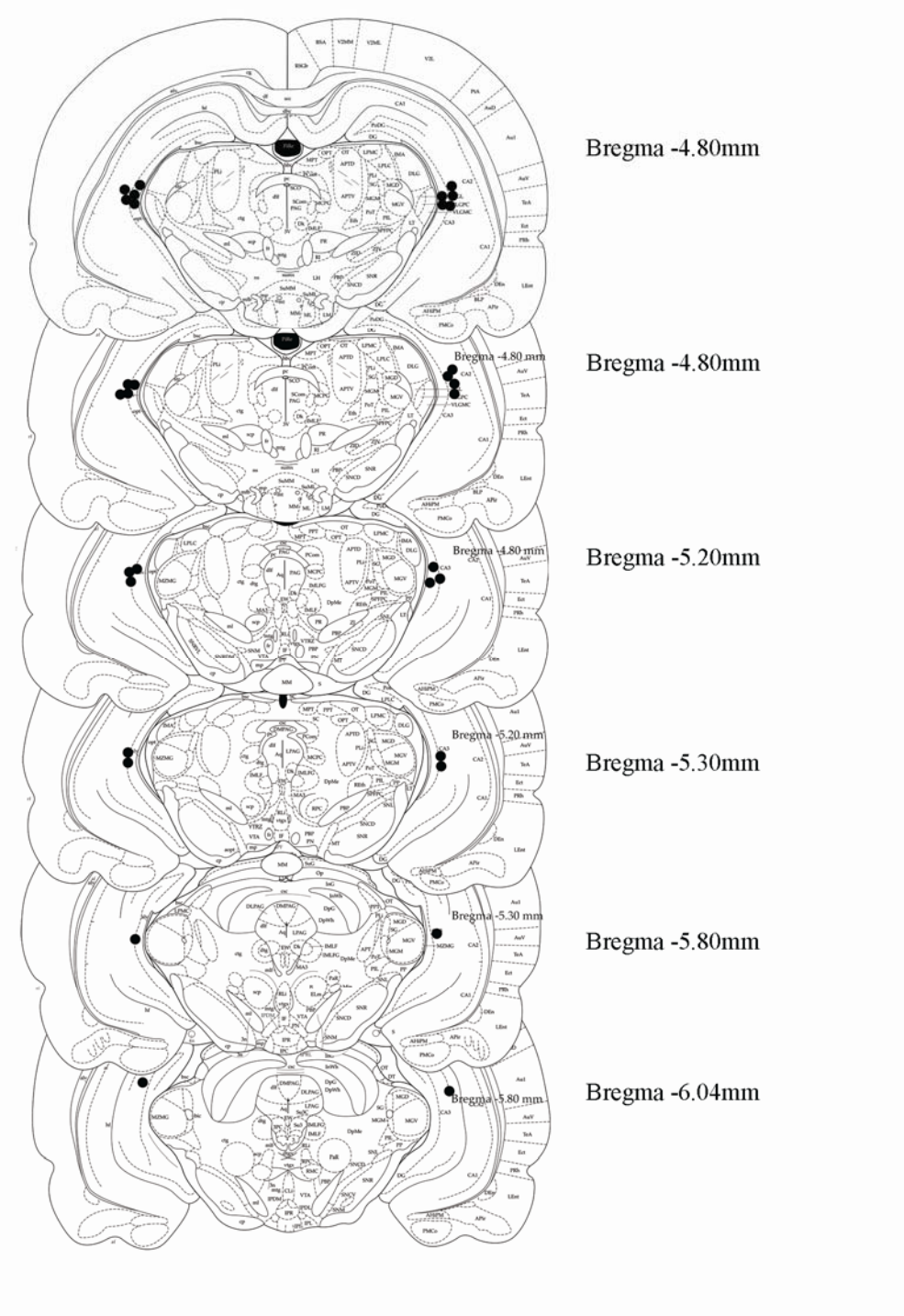

Figure 9. A schematic representation of the guide cannula locations for the Saline (N = 8), Muscimol 0.1ug (N = 8), and Muscimol 0.01ug (N = 8) infusion groups. Guide cannula placements were implanted and aimed 1mm above the Ventral Hippocampus (co-ordinates AP – 5.6mm from bregma, ML ± 4.6mm from the sagital suture, DV- 6.4mm from the skull surface). The representative sections (-4.80mm - -6.04mm from bregma) were taken from The Rat Brain in Stereotaxic Coordinates, by Paxinos and Watson (1998).

3.3.2 Acoustic Startle and Selected Decibel Levels

The acoustic startle levels of the individual animals did not differ significantly across the saline and ventral hippocampus groups during the baseline screening session, $F(1,21) = 1.37$, $p = 0.26$, ns. Therefore, any differences seen in the startle levels of the animals post sensitization were not a result of variable baseline levels. The decibel levels selected for each subject also did not differ significantly between the Saline and muscimol groups, $F = (1,22) = 0.13$, $p = 0.72$, ns. The noise burst decibel level varied between 91 – 99 dB, with an average of 94.67 dB.

3.3.3 Effects of Muscimol Drug Infusion into the Ventral Hippocampus for Shock Sensitization

In this experiment subjects firstly experienced a baseline session of 20 white noise bursts, followed quickly by the infusion of muscimol 0.1ug, 0.01ug, or saline, and were then placed back into the experimental chamber for the sensitization experiment of 20 white noise bursts, 10 footshock, and lastly 20 white noise bursts. The behavioural data are shown in Figure 10. Subjects that received intracranial infusions of saline served as a control group (N=8) for the animals that received intracranial infusions of muscimol 0.1ug or 0.01ug prior to the sensitization session. A repeated measures ANOVA of the acoustic startle data for the muscimol drug group 0.1ug revealed main effects of startle session, $F(2,28) = 22.89$, $p < 0.000001$ and a significant drug treatment x startle session interaction $F(2,28) = 5.56$, $p < 0.009$. A repeated measures ANOVA of the acoustic startle data for the muscimol drug group 0.01ug revealed main effects of startle session,

$F(2,28) = 15.77$, $p < 0.00003$ and a significant drug treatment x startle session interaction $F(2,28) = 6.06$, $p < 0.006$. Figure 10 depicts that the intra ventral hippocampus muscimol infusion did not significantly affect acoustic startle amplitudes (baseline versus postdrug). Bonferroni post-hoc analysis revealed that both doses of muscimol (0.1ug and 0.01ug) infused into the ventral hippocampus blocked the shock sensitization of the acoustic startle response (see figure 11). Therefore, muscimol infusion into the ventral hippocampus blocked the fear potentiating effects of footshock on the amplitude of the acoustic startle response. A one-way ANOVA of the difference scores (post shock – post drug acoustic startle scores) was carried out between the muscimol 0.1ug and 0.01ug (experimental) and saline (control) groups (see figure 11). ANOVA of the difference scores revealed a significant main effect for drug treatment $F(1,14) = 6.54$, $p<0.02$ (muscimol 0.1ug vs. saline), and $F(1,14) = 6.77$, $p<0.02$ (muscimol 0.01ug vs. saline) represented in Figure 11. The results reveal that infusions of muscimol 0.1ug and 0.01ug into the ventral hippocampus significantly inhibited the shock-enhanced acoustic startle amplitudes of the subjects. Post-hoc analysis using the Bonferroni test confirmed that the two varying doses of muscimol used in the current study significantly inhibited sensitization relative to the saline-treated control group. Therefore, the subjects in the two varying drug groups failed to demonstrate sensitization after receiving intra-ventral hippocampus drug infusions of muscimol in comparison with the control group.

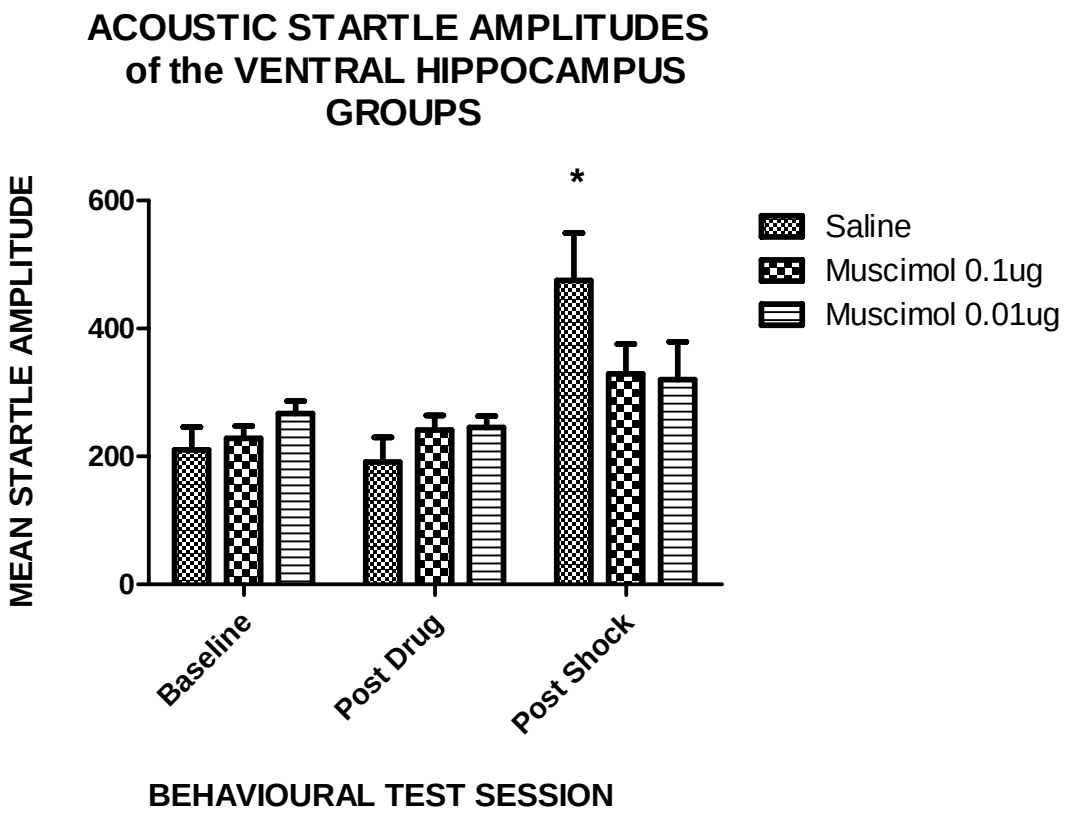

Figure 10. Mean (±S.E.M.) acoustic startle amplitude as a function of test session (baseline, postdrug infusion, and postshock) following intra ventral hippocampus infusions of saline (N=8) or muscimol 0.1ug (N=8), 0.01ug (N=8) (*$p < 0.01$).

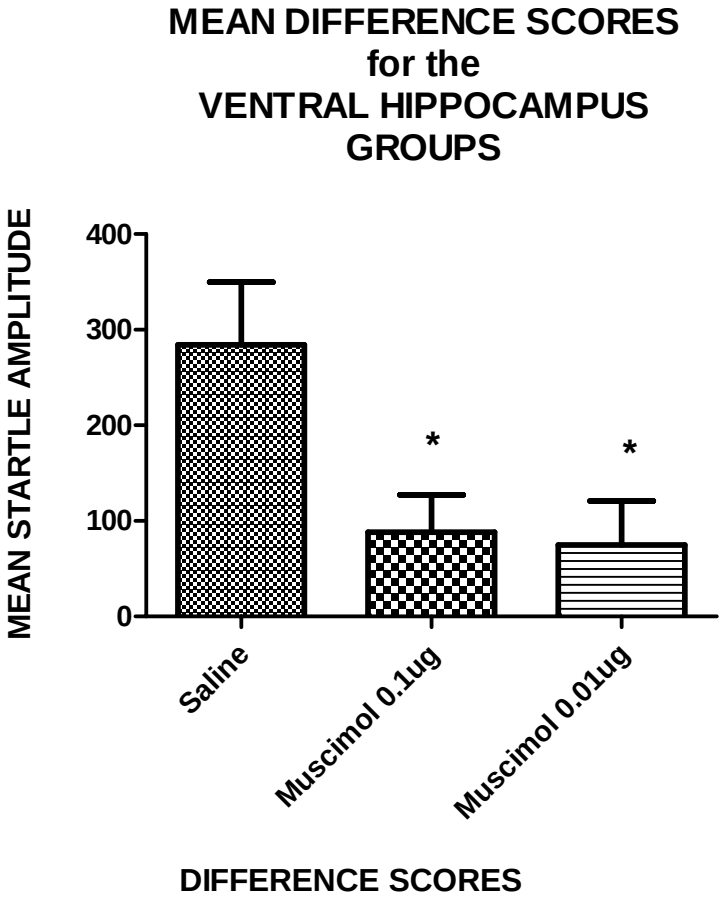

Figure 11. Mean difference score (post shock – post drug) following infusion of saline (N=8) or muscimol 0.1ug (N=8), 0.01ug (N=8) into the ventral hippocampus.

3.3.4 Shock Reactivity

In order to demonstrate that drug infusions did not significantly attenuate the shock reactivity of the subjects a one-way ANOVA was used. There was no significant difference in shock reactivity between the muscimol drug group and the saline group, $F(1,22) = 1.85$, $p<0.19$, ns. Therefore, the anxiolytic actions of muscimol infused into the ventral hippocampus cannot be effected by possible drug effects on sensorimotor responding as a suppression of movement amplitude was not induced by the muscimol infusion (see Figure 12).

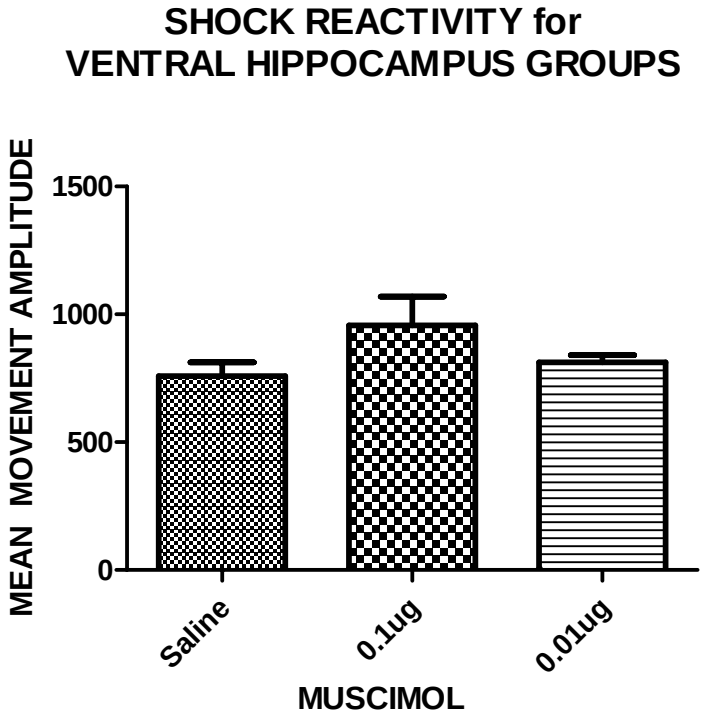

Figure 12. Mean movement amplitude recorded 100ms before shock and 100ms after shock onset following infusion of Saline (N = 8), Muscimol 0.1ug (N = 8), or Muscimol 0.01ug (N = 8) into the ventral hippocampus. ANOVA did not reveal any significant difference between the groups.

4.0 DISCUSSION

4.1 Main Findings

There was no significant effect of drug infusion on the acoustic startle levels (baseline vs postdrug) in the saline control animals and the muscimol drug groups for all three brain regions of the BLA, dorsal and ventral hippocampus. Therefore, the drug muscimol did not impair sensorimotor reflexes of the animals used in this study. This is important as the results demonstrate that the differences found in the startle levels of the animals post sensitization was not a result of variable baseline levels.

4.2 Amygdala

Intra BLA infusions of the drug muscimol (GABA A receptor agonist) at measures of 0.1ug and 0.01ug significantly attenuated the shock sensitization of the acoustic startle response relative to the saline control animals. An ANOVA of the difference scores (post-shock – postdrug) confirmed the results of the repeated measures ANOVA in that the saline control group demonstrated significantly enhanced shock sensitization of the ASR in comparison with the 0.1ug and 0.01ug muscimol groups. An ANOVA of the shock reactivity between each group revealed no group differences in mean movement amplitudes (movement 100ms before and 100ms after receiving footshock). Therefore, the infusion of muscimol did not affect the animals' ability to respond appropriately to the footshock, nor did it impair their ability to perceive the footshock as an aversive event.

4.3 Ventral Hippocampus

Intra ventral hippocampal infusions of the drug muscimol (GABA A receptor agonist) at doses of 0.1ug and 0.01ug significantly attenuated the shock sensitization of the acoustic startle response relative to the saline control animals. An ANOVA of the difference scores (post-shock – postdrug) confirmed the results of the repeated measures ANOVA in that the saline control group demonstrated significantly enhanced shock sensitization of the ASR in comparison with the 0.1ug and 0.01ug muscimol groups. An ANOVA of the shock reactivity between each group revealed no group differences in mean movement amplitudes (movement 100ms before and 100ms after receiving footshock). Therefore, the infusion of muscimol did not affect the animals' ability to respond appropriately to the footshock, nor did it impair their ability to perceive the footshock as an aversive event.

4.4 Dorsal Hippocampus

Intra dorsal hippocampal infusions of the drug muscimol (GABA A receptor agonist) at the measure of 0.1ug significantly attenuated the shock sensitization of the acoustic startle response relative to the saline control animals. An ANOVA of the difference scores (post-shock – postdrug) confirmed the results of the repeated measures ANOVA in that the saline control group demonstrated significantly enhanced shock sensitization of the ASR in comparison with the 0.1ug and 0.01ug muscimol groups. An ANOVA of the shock reactivity between each group revealed no group differences in mean movement amplitudes (movement 100ms before and 100ms after receiving

footshock). Therefore, the infusion of muscimol did not affect the animals' ability to respond appropriately to the footshock, nor did it impair their ability to perceive the footshock as an aversive event.

The present results demonstrated that the inactivation of the BLA, and dorsal and ventral hippocampus with muscimol caused the attenuation of shock sensitization without any significant changes in shock reactivity. The drug muscimol is a direct agonist for the gamma – aminobututyric acid (GABA) binding site on the GABA A receptor (Nicoll, Malenka, & Kauer, 1989). GABA is an amino acid and is the most important inhibitory neurotransmitter in the CNS (Stachowicz, Klak, Klodińska, Chojnacka-Wójcik, & Pilc, 2006). GABA is produced from the glutamic acid action of the enzyme glutamic acid decarboxylase (GAD) that removes a carboxyl group. There are two GABA receptors namely, GABA A and GABA B. GABA A is ionotropic and controls the chloride channel, the GABA B receptor is metabotropic and controls the potassium channels. The GABA A receptors are quite complex in their design and contain at least five different binding sites. Muscimol is derived from the Ach agonist muscarine and serves as a direct agonist from the GABA site. Barbiturates, drugs that bind to the steroid site, and benzodiazepines all promote the activity of the GABA A receptor, and are therefore indirect agonists. Benzodiazepines are very effective anxiolytics and are termed 'anxiety dissolving' drugs which are used to treat patients suffering from severe anxiety disorders (Delaney & Sah, 1999; Marowsky, Fritschy, & Vogt, 2004).

The inhibitory action of GABA has been identified as playing an important role in mediating neuronal excitation of the amygdala (Delaney & Sah, 1999; Marowsky et al., 2004) and the hippocampus (Stachowicz et al., 2006; Berretta, Munno, & Benes, 2001; Bannerman et al., 2004; Corcoran & Maren, 2001; Bast et al., 2001). The present study's results revealed an important finding that the infusion of the GABA A receptor agonist muscimol into the BLA, and dorsal and ventral hippocampus blocked the ability of footshock to increase the mean amplitude of the ASR in shock sensitization. The amount of drug diffusion that may have occurred with the drug muscimol was dependent on the volume, concentration, and time after the infusion was administered. The present study employed similar parameters to those used by Martin (1991) who reported drug diffusion of 1.7mm from the forebrain tissue injection site, subsequent to a 1.0ul infusion of 1.0ug of muscimol. The present experiment utilised volumes of 0.1ug and 0.01ug of the drug muscimol, which are one half of the volume used in the study carried out by Martin (1991). The behavioural effects were apparent with both low doses of muscimol in the amygdala, and dorsal and ventral hippocampus. Furthermore, it is important to note that the efficacy of low dose muscimol infusion in inhibiting shock sensitization of the ASR supports previous research that demonstrated reliable conditioned place preference after infusion of 0.005ug muscimol into the VTA (Laviolette & Van der Kooy, 2001; 2004).

4.5 The Amygdala and GABA A's Involvement in Fear Arousal

The present study assessed the effects of rapid footshock on the ASR as a measure of fear arousal and the results confirmed the hypothesis that shock-associated fear involves a GABA mechanism in the BLA. The amygdala has been identified as an important structure for receiving important information regarding aversive events (such as footshock), interpreting that information, and the resultant expression of fear arousal is expressed. It has also been identified as an important structure for the acquisition and expression of fear and is widely accepted as playing an important role in the neurocircuitry involved in fear and anxiety. Research investigating the underlying neural networks of the ASR has implicated a number of brain areas involved. For example, lesions of the caudal granular, dysgranular cortex and the posterior intralaminar thalamic nuclei in Davis and Shi's (1999) experiment demonstrated blocked shock sensitization of the ASR. Lesions of the periacqueductal grey (Fendt et al., 1994) and infusions of substance P (antagonist) into the caudal pontine reticular nucleus have also demonstrated attenuation of shock-potentiated startle which provides more insight into the startle circuitry within the brain stem (Krase, Koch, & Schnitzler, 1994). Both the periacqueductal grey (Gebhart, 2004) and the amygdala have been identified as modulators of pain (Helmstetter & Bellgowan, 1993). Differing tests of nociception, excitotoxic lesions, and muscimol infusions have demonstrated various effects revealing that the central nucleus of the amygdala, not the BLA is involved in processing information regarding fear from noxious stimuli (Manning, 1998; Manning, Martin, & Meng, 2003). Research involving lesions of the central and BLA have blocked shock sensitization of the ASR (Hitchcock, Sananes, & Davis, 1989; Sananes & Davis, 1992),

while excitotoxic lesions of the central amygdala attenuated shock induced hyperalgesia (Crown, King, Meagher, Grau, 2000). However, excititoxic lesions of the BLA do not show similar results.

Research involving electrolytic lesions of the central amygdala have shown reductions in the shock reactivity of rats after footshock (Hitchcock et al., 1989). The two doses of 0.1ug and 0.01ug of muscimol used in the present study did not have any effect on the animals' footshock reactivity suggesting that shock perception in the BLA does not involve GABA neurotransmission. Previous research has shown that after classical fear conditioning takes place, the CS activates the neural representation of an US (Wagner & Brandon, 1989). Stork and colleagues (2002) found that lab mice that were exposed to a fear evoking CS exhibited a markedly reduced level of extracellular GABA in the amygdala. This observation lent further support to the view that there is a causal relationship between a decrease in GABA activity within the amygdala and during enhancement of fear. The results of the present study revealed that the GABA A receptor agonist muscimol attenuated the amplitude of the ASR after receiving fear provoking footshock, therefore suggesting that the amygdala and GABA are important underlying neural mechanisms that regulate the emotion of fear, which is activated by an aversive stimulus. The results of the experiment also lend further support to previous research demonstrating similar results (Van Nobelen & Kokkinidis, 2006).

Research carried out by Liu and colleagues (2007) investigated the neurochemical mechanisms that underlie the effects of fearful cue presentations through the release of norepinepherine (NE) and GABA within the amygdala. Their results revealed that fearful cues such as footshock produce an altered release of NE and GABA within the amygdala two hours after initial presentation, which later recovered to normal levels four hours after the presentation of the fearful cue. Noradrenalin levels increased when a stressful event occurred, which in turn caused an increase in the release of NE within the amygdala. Intense footshock altered the release of NE after the presentation of the CS, while the administration of muscimol decreased the level of NE within the amygdala (Hatfield, Spanis, & McGaugh, 1999), suggesting that GABA regulates NE release. The results of Liu et al., (2007) demonstrated that fear-induced alterations exhibited in NE and GABA release in multiple brain areas influenced fear-induced changes in neurotransmitter release systems within the amygdala. In the current experiment animals received ten rapid presentations of mild electric footshock during the shock sensitization procedure. The animals experienced a stressful event in the chamber which increased their noradrenalin levels, resulting in an increase in NE within the amygdala. The infusion of muscimol into the amygdala decreased the level of NE within the amygdala suggesting that GABA regulates NE release. The GABA A receptor agonist muscimol blocked the increase of the ASR after receiving fear inducing footshock in the present study, therefore supporting Liu's et al., (2007) research which suggests that the amygdala and GABA are important underlying neural mechanisms for regulating the emotion of fear and anxiety when activated by an aversive event.

4.6 The Dorsal and Ventral Hippocampus and GABA A's Involvement in Fear Arousal

GABA has been shown to play an important role in the inhibition of neurotransmitters in the hippocampus (Bast et al., 2001; Buzsaki, Horvath, Urioste, Hetke, & Wise, 1992 Cobb, Buhl, Halasy, Paulsen, & Somogyi, 1995). The present study revealed an important finding, namely, that the infusion of the GABA A receptor agonist muscimol into both the dorsal and ventral hippocampus blocked the magnitude of the ASR after the presentation of fear-provoking footshock. The majority of research has investigated involvement of the hippocampus in learning and memory and has concentrated on its role in Pavlovian fear conditioning. Research has demonstrated that lesions of the hippocampus reduce contextual freezing (Phillips & LeDoux 1992; LeDoux, 1993, Fendt & Fanselow, 1999; Antoniadis & McDonald, 2000; Bannerman et al., 2003; Bast et al., 2001) but not the fear-potentiated startle response (McNish et al., 1997).

Major current views in understanding the roles of both the amygdala and hippocampus in fear conditioning to a context, include the first view states that the hippocampus is critical for stimulus selection during learning (Winocur, Rawlins, & Gray, 1987). Rats with hippocampal lesions tend to show an enhanced fear to a context after fear conditioning to a context takes place. A second view posits that the hippocampus relays contextual information through the amygdala's fear circuitry that is associated with the unconditioned response to shock. This view suggests that the amygdala and hippocampus are interconnected (McDonald & White, 1993). Experiments

have shown that rats with damage to the amygdala are unable to acquire and retain conditioned fear to a cue and context paired with footshock (Kim & Fanselow, 1992; Phillips and LeDoux, 1992). Rats with hippocampal damage exhibit impairment in their ability to acquire conditioned fear to a context but not a cue paired with footshock (Blanchard & Fial, 1968).

Other researchers have suggested that the hippocampus and amygdala work independently to access unconditioned fear responses to footshock and that the ability to identify one's environment and the context is retrieved by neurocircuitry of the hippocampus or amygdala. Supporting evidence for this assumption stems from research revealing that rats with hippocampal damage, demonstrate impairment in the acquisition of contextual fear conditioning (Helmestetter et al., 1993; Kim & Fanselow, 1992; Selden, Everitt, Jarrard, & Robbins, 1991; Sutherland & McDonald, 1990). The final view suggests that the hippocampus has a very limited role in contextual fear conditioning. Phillips and LeDoux (1994) state that the hippocampus is only necessary for contextual fear conditioning when there is a demand between a static context and predictive phasic cue. Research with rats that have hippocampal damage demonstrate impairments in contextual fear conditioning when there is competition between a cue and its context, but when contextual conditioning is static they show normal acquisition (Penick & Solomon, 1991; Phillips & LeDoux, 1994). Antoniadis and McDonald (2000) investigated eight different response measures of fear in order to assess the roles of the amygdala and hippocampus in fear conditioning to a context. Their results revealed that the amygdala is involved in learning and memory of fear conditioning to a context and to

the conditioning of heart rate. The hippocampus was identified in their experiment as a participant in learning and memory of fear conditioning to a context, and also to conditioned defecation and body temperature. Both the amygdala and hippocampus were identified as important for the conditioning of freezing, ultrasonic vocalizations, locomotion, and preference.

Antoniadis and McDonald (2000) proposed a different view of involvement of the amygdala and hippocampus in contextual fear conditioning. They suggested that the fear responses of freezing, urination, locomotion, and preference are 'fast' measures of fear which have been shown to discriminatively condition to a context after a single training session. On the other hand, ultrasonic vocalisations and defecation were seen as 'slow' measures of fear as they tend to require multiple training sessions to exhibit fear of a context. Heart rate was described as an 'intermediate' measure of fear that takes 20 minutes to become apparent. Defecation and body temperature that are mediated by the hippocampus were viewed as 'slow' measures of fear. All these different measures of fear require the participation of both the hippocampus and amygdala. A pattern seems to emerge from Antoniadis and McDonald's (2000) research which suggests that the hippocampus selectively regulates the 'slow' fear measures, while the amygdala selectively mediates 'intermediate' fear measures. However, both of these memory structures interact together in order to modulate the 'fast' measures of locomotion, freezing, and preference, as well as the 'slow' measure of ultrasonic vocalisations. The present study demonstrated that a few presentations of mild footshock attenuated the ASR of rats after muscimol infusions into the amgydala, and dorsal and ventral

hippocampus. We propose that the shock sensitization paradigm used in this study can be viewed as a 'fast' measure of fear which provides further support for Antoniadis and McDonald's (2000) suggestion that the hippocampus and amygdala interact in order to modulate 'fast' measures of fear; that is the shock sensitization paradigm in the current experiment.

Research involving microinfusions of various drugs including muscimol into either the dorsal or ventral hippocampus have tended to attenuate the startle response, block freezing after receiving contextual fear conditioning (Bast et al., 2001; Wan et al., 1996; Zhang et al., 2000; Zhang et al;., 2002), and decrease the rate of extinction after receiving Pavlovian fear conditioning (Corcoran & Maren, 2001; Cocoran, Desmond, Frey, & Maren, 2005). Bast and his colleagues (2001) found that the inhibitory action of GABA A receptors via muscimol infusion into the ventral hippocampus disrupted the ability for laboratory rats to fear condition to a context. Therefore, the current study lends further support for the view that a there is a causal relationship between a decrease in GABA activity within the hippocampus and the enhancement of fear. This is because rats with muscimol microinfusions into the dorsal and ventral hippocampus exhibited a decrease in their ASR amplitudes after receiving fear inducing electric footshock.

4.7 Is sensitization a conditioned or unconditioned form of aversive information processing?

Sensitization is thought to be a process of non-associative learning because the animal does not associate a specific event with an aversive stimulus. However, several researchers have suggested that some sort of associative learning takes place, such as a rapid conditioning to the context or background cues (Pilz & Schnitzler, 1996). Davis designed a specific experiment for investigating the conditioning of background cues and how they affected the ASR. The experiment made use of varied and constant lighting conditions. His results demonstrated that both groups of rats exhibited similar enhanced ASR potentiation after receiving footshock (Davis, 1989). Davis concluded that background conditioning does not contribute significantly to the enhancement of the ASR by footshock (Davis, 1989).

In contrast, research carried out by Richardson and Elsayed (1998) demonstrated that shock sensitization of the ASR is mediated by contextual conditioning. Therefore, the varying sensitization experiments have produced mixed results as to whether sensitization is a process of non-associative or associative learning. However, Van Nobelen and Kokkinidis (2006) examined the shock sensitization paradigm using laboratory rats that received infusions of saline, AP5, CNQX, anisomycin, and muscimol (0.0001ug) into the amygdala. They demonstrated robust shock sensitization of the ASR despite pre-exposure to the apparatus in dB selection testing, pre-drug testing, and post-drug testing prior to receiving the presentation of footshock.

Currently there is no literature demonstrating that fear-potentiated startle rapidly conditions to an explicit CS after a single pairing of the CS-US. This is unlike the fear response of defensive freezing that is proposed to be a learned response to a context which is evident after a single pairing of the CS-US (Fanselow, 1980; LeDoux, 2000). The present study used a similar paradigm to that of Van Nobelen and Kokkinidis (2006) in that laboratory rats in saline and muscimol groups (0.1ug & 0.01ug) exhibited robust shock sensitization amplitudes following pre-exposure to the procedural apparatus during dB selsction testing, predrug baselining testing, and postdrug testing. Therefore, it is proposed that the experimental procedure used in the present experiment did not result in rapid fear conditioning to the context, suggesting that the observed shock augmentation of startle amplitudes reflected an objective measure of 'fast' or rapid fear arousal brought on by the US.

The amygdala has been strongly implicated in the acquisition, expression and conditioning of fear. If sensitization is a form of associative learning or contextual conditioning then we would not have expected to observe attenuation of dorsal and ventral hippocampus with the drug muscimol after shock sensitization as the hippocampus has not been shown to be involved in fear potentiation of the ASR. The current study demonstrated that muscimol infusions into the amygdala, and dorsal and ventral hippocampus blocked shock sensitization thereby providing further support for the premise that sensitization is a non-associative form of learning and may involve both the amygdala and hippocampus (Van Nobelen and Kokkinidis, 2006).

4.8 Clinical Implications

The present study demonstrated that muscimol infusions into the amygdala, and dorsal and ventral hippocampus attenuated shock sensitization of the ASR, which may further the understanding of the neural basis of psychological disorders associated with disturbances in fear and anxiety. The development of many psychological disorders is influenced by anxiety-related stressful experiences (Davidson, Tupler, Wilson, & Comer, 1998). The inhibitory actions of GABA interneurons have been implicated in the physiology of the hippocampus (Buzsaki et al., 1995; Cobb et al., 1995; Whittington, Traub, & Jefferys, 1995; Freund & Gulyas, 1997; Sik, Penntonen, & Buzsaki, 1997; Zhang et al., 1998; Csicsvari, Hirase, Czurko, Mamiya, & Buzsaki, 1999). The interneurons in the hippocampus have been suggested to control the long-term modifications of synaptic transmission and the regulation of phenotypical differentiation during development of the brain (Marty, Berninger, Carroll, Thoenen, 1996). Therefore, it is thought that pathological alterations of GABA neurons within the hippocampus are associated with its physiological functions, and as a result be connected with other structures within the corticolimbic system (Berretta et al., 2001).

Schizophrenia is a human brain disorder associates with corticolimbic regions, most commonly the hippocampus where complex neurocircuitry changes occur (Arnold & Trojanowki, 1996; Benes, 1999). Abnormalities identified in the schizophrenic's hippocampus are a general decrease in GABA neuronal activity in the CA4, CA3, CA2, but not the CA1 regions. A similar finding is observed in neuroleptic free patients. Therefore, the decrease in GABA activity is unlikely to be brought on by the use of anti-

psychotic drugs. The amygdala and hippocampus have been proposed as the strongest candidates for abnormal activity within these structures of schizophrenic sufferers. The amygdala and hippocampus are intimately connected by coherent theta oscillations, therefore suggesting an interaction between the two structures (Paré & Gaudreau, 1996). Imaging studies have consistently found changes in both the hippocampus and amygdala in schizophrenic patients (Shenton, Kikinis, Jolesc, Pollak, LeMay, Wible, Hokama, Martin, Metcalf, Coleman, & McCarley, 1992; Bogerts, Lieberman, Ashtari, Bilder, Degreef, Lerner, Johns, & Masiar, 1993; Marsh, Suddath, Higgins, & Weinberger, 1994). Therefore, research supports the hypothesis that the amygdala may influence the abnormalities found in the hippocampus of the schizophrenic brain. The results of the present study may help further understand the mechanisms underlying the symptoms and etiology of schizophrenia. Berretta and his colleagues (2001) suggest that the hippocampus and amygdala are interconnected especially in regards to GABA activity which in turn supports the results of the present experiement that demonstrated GABA A activity within the amygdala and hippocampus. Accumulating evidence supports the view that changes in the schizophrenic hippocampus in early adulthood maybe due to abnormal GABA activity in the amygdala.

GABA is a major inhibitory neurotransmitter in the CNS (Nicoll et al., 1989). GABA A receptors gate the chloride ionophore channels and have binding sites for benzodiazepines, barbiturates, and anaesthetics which potentiate the GABA response (MacDonald & Olsen, 1994; Johnston, 1996). Benzodiazepines, which are commonly used to treat patients suffering from anxiety, are thought to enhance the action of GABA

in the brain (Tallman & Gallager, 1985; Costa & Guidotti, 1996). Benzodiazepines are the most popular medications used to treat anxiety-related disorders and are thought to act on GABA at the GABA A receptors. The amygdala is the key structure in the brain that has been identified as processing emotional information and generating fear arousal and responding (LeDoux, 1996). A dysfunction in the amygdala has been proposed to be the causal mechanism underlying anxiety-related disorders (Tallman & Gallager, 1985; Costa & Guidotti, 1996; Davis, 1992; LeDoux 1995). The present results establish the importance of the amygdala, hippocampus and GABA activation in fear inducing events which indicate that the amygdala and hippocampus may be potential sites for further investigation of the neural mechanisms involved in anxiety-related psychological disorders.

4.9 Future Research

Future research should further investigate effects of lower doses of muscimol on the hippocampus and amygdala in order to determine the role that GABA neurotransmission plays within the neural circuitry of fear learning and memory. Further research could also study the possible relationship between the amygdala, hippocampus and GABA in the fear-potentiated startle response following Pavlovian conditioning, as most research involving fear conditioning has been confined to the fear measure of freezing. It would be interesting to see if the results would be similar to those demonstrated in the present study.

Disorders of fear acquisition and expression are thought to underlie mental complaints such as panic attacks, anxiety, schizophrenia, depression, and PTSD. Research involving various anxiolytic drugs such as benzodiazepines, has not resulted in any major breakthroughs since first being introduced 50 years ago. Benzodiazepines used for treating anxiety are thought to enhance the inhibitory action of GABA in the brain. Previous research has resulted in a focus on two major brain transmitters GABA (inhibitory) and glutamate (excitatory). The hippocampus has been identified as being involved in the regulation of anxiety-related behaviour (Gray, 1982; Stachowicz et al., 2006). The present study revealed that the amygdala and hippocampus have varying GABA actions in the brain. Therefore further research needs to be carried out in order to understand GABA's actions within the amygdala and hippocampus thereby advancing understanding of the nature, development, and treatment of anxiety-and fear-related disorders.

4.10 Summary

Previous research has demonstrated that pretraining infusion of muscimol into the lateral and basolateral amygdala interferes with Pavlovian fear conditioning (Helmstetter & Bellgowan, 1994). The present study found that postshock fear can be reliably inhibited by administration of the GABA A agonist muscimol into the BLA, and dorsal and ventral hippocampus. As GABA A receptors did not mediate shock reactivity or the amplitude of the ASR in the BLA, dorsal, and ventral hippocampus, it can be reasonably concluded that GABA neurotransmission within the amygdala and hippocampus may be

an essential component modulating the fear arousal produced by footshock. The results of the current experiment suggest that both the dorsal and ventral hippocampus play a larger role in the emotion of fear than initially believed. It would appear that the amygdala and its limbic counterparts all take part in the initial stages of fear arousal. The amygdala, dorsal and ventral hippocampus have been identified as critical brain structures involving GABA neurotransmission in the unconditioned shock sensitization paradigm. Shock sensitization has been represented as a rapid state of fear that can be expressed and measured through an animal's enhancement of the ASR.

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