THE LATERAL SEPTUM AND THE REGULATION OF ANXIETY

by

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Abstract

Compared to other structures, such as the amygdala, the lateral septum’s (LS) role in the regulation of anxiety and/or behavioural defense is relatively understudied. Thus, the overarching goal of this thesis was to further investigate its contribution to rats’ anxiety-related behaviours. In Chapter 2, we demonstrate, for the first time, that while the dorsal LS does not mediate rats’ appetitive motivation or anxiety in the novelty induced suppression of feeding (NISF) paradigm, it does modulate their defensive behaviours in the elevated plus maze (EPM) and shock probe burying tests (SPBT). In Chapter 3, we are the first to show that bilateral infusions of histamine, a neurochemical previously linked to anxiety, into the LS reduce rats’ anxiety-related behaviours in the EPM and NISF. In addition, we report a novel double dissociation between lateral septal H₁ and H₂, and H₃ receptors in their regulation of rats’ defensive behaviours in those two paradigms. More specifically, the H₁ and H₂ receptors contribute to rats’ hyponeophagia in the NISF but not their open arm exploration in the EPM, while the H₃ receptors modulate rats’ defensive behaviors in the EPM but not in the NISF. Finally, in Chapter 4, we report for the first time that infusions of histamine into the LS, which produce behavioural anxiolysis, increase rather than decrease the frequency of reticular-elicited hippocampal theta activity, a putative neurophysiological correlate of anxiolytic-drug action. Altogether, the data in this thesis increase our understanding of how the LS contributes to rats’ defensive behaviours and adds to the existing literature regarding the neurobiology of fear/anxiety. More importantly though, the data presented here could ultimately aid in the development of novel drugs to treat anxiety disorders in humans.
Co-Authorship

In all cases, I (San-San Chee) participated in the study design, data acquisition, data analysis, and manuscript participation. Janet L. Menard contributed to the study design, data analysis, and manuscript participation for Chapters 2 and 3 and provided editorial advice for Chapters 1 and 5. Hans C. Dringenberg contributed to the study design and data analysis for Chapter 4. Both Janet L. Menard and Hans C. Dringenberg contributed to the manuscript preparation for Chapter 4.

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Chapter 4 has been submitted for publication, and is under review:

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List of Abbreviations

5-HT  serotonin (5-hydroxytryptamine)
5-HT$_{1A}$  serotonin 1A receptor subtype
5-HT$_{2}$  serotonin 2 receptor subtype
5-HT$_{3}$  serotonin 3 receptor subtype
AA  arachidonic acid
AHN  anterior hypothalamic nucleus
Akt  protein kinase B
ANOVA  analysis of variance
AP  anterior-posterior
ATP  adenosine triphosphate
cAMP  cyclic adenosine monophosphate
CIP/HIS  ciproxifan/histamine
CIP/SAL  ciproxifan/saline
CREB  cAMP response element binding-protein
CRF  corticotropin releasing factor
DAG  diacyl glycerol
DBB  diagonal band of Broca
DV  dorsal-ventral
EPM  elevated plus maze
FFT  Fast Fourier Transform
GABA  γ-aminobutyric acid
GABA$_{A}$  GABA A receptor subtype
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSK-3</td>
<td>glycogen synthase kinase-3</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>intracerebroventricular</td>
</tr>
<tr>
<td>i.p.</td>
<td>intraperitoneal</td>
</tr>
<tr>
<td>IP$_3$</td>
<td>inositol 1,4,5-trisphosphate</td>
</tr>
<tr>
<td>IPSP</td>
<td>inhibitory post-synaptic potential</td>
</tr>
<tr>
<td>LH</td>
<td>lateral hypothalamus</td>
</tr>
<tr>
<td>LS</td>
<td>lateral septum</td>
</tr>
<tr>
<td>LSd</td>
<td>lateral septum, dorsal</td>
</tr>
<tr>
<td>LSi</td>
<td>lateral septum, intermediate</td>
</tr>
<tr>
<td>LSv</td>
<td>lateral septum, ventral</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>MR</td>
<td>median raphe nucleus</td>
</tr>
<tr>
<td>MS</td>
<td>medial septum</td>
</tr>
<tr>
<td>NA</td>
<td>nucleus accumbens</td>
</tr>
<tr>
<td>NeuN</td>
<td>neuronal nuclei</td>
</tr>
<tr>
<td>NISF</td>
<td>novelty induced suppression of feeding</td>
</tr>
<tr>
<td>NO</td>
<td>nitrous oxide</td>
</tr>
<tr>
<td>NPY</td>
<td>neuropeptide Y</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PKA</td>
<td>protein kinase A</td>
</tr>
<tr>
<td>PIP$_2$</td>
<td>phosphatidylinositol 4,5-bisphosphate</td>
</tr>
<tr>
<td>PYR/HIS</td>
<td>pyrilamine/histamine</td>
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<tr>
<td>PYR/SAL</td>
<td>pyrilamine/saline</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>RAN/HIS</td>
<td>ranitidine/histamine</td>
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<tr>
<td>RAN/SAL</td>
<td>ranitidine/saline</td>
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<tr>
<td>RF</td>
<td>reticular formation</td>
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<td>RPO</td>
<td>nucleus pontis oralis</td>
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<tr>
<td>SAL/HIS</td>
<td>saline/histamine</td>
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<tr>
<td>SAL/SAL</td>
<td>saline/saline</td>
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<tr>
<td>s.c.</td>
<td>subcutaneous</td>
</tr>
<tr>
<td>SUM</td>
<td>supramammillary nucleus</td>
</tr>
<tr>
<td>SPBT</td>
<td>shock probe burying test</td>
</tr>
<tr>
<td>SSRI</td>
<td>selective serotonin reuptake inhibitor</td>
</tr>
<tr>
<td>TM</td>
<td>tuberomammillary nucleus of the posterior hypothalamus</td>
</tr>
<tr>
<td>V$_{1A}$</td>
<td>vasopressin 1A receptor subtype</td>
</tr>
<tr>
<td>VTA</td>
<td>ventral tegmental area</td>
</tr>
</tbody>
</table>
Chapter 1. **General Introduction**

The lateral septum (LS) is a structure found in the subcortical forebrain; it is rostrodorsal to the hypothalamus and the anterior commissure and dorsocaudal to the nucleus accumbens (Sheehan et al., 2004). In humans, the LS borders the ventral aspects of the lateral ventricles, with the LS of each hemisphere separated by the septum pellucidum, a thin membrane containing mostly fiber tracts and glia that connects the dorsal parts of the LS to the base of the corpus callosum (Sheehan et al., 2004). In rodents, the LS is situated between the lateral ventricles and is directly ventral to the corpus callosum (Sheehan et al., 2004).

The LS is implicated in fear/anxiety/behavioural defense (Sheehan et al., 2004). Interest in the LS has grown since the 1970s (see Figure 1.1); however, articles specific to the LS and its role in fear, anxiety, or defense are surprisingly few, especially when compared to the number of articles available on the amygdala and *its* relation to fear, anxiety, or defense. While the disproportionate focus on the amygdala might suggest that it is critical or more important than other brain regions (e.g. the LS) in regulating anxiety, a more likely explanation is that the amygdala has simply received more attention, and that the LS (and other regions) are under studied (Menard and Treit, 1999). Indeed, the amygdala and the septum have been shown to mediate different fear reactions, indicating that the amygdala does not globally regulate fear, anxiety, or defense (Pesold and Treit, 1994; Treit et al., 1993b). Accordingly, anxiety, fear, or defense as a whole cannot be attributed to a single brain area (Menard and Treit, 1999), and the contributions of other structures such as the LS to these affective states or behaviours need to be studied.
Figure 1.1. The number of citations available from 1961 to 2013 following a Medline database search using the following search terms: “amygdala and (fear or anxiety or defense)”, “lateral septum”, and “lateral septum and (fear or anxiety or defense)”. 
Thus, the goal of this thesis is to further investigate the lateral septum and its regulation of fear/anxiety/defensive behaviours. Chapter 2 focuses on the role of the LS in modulating rats’ defensive behaviours in novelty-induced suppression of feeding. Chapter 3 explores the neuropharmacology of the LS; more specifically, whether the histaminergic system of the LS is involved in rats’ anxiety-like responses. In Chapter 4, the findings from Chapter 3 are extended by determining whether the behavioural effects of histamine in the LS are associated with a reduction in hippocampal theta frequency, a putative neurophysiological correlate of anxiolytic drug action. To provide background information for these three empirical chapters, the remainder of this chapter will be a literature review.

1.1. Fear and anxiety

Fear and anxiety are basic, primitive emotions that aid survival (Sylvers et al., 2011). Fear is often described as an aversive reaction to a specific, perceived threat, while anxiety is usually defined as prolonged hypervigilance in response to, or in anticipation of, a diffuse threat in which the danger is not clearly imminent (Sylvers et al., 2011). In general, fear tends to be shorter lived than anxiety (Sylvers et al., 2011). Normally, fear and anxiety are adaptive states that help maintain an organism’s safety and well-being (Lang et al., 2000). However, when over-expressed, these adaptive traits can become maladaptive and detrimental, either physically (e.g. cardiovascular disease) or psychologically (e.g. phobias) (Sylvers et al., 2011). In humans, inappropriate expressions or levels of trait-like fear and/or anxiety result in psychopathologies that are largely categorized as anxiety disorders (Sylvers et al., 2011). Globally, the prevalence of anxiety disorders is estimated to be 7.3% (Baxter et al., 2013). In Canada alone, anxiety disorders are estimated to cost $14.4 billion per year when indirect costs (e.g. disability) are
included (Stephens and Joubert, 2001). Clearly, more effective treatments for these disorders are needed.

1.2. Animal models of anxiety

In anxiety research, animal models are employed as screening tools for novel therapeutic compounds and as simulations to study the neurobiology of fear or anxiety-related behaviours (Rodgers et al., 1997). Some animal models of anxiety are based on stress responses, either physiological (e.g. hypothermia) or endocrinological (e.g. plasma corticosterone) (Rodgers et al., 1997). The vast majority, however, are behavioural in nature and are broadly categorized as to whether they involve conditioned or unconditioned responses to fear or anxiety-eliciting stimuli (Rodgers et al., 1997). Conditioned models involve the animal’s conditioned responses to stressful (and often painful) events, such as an electric foot shock (Bourin et al., 2007). These models allow the experimenter relative control over behavioural baselines, but they often require food or water deprivation, electric shock, as well as time spent in training subjects (Rodgers et al., 1997). Conversely, models utilizing unconditioned responses measure the animal’s spontaneous or natural responses (e.g. avoidance, flight, freezing) to stimuli not overtly involving pain or discomfort (e.g. exposure to open space or to a predator) (Bourin et al., 2007). While behavioural baselines are more variable, unconditioned models are less vulnerable to confounds such as learning and memory, pain, hunger, or thirst (Rodgers et al., 1997). These models are also generally considered to have more ethological relevance than conditioned models (Bourin et al., 2007; Rodgers et al., 1997).

1.2.1. Ethological relevance

In recent years, research in the neurobiology of anxiety has shifted towards the use of more ethological or “ecologically valid” animal models (McGregor et al., 2002; Rodgers, 1997).
Many of these models expose laboratory animals to stimuli closely resembling those encountered in their natural environments instead of the more artificial stimuli traditionally used in the laboratory (Dielenberg et al., 2001; McGregor et al., 2002). For example, rather than receiving footshocks or being restrained, the animals are exposed to an aggressive conspecific (Chung et al., 2000), a live predator (e.g., a cat) (Blanchard and Blanchard, 1972), a predatory cue (e.g., owl call or cat odor) (Blanchard et al., 1990; Dielenberg et al., 1999), or alarm signals (e.g., ultrasonic vocalizations) (Beckett et al., 1997; Brudzynski and Chiu, 1995). An advantage of using naturalistic stimuli is that more ethological defensive behaviours can be evoked, leading to improved modeling and analysis of anxiety states (Dielenberg et al., 2001). Other ethologically relevant models, such as the shock probe burying test, do use artificial stimuli (e.g., a shock probe) but tap into defensive behaviours (e.g., defensive burying) observed in the wild. Notably, ecologically valid models tend to study behavioural patterns instead of a single response (Blanchard et al., 1993); this approach is more likely to encompass the complexities of the defense system and allow the detection and identification of specific anxiety subsystems (Staples, 2010). In addition, the neural correlates underlying anxiety are more efficiently mapped because the animals’ responses are not limited to stimuli having little relevance to their natural environments, and moreover, are not influenced by extraneous factors such as pain reactivity or hunger/thirst (Staples, 2010). In this thesis, I use three ethologically relevant animal models of anxiety: the elevated plus maze, the novelty-induced suppression of feeding paradigm, and the shock probe burying test.

1.2.2. Elevated plus maze (EPM)

The elevated plus maze (EPM) is one of the most popular and commonly used animal models of anxiety (Carobrez and Bertoglio, 2005; Hogg, 1996). The apparatus is cross-shaped
and consists of two opposing open arms and two opposing enclosed arms; all arms have open roofs (Pellow et al., 1985). The maze is elevated above the ground (Pellow et al., 1985). Rodents are placed into the center of the maze, facing a closed arm, for a free-exploration test; they preferentially explore the closed arms and avoid the open arms (Hogg, 1996; Pellow et al., 1985). Compared to rats confined to the closed arms, rats confined to the open arms show increased levels of anxiety-related behaviours, such as freezing, defecation, and immobility, as well as significantly higher plasma corticosterone levels (Pellow et al., 1985). Thus, the open arms are believed to elicit fear or anxiety, and, as a consequence, the rats avoid them (Pellow et al., 1985). Interestingly, rats show similar levels of open arm avoidance even if the maze height is decreased from 50cm to 6cm (Treit et al., 1993a). Additionally, they spend more time on an open arm with a raised Plexiglas edge than an open arm with the standard flat edges even if both arms have the same height (Treit et al., 1993a). These findings suggest that the anxiogenic stimulus in the EPM is the open space rather than the height, and that rats’ open arm avoidance reflects thigmotaxis, a natural defensive behaviour in which rodents stay close to vertical spaces to avoid detection by predators (Treit et al., 1993a). As rats naturally display thigmotaxis, the open arm aversion elicited during the test is thought to have ecological relevance to the animal and as such, the EPM is considered to be an “ethological” animal model of anxiety (Rodgers et al., 1997).

The primary index of anxiety in the EPM is open arm activity, which is expressed as the percentage of open arm entries (open/open + closed) and/or as the percentage of open arm time (open/open + closed) (Pellow et al., 1985; Hogg, 1996). Decreases in open arm activity indicate increases in anxiety; while conversely, increases in open arm activity signify decreases in anxiety.
Locomotor activity is measured with the number of total and/or closed arm entries (Hogg, 1996).

Pharmacological studies have reported that clinically effective anxiolytics (chlordiazepoxide, diazepam) specifically increase open arm activity, while yohimbine, which increases anxiety in humans, selectively decreases open arm activity (Pellow et al., 1985). Haloperidol, which is not clinically established to decrease anxiety, does not specifically affect open arm activity (Pellow et al., 1985). Similar null effects are found following the administration of the antidepressants imipramine and mianserin (Pellow et al., 1985). Inverse agonists of the benzodiazepine receptor, such as FG 7142 and CGS 8216, produce anxiogenic-like effects in the EPM: rats given these compounds show decreased open arm activity relative to controls (Pellow and File, 1986). Notably, injections of the benzodiazepine receptor antagonists Ro 15-1788 and ZK 93426 do not affect rats’ open arm exploration (Pellow and File, 1986).

The EPM has multiple advantages – simplicity of the design, bidirectional drug sensitivity, economy, and is quick – each test trial only takes five minutes to complete (Carobrez and Bertoglio, 2005). Moreover, the EPM does not require lengthy training, food/water deprivation, or electric shock (Pellow et al., 1985; Rodgers et al., 1997). However, many variations of the EPM paradigm exist, and a wide range of variables can influence behavioural or pharmacological responses to the test (Hogg 1996; Rodgers et al., 1997). For example, species (mouse versus rat), strain, gender, housing condition, or prior test experience can all affect responding in the EPM (Carobrez and Bertoglio, 2005). One major limitation of the EPM is that treatment-induced changes in open arm activity may reflect changes in appetitive motivation rather than changes in anxiety levels, per se (Wall and Messier, 2001). For instance, food deprivation increases rats’ open arm activity without affecting their defensive behaviours in non-
exploration based models of anxiety (Geen et al., 2003a; Geen at al., 2003b; Inoue et al., 2004). Thus, an additional paradigm is needed that can differentiate between the effects of anxiety and those of appetitive motivation – the novelty induced suppression of feeding paradigm.

1.2.3. Novelty-induced suppression of feeding (NISF)

In 1934, Hall reported that rats decreased their feeding when placed into a novel environment (Hall, 1934). This suppression of feeding in response to novelty is referred to as “hyponeophagia” (Dulawa and Hen, 2005). Hyponeophagia-based models are conflict-based where animals encounter a choice between approaching and ingesting desirable food and avoiding the anxiogenic stimulus, the novel environment (Dulawa and Hen, 2005). These paradigms are considered ethologically relevant and do not involve painful stimuli or require complex training procedures (Dulawa and Hen, 2005). In addition, they are economical and simple to conduct (Dulawa and Hen, 2005). Currently existing hyponeophagia paradigms involve taking the animals to an unfamiliar novel environment and measuring the latency to consume and/or the amount eaten by either food-deprived animals given lab chow or by satiated animals given highly palatable food (Dulawa and Hen, 2005).

Pharmacological studies have shown that hyponeophagia models have a high predictive validity for the anxiolytic effects of drugs and the time-course of their effects (Dulawa and Hen, 2005). More specifically, acute treatment with a variety of benzodiazepines, including diazepam, adinazolam, and chlordiazepoxide, decreases rodents’ hyponeophagia (Bodnoff et al., 1988; Merali et al., 2003; Shepherd and Broadhurst, 1982; Shepherd and Estall, 1984). Picrotoxin, a GABA antagonist with anxiogenic properties, increases hyponeophagia (File and Lister, 1984; Shepherd et al., 1985), while the benzodiazepine antagonist Ro-15-1788 has no effect (Shepherd et al., 1985). In humans and animals, tolerance to the sedative (but not the anxiolytic) effects of
benzodiazepines develops after repeated administration (Dulawa and Hen, 2005). As such, benzodiazepines should reduce hyponeophagia irrespective of whether they were administered once or repeatedly. Accordingly, chronic treatment with adinazolam (Bodnoff et al., 1988) or diazepam (Bodnoff et al., 1989; Shepherd and Broadhurst, 1982) decreases rats’ hyponeophagia. A number of serotonergic (5-HT) compounds have also been tested in hypophagia-based models. For example, the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT, the 5-HT<sub>2</sub> antagonist ritanserin, and two 5-HT<sub>3</sub> antagonists, ondansetron and tropisetron, all reduce hyponeophagia when given acutely (Rex et al., 1998). All these compounds have anxiolytic (and antidepressant) properties (Dulawa and Hen, 2005). Buspirone, a 5-HT<sub>1A</sub> agonist and clinically effective anxiolytic, takes about 1-2 weeks before patients experience its anxiolytic effects (Gao and Ward, 1986). This “lagtime” is mirrored in animal studies: in both the EPM and Vogel water licking tests, buspirone given chronically yields stronger anxiolytic effects than when it is given acutely (Cole and Rodgers, 1994; Yamashita et al., 1995). Similarly, chronic, but not acute, buspirone treatment decreases hyponeophagia (Bodnoff et al., 1989; Merali et al., 2003). Notably, dopaminergic compounds do not affect hyponeophagia. For example, haloperidol, a dopamine antagonist, and sulpiride, a D<sub>2</sub> antagonist, two compounds that do not affect anxiety, also do not affect rats’ hyponeophagic responses (Merali et al., 2003; Rex et al., 1998). These findings increase the construct validity of the hyponeophagia paradigm, i.e., that it actually measures anxiety (Dulawa and Hen, 2005).

A major methodological issue with currently existing hyponeophagia models is that many of them do not control for the effects of appetite on animals’ feeding behaviours (Dulawa and Hen, 2005). Many drugs can affect appetite (van der Hoek and Cooper, 1994) or food preferences (Heisler et al., 1997; Leibowitz et al., 1993). For example, benzodiazepines, especially at higher doses, can enhance appetite (Cooper et al., 1981; Wise and Dawson, 1974).
Moreover, drugs can be stimulating or sedative, both of which can influence appetite (Dulawa and Hen, 2005). Another concern involves the use of food-deprived animals in some hyponeophagic models – food deprivation can potentiate the effects of drugs on appetite (Dulawa and Hen, 2005). Food deprivation in itself is also stressful to animals, and moreover, individual animals can experience different hunger levels (Dulawa and Hen, 2005). Hyponeophagic paradigms that offer satiated animals a familiar, palatable food also need to control for appetitive motivation, as different treatments can affect the rate that the animal learns to eat the unfamiliar food (Dulawa and Hen, 2005).

The hyponeophagia paradigm specifically used in this thesis, the novelty-induced suppression of feeding (NISF) paradigm, is derived from Merali et al. (2003)’s pharmacologically validated version and controls for appetitive motivation. Here, rats are offered graham crackers, a highly palatable snack, in a familiar environment (i.e., their home cage) for six consecutive days. The first five days serve to habituate the rats to the unfamiliar food, while the sixth day is the home cage test. On the seventh day, the novel cage test, the rats are taken to an unfamiliar testing room, where each rat is placed into an opaque cage lined with fresh bedding and subsequently given the graham crackers. On all days, the latency to initiate snack consumption is measured. Anxiolytic effects in the NISF are indicated by a decrease in the latency to initiate snack consumption in the novel cage without affecting response latencies in the home cage. In contrast, treatment-induced increases in appetitive motivation would decrease latency scores in both the home and novel cages.

The NISF paradigm offers several advantages over other hyponeophagia-based paradigms. First, the inclusion of the home cage test allows one to determine whether any anxiolytic effects detected in the novel cage are truly due to treatment-induced changes in
anxiety or rather reflect changes in appetitive motivation (Dulawa and Hen, 2005). Second, the NISF does not involve food deprivation, which is stressful to animals, and as mentioned above, food-deprived animals can show different hunger levels (Dulawa and Hen, 2005). One disadvantage of the NISF is that the rats have to be habituated to the novel food over five days and therefore requires more time to conduct, but the advantages of the NISF over past hyponeophagia-based models clearly outweigh any disadvantages.

1.2.4. Shock probe burying test (SPBT)

Defensive burying describes the rodent behaviour of using quick, forward-reaching movements of their front paws or head to push bedding material towards a localized aversive stimulus or threat (de Boer and Koolhaas, 2003; Pinel and Treit, 1978). For example, rats have been shown to bury electrified prods (Pinel and Treit, 1978), striking mouse traps, discharging flashcubes (Terlecki et al., 1979), and tubes directing airbursts into their faces (Pinel et al., 1994) or that emit predator odor (Holmes and Galea, 2002). Defensive burying is specific to an aversive stimulus, as rats will bury spouts filled with Tabasco sauce or rat chow pellets coated with quinine, a bitter substance, but will not bury spouts filled with water or apple juice (Poling et al., 1981). It is thought that by burying harmful and/or unfamiliar objects, the animal can avoid or remove aversive stimuli or threats from their environment (de Boer and Koolhaas, 2003). As a term, “defensive burying” was first coined by Pinel and Treit (1978), who took advantage of this behaviour to develop the shock probe burying test (SPBT).

In the SPBT, rodents are placed into a Plexiglas test chamber that they had been previously habituated to. The test chamber is lined with fresh bedding with a wire-wrapped probe inserted through a small hole 2cm above the bedding. The wires are uninsulated and connected to a shock source. Whenever a subject contacts the probe with its forepaws or nose, it
receives a brief, contact-induced electrical shock (de Boer and Koolhaas, 2003). After the subject receives its first shock, its behaviour is observed/recorded for 15 minutes. The primary index of anxiety in the SPBT is defensive burying, which is operationally defined as the act of pushing, digging, flicking, or shovelling material (e.g. bedding or sawdust) towards and around the shock probe with quick, rapid movements of the forepaws and snout (Pinel and Treit, 1978). Decreases in defensive burying indicate reduced anxiety; while conversely, increases in defensive burying suggest increased anxiety. Notably, treatment-induced changes in shock probe burying can also reflect treatment-induced changes in associative learning (i.e. associating the probe with shock/pain) or pain sensitivity. As such, the number of shocks that the animal receives is used as a proxy for associative learning (Treit and Menard, 1997). Similarly, to measure pain sensitivity, a mean reactivity score is calculated for each subject (Treit and Menard, 1997). Each time the animal receives a shock, its shock reactivity is scored using a four-point scale (as defined in Menard and Treit, 1996): (1) flinch of the forepaw or head only; (2) whole body flinch, without locomotion away from the probe; (3): whole body flinch with movement and/or jumping away from the probe; (4): whole body flinch and/or jumping followed by running to the opposite end of the test chamber. Each individual shock reactivity score is summed, and the total is divided by the number of shocks to yield a mean shock reactivity score (Treit and Menard, 1997).

A number of anxiolytic compounds have been shown to selectively suppress defensive burying in the SPBT without affecting associative learning, pain sensitivity, or general locomotor activity (de Boer and Koolhaas, 2003). For example, benzodiazepine anxiolytics, such as diazepam (Blampied and Kirk, 1983; Treit, 1985; Treit et al., 1981), chlordiazepoxide, or midazolam (Treit, 1990; Treit et al., 1981), dose-dependently suppress defensive burying. Chlordiazepoxide-induced suppression of burying can be blocked with GABA_A-receptor or
benzodiazepine antagonists (Treit, 1987). Reductions in shock-probe burying are found after administration of serotonergic anxiolytics, such as buspirone, flesinoxan, and ipsapirone (Groenink et al., 1995; Korte and Bohus, 1990; Treit and Fundytus, 1988). In contrast, anxiogenic compounds, including yohimbine (Treit and Fundytus, 1988), the benzodiazepine receptor inverse-agonists β-CCE (Tsuda et al., 1989) and DMCM (de Boer and Koolhaas, 2003), and corticotropin-releasing hormone (Diamant et al., 1992) increase shock probe burying. More importantly, non-anxiolytic compounds do not significantly affect shock probe burying (de Boer and Koolhaas, 2003). For example, the antipsychotics chlorpromazine (Craft et al., 1988; Treit, 1990) and haloperidol (Sakamoto et al., 1998), and morphine, an opiate analgesic, (Craft et al., 1988; Treit et al., 1981) do not affect shock probe burying. While injections of D-amphetamine, a psychostimulant, increase shock probe burying, the increase is not significant (Treit et al., 1981). Finally, scopolamine, a muscarinic antagonist, was not found to significantly alter defensive burying (Treit, 1990). Altogether, these pharmacological experiments indicate that the suppression of burying in the SPBT is selective to anxiolytic compounds.

Although the SPBT has been extensively used as an index of fear/anxiety in a fair number of studies, the paradigm itself has several disadvantages. Like the EPM, the SPBT is susceptible to a wide range of organismic and procedural variables (de Boer and Koolhaas, 2003). For example, the degree of defensive burying can be affected by the type and availability of the bedding material, the animal’s strain or genotype, or lighting levels (de Boer and Koolhaas, 2003). Burying itself is highly variable between individual subjects and can differ between experiments and between subjects (de Boer and Koolhaas, 2003).
1.2.5. The combined use of multiple models

Each of the three models mentioned above utilizes a different type of anxiogenic stimulus, and each one therefore elicits a defensive response specific to that stimulus. In the EPM, animals passively avoid the open environment of the open arms. In the NISF, the novel environment elicits hyponeophagia. In the SPBT, rodents actively bury a discrete painful stimulus – the shock probe. The idea that these different behavioural tests, at least the EPM and SPBT, evoke different types of anxiety-related behaviours is supported by multiple studies that fail to find a correlation between measures of anxiety in those two paradigms (Beardslee et al., 1989; Treit et al., 1993b; Treit and Menard, 1997). The combined use of the three models allows for the expression of different defensive behaviours and therefore leads to a more comprehensive study of rodents’ behavioural defense repertoire.

The combined use of the EPM, NISF, and SPBT also allow the limitations of one model to be complemented by the strengths of another. For example, as mentioned above, one limitation of the EPM is that open arm exploration can be influenced by appetitive motivation (Wall and Messier, 2001). By including the NISF, treatment-induced increases in open arm activity can be assayed as to whether these increases truly indicate decreased anxiety or instead reflect increased appetitive motivation. Another example is that open arm exploration in the EPM and shock probe burying in the SPBT are susceptible to changes in general activity. As discussed above, anxiolytic effects in the EPM are indicated in an increase in a particular behaviour (open arm exploration), while in the SPBT, anxiolytic effects are indicated by a decrease in a particular behaviour (shock probe burying) (Treit et al., 1993b). Given the bidirectional nature of these two paradigms, anxiolytic effects found in both tests cannot be
easily explained in terms of nonspecific effects on general activity, arousal, response inhibition, or pain sensitivity (Treit et al., 1993b).

1.3. Neural substrates of fear/anxiety

The study of the neural substrates of anxiety has largely, for ethical and technical reasons, been performed in nonhumans (Canteras et al., 2010). This body of research has implicated a variety of brain regions in the regulation of fear/anxiety. Some of these regions include the amygdala (Wilensky et al., 2006; Anglada-Figueroa and Quirk, 2005; LeDoux, 2000), hypothalamus (Canteras, 2002; Schwerdtfeger and Menard, 2008), periaqueductal gray (Canteras et al., 2010), hippocampus (Bannerman et al., 2004), prefrontal cortex (Davidson, 2002), and, as the focus of this thesis, the lateral septum (Sheehan et al., 2004).

1.4. The lateral septum (LS)

The LS, together with the medial septum (MS), form the septum (Sheehan et al., 2004). As mentioned above, the septum is located in the subcortical forebrain, rostroventral to the hypothalamus and anterior commissure, and slightly dorsal to the nucleus accumbens (Sheehan et al., 2004). As their names suggest, the LS forms the lateral aspects of the septum, while the MS comprises its medial, more ventral aspects (Paxinos and Watson, 1998). The LS and MS differ in their neurochemistry and in whether they primarily receive input from or send input to the hippocampus (Sheehan et al., 2004). More specifically, the MS, together with the diagonal band of Broca, send GABAergic and cholinergic projections to the hippocampus, allowing it to regulate hippocampal function, such as the theta rhythm (Vertes and Koscis, 1997; see section 1.6.). In contrast, the LS receives substantial glutamatergic input from the hippocampus and is primarily composed of GABAergic projection neurons that are interconnected with lateral and medial hypothalamic nuclei and midbrain areas (e.g.
periaqueductal gray) implicated in drive states and motivated responses (Risold and Swanson, 1997b; Sheehan et al., 2004). It also receives input from regions involved in emotional states, such as the medial prefrontal cortex, entorhinal cortex, amygdala, and bed nucleus of the stria terminalis (Risold and Swanson, 1997b; Sheehan et al., 2004). Thus, the LS is thought to function as a nodal point that integrates affective information from the amygdala, hypothalamus, and bed nucleus with cognitive information from the prefrontal cortex, hippocampus, and entorhinal cortex, and sends the processed information to areas (e.g. hypothalamic nuclei) directly controlling the behavioural responses appropriate to a specific environmental stimulus (Sheehan et al., 2004).

The LS is clearly involved in fear/anxiety. Exposure to anxiety-provoking or stressful stimuli, such as restraint, cat odor, foot shock, air-puff, or open space, increase neural activity, as indicated by enhanced c-fos expression, in the LS (Cullinan et al., 1995; Dielenberg et al., 2001; Duncan et al., 1996; Nagahara and Handa, 1997). Inactivation of the LS, whether permanently (e.g. electrolytic lesions) or temporarily (e.g. intra-LS infusions of midazolam or muscimol), reduces rats’ defensive behaviours in the EPM and SPBT (Menard and Treit, 1996; Pesold and Treit, 1996; Degroot and Treit, 2001). The hippocampus works with the LS to modulate anxiety: blocking the glutamatergic input from the hippocampus to the LS, via intra-septal infusions of CNQX, a non-NMDA receptor antagonist, reduces defensive behaviours in the EPM and SPBT (Menard and Treit, 2000). Moreover, while intra-hippocampal infusions of midazolam increase open arm activity in the EPM, this anxiolytic-like effect is reversed if L-glutamate is co-infused into the LS (Menard and Treit, 2001). Tetrodotoxin lesions of the fimbria fornix, which contains the glutamatergic projection fibers from the hippocampus to the LS, reduces anxiety-related behaviours in the EPM (Degroot and Treit, 2004). Finally, the ventral hippocampus and LS have
been shown to work in tandem to modulate rats’ open-arm exploration in the EPM (Trent and Menard, 2010).

1.4.1. The LS, motivation, and hyponeophagia – Chapter 2

In addition to anxiety, the LS, especially its dorsocaudal aspects, is implicated in reward and motivation (Sheehan et al., 2004). The LS coordinates drive states associated with natural rewards such as thirst and appetite and is involved in pair-bond formation and parental behaviour (Morgenson, 1976; Sheehan et al., 2004; Liu et al., 2001; Sheehan et al., 2000). Moreover, exposure to stimuli that had been previously paired with positive reinforcers, such as food or cocaine, increases LS neural activity (Yadin and Thomas, 1981; Brown et al., 1992; Franklin and Druhan, 2000). Anatomically, the LS is connected with the mesocorticolimbic dopamine system (Sheehan et al., 2000). The LS projects to the ventral tegmental area (VTA) and nucleus accumbens (NA), and receives input from the medial prefrontal cortex (Sheehan et al., 2000). The LS also sends strong projections to the lateral hypothalamus, an area known for its role in appetitive motivation (Risold and Swanson, 1997b; Sheehan et al., 2004). Behaviourally, pharmacological manipulations of the LS affect feeding behaviour (Wang and Kotz, 2002; Bakshi et al., 2007).

As discussed above, one limitation of the EPM is that open arm exploration can be affected by appetitive motivation (Wall and Messier, 2001). The LS’s role in hyponeophagia is currently unclear. While one study found that septal lesions decreased the latency to initiate snack consumption in the novel cage without affecting home cage behaviour (Ross et al., 1975), another failed to find any effects (Devenport and Balagura, 1971). In both of these studies, the entire septum, which includes the MS, was lesioned. The MS’s role in anxiety is equivocal. While electrolytic lesions of the MS reduce rats’ anxiety-related behaviours in the EPM and
SPBT (Menard and Treit, 1996), infusions of the benzodiazepine midazolam into the MS have no effect (Pesold and Treit, 1996). However, intra-MS infusions of the GABA<sub>A</sub> agonist muscimol have been found to increase open arm exploration in the EPM and decrease shock probe burying in the SPBT (Degroot et al., 2001). Thus, the goal of Chapter 2 was to determine whether excitotoxic lesions of the LS affect anxiety or appetitive motivation in the NISF. The EPM and SPBT were included as positive controls, as lesions or chemical perturbations of the LS have been reported to reduce defensive behaviours in these two paradigms (Menard and Treit, 1996; Pesold and Treit, 1996). To limit damage to the MS, the dorsal LS was lesioned using the excitotoxic compound ibotenic acid, which, unlike in electrolytic lesions, destroy cell bodies while leaving fibres of passage intact (Sheehan et al., 2004).

**1.4.2 Neurochemical systems of the LS involved in fear/anxiety**

With respect to neurochemistry, the LS is innervated with axon terminals containing, and expresses receptors for, a variety of neurochemicals implicated in fear/anxiety (Sheehan et al., 2004). For example, corticotropin releasing factor (CRF), and the closely related urocortins I, II, and III, have been reported to increase fear and anxiety (Dunn and Berridge, 1990; Skelton et al., 2000). Urocortin III-immunoreactive fibres, likely originating from the medial hypothalamus (Risold and Swanson, 1997b; Sakanaka et al., 1988), are found in the intermediate and ventral aspects of the LS, the LSi and LSv, respectively (Li et al., 2002). High mRNA expression levels of the CRF<sub>2</sub> receptor, which urocortins show a high affinity for, are found in the LSi, with lower levels detected in the dorsal LS (LSd) (Van Pett et al., 2000). Acutely blocking these receptors with αhCRH, a CRF<sub>1</sub>/CRF<sub>2</sub> antagonist, attenuates shock-induced freezing in rats without affecting locomotor activity or pain sensitivity (Bakshi et al., 2002). Correspondingly, infusions of CRF into the LS reduce mice’s open arm activity in the EPM (Radulovic et al., 1999).
Another neurochemical that plays a large role in modulating anxiety is serotonin (Akimova et al., 2009). Selective 5-HT reuptake inhibitors (SSRIs) and 5-HT$_{1A}$ agonists, such as the atypical anxiolytic buspirone, have potent anxiolytic effects (Bagdy, 1998). The raphe nuclei send serotonin-containing terminals to the LS (Gall and Moore, 1984), and the LS expresses high 5-HT$_{1A}$ receptor mRNA levels (Pompeiano et al., 1992). Depending on the laboratory, intra-LS infusions of 5-HT$_{1A}$ agonists yield both anxiogenic and anxiolytic like effects, probably due to the complex electrophysiological effects of 5-HT$_{1A}$ agonists on LS neurons (Sheehan et al., 2004). A third neurochemical implicated in anxiety is Neuropeptide Y (NPY) (Heilig, 2004). When administered centrally, NPY has anxiolytic effects (Griebel 1999), and it is hypothesized that endogenous NPY offsets stress and anxiety (Heilig et al., 1994). NPY is expressed in the LS, as are the NPY Y$_1$ and Y$_2$ receptors (Kask et al., 2001; Dumont et al., 2000). Prior work in our laboratory found that infusions of NPY into the LS reduce rats’ defensive behaviours in the NISF and SPBT but not in the EPM (Trent and Menard, 2011). Moreover, NPY-induced anxiolysis in the NISF but not in the SPBT is attenuated if the Y$_1$ receptor is blocked (Trent and Menard, 2011). Later experiments found that selective activation of the Y$_2$ receptor decreases rats’ defensive behaviours in the EPM, and blockade of the Y$_2$ receptor abolishes this anxiolytic-like effect (Trent and Menard, 2013). Together, these findings suggest that the Y$_1$ and Y$_2$ receptor subtypes differentially mediate NPY-induced anxiolysis. Another neurochemical implicated in anxiety present in the LS is histamine, which forms the basis for the experiments detailed in Chapter 3.

1.5. Histamine and anxiety

Histamine is synthesized from L-histidine by histidine decarboxylase and is subsequently metabolized by histamine $N$-methyltransferase (Brown et al., 2001). Histaminergic neurons are
localized to the tuberomammillary nucleus of the posterior hypothalamus (TM) and innervate almost all areas of the brain and parts of the spinal cord (Panula et al., 1984; Watanabe et al., 1984). Histamine is involved in a variety of physiological processes, including arousal, sleep, homeostasis, pain, learning and memory, and anxiety (Brown et al., 2001). Rodents exposed to stressful stimuli, such as restraint, cold, air blasts, and foot shocks, show increased brain histamine turnover (Mazurkiewicz-Kwilecki, 1980; Mazurkiewicz-Kwilecki and Prell, 1986; Taylor and Snyder, 1971; Yoshitomi et al., 1986), while anxiolytic drugs, such as diazepam or buspirone, reduce it (Oishi et al., 1986; Oishi et al., 1992). Lesions of the rat TM increase open arm activity in the EPM, alluding that a reduction of histaminergic activity can produce anxiolytic-like effects (Frisch et al., 1998). On the flip side, intraperitoneal (i.p.) injections of L-histidine, a histamine precursor, dose-dependently reduce murine open arm exploration (Kumar et al., 2007). Comparable anxiogenic-like effects are found in the EPM following intracerebroventricular (i.c.v.) injections of histamine into rats (Zarrindast et al., 2005). These last three studies suggest that neural histamine overall enhances anxiety. Intra-cerebral infusion studies, however, show that the effects of histamine on anxiety-related behaviours are specific to the brain region. For example, histamine infusions into the rat dorsal hippocampus increase open arm exploration in the EPM (Zarrindast et al., 2006), while histamine infusions into the ventral hippocampus decrease rats’ open arm exploration (Rostrami et al., 2006). Similarly, intracerebral infusions of histamine into the rat central amygdala reduce open arm activity in the EPM (Zarrindast et al., 2005).

Histamine exerts its effects through four distinct G-protein coupled receptors: H₁, H₂, H₃, and H₄. The H₁ and H₂ receptors are mainly postsynaptic, while the H₃ receptor is exclusively presynaptic (Brown et al., 2001). Little is currently known regarding the H₄ receptor; it was only
recently discovered to be functionally expressed in the brain (Connelly et al., 2009). Thus, only the \( H_1 \), \( H_2 \), and \( H_3 \) receptors will be reviewed.

**1.5.1. \( H_1 \) receptor**

The \( H_1 \) receptor is widely distributed throughout the rat brain, with the bed nucleus of the stria terminalis, the ventromedial nuclei of the hypothalamus, pontine nuclei, and the polymorphic layer of the hilus of the dentate gyrus showing particularly high \( H_1 \) receptor densities (Palacios et al., 1981). In the LS, the \( H_1 \) receptor is expressed at low-moderate levels (Palacios et al., 1981).

The primary effect of \( H_1 \) receptor activation is the stimulation of phospholipase C via a pertussis toxin-insensitive G-protein from the \( G_{q/11} \) family of G-proteins (Hill et al., 1997). Phospholipase C hydrolyzes the phospholipase phosphatidylinositol 4,5-bisphosphate (PIP\(_2\)) into diacyl glycerol (DAG) and inositol 1,4,5-trisphosphate (IP\(_3\)) (Alberts et al., 2007). DAG activates protein kinase C, which then phosphorylates a variety of other proteins, including neural ion channels (Alberts et al., 2007). IP\(_3\) increases intracellular calcium (Ca\(^{2+}\)) ion levels, which then activate downstream calcium-dependent pathways (Alberts et al., 2007). \( H_1 \) receptor activation can also increase nitric oxide (NO) synthase activity via a Ca\(^{2+}\)/calmodulin-dependent pathway, which activates guanylyl cyclase (Hill et al., 1997). As well, \( H_1 \) receptor activation can enhance arachidonic acid (AA) release and the synthesis of its metabolites such as thromboxane A\(_2\) and prostacyclin (Hill et al., 1997). As NO and AA have been proposed to act as retrograde messengers, \( H_1 \) receptor activation could potentially modulate presynaptic transmitter release (Brown et al., 2001).

The effect of \( H_1 \) receptor activation in the brain is region-specific. \( H_1 \) receptor activation has been shown to depolarize striatal cholinergic interneurons (Munakata and Akaike, 1994),
cholinergic cells of the medial septum (Gorelova and Reiner, 1996), vasopressin-containing supraoptic neurons (Smith and Armstrong, 1996), and cells in the cortex (Reiner and Kamondi, 1994) and thalamus (McCormick and Williamson, 1991). However, \( \text{H}_1 \) receptor stimulation has also been reported to hyperpolarize and/or inhibit the firing of cells in the hippocampus and cortex (Haas, 1981; Brown et al., 2001). Similarly, in olfactory bulb interneurons, \( \text{H}_1 \) receptor activation causes an outward current, likely reflecting a calcium-dependent potassium efflux (Jahn et al., 1995).

The \( \text{H}_1 \) receptor is linked to anxiety. For example, pre-treatment with pyrilamine (i.p.), a \( \text{H}_1 \) receptor antagonist, attenuates the anxiogenic-like effects of peripherally administered L-histidine (Kumar et al., 2007). \( \text{H}_1 \) receptor activation overall seems to increase anxiety, as i.c.v. administration of the selective \( \text{H}_1 \) receptor agonist 2-(3-trifluoromethylphenyl)histamine increases mice’ defensive behaviours in the light/dark box test (Malmberg-Aiello et al., 2002). Correspondingly, \( \text{H}_1 \) receptor knockout mice display less anxiety-related behaviours in the EPM than their wild-type counterparts (Yanni et al., 1998). As well, i.c.v. infusions of the \( \text{H}_1 \) receptor antagonist chlorpheniramine decrease rats’ defensive behaviours in the open field and light/dark box tests (Hasenöhrl et al., 1999). Note, however, that intracerebroventricular injections of pyrilamine have been reported to increase rats’ defensive behaviours in the EPM (Zarrindast et al., 2005), suggesting that the \( \text{H}_1 \) receptor’s role in anxiety regulation may be more complex. Indeed, local intracerebral infusion studies indicate that the effects of \( \text{H}_1 \) receptor antagonism on anxiety are region-specific. That is, infusions of pyrilamine into the dorsal hippocampus or basolateral amygdala increase rats’ open arm exploration in the EPM (Zarrindast et al., 2006; Hajizadeh et al., 2008). Comparable effects are found following injections of chlorpheniramine into the nucleus basalis mangocellularis region (Privou et al., 1998). In contrast, pyrilamine
infused into the ventral hippocampus increase rats’ open-arm avoidance in the EPM, suggesting an anxiogenic-like effect (Rostami et al., 2006), while pyrilamine injected into the central amygdala has no effects on rats’ defensive behaviours in the EPM (Zarrindast et al., 2005).

1.5.2. H$_2$ receptor

Like the H$_1$ receptor, the H$_2$ receptor is widely expressed and evenly distributed throughout the rat brain; however, most nuclei express it in low amounts (Karlstedt et al., 2001). The areas with the greatest H$_2$ receptor expression include the pyramidal cell layers of the hippocampal CA1-CA3 regions, the dentate gyrus of the hippocampus, the piriform cortex, the red nucleus of the thalamus, and the suprachiasmatic nucleus of the hypothalamus (Karlstedt et al., 2001). Similar to the H$_1$ receptor, the H$_2$ receptor is expressed at low-moderate levels in the LS (Karlstedt et al., 2001).

The H$_2$ receptor is generally accepted to be coupled to adenylyl cyclase via the G$_s$ G-protein (Hill et al., 1997). The activation of adenylyl cyclase results in the production of cyclic adenosine monophosphate (cAMP) from adenosine triphosphate (ATP) (Brown et al., 2001). cAMP activates protein kinase A (PKA), which then phosphorylates various protein targets, including ion channels (Brown et al., 2001). PKA can also activate CREB (cAMP response element binding-protein) transcription factors, which modulate the transcription of downstream genes (Alberts et al., 2007). In addition to adenylyl cyclase, the H$_2$ receptor has been reported to be coupled to other signaling systems (Hill et al., 1997); for example, its activation has been reported to inhibit arachidonic acid release (Traiffort et al., 1992).

Like the H$_1$ receptor, the effects of H$_2$ receptor stimulation in the brain are site-specific. H$_2$ stimulation excites granule and pyramidal cells of the dentate gyrus and hippocampus, respectively, as well as thalamic neurons (Brown et al., 2001; McCormick and Williamson,
In the striatum, $H_2$ receptors can act synergistically with $H_1$ receptors to depolarize cholinergic aspiny interneurons through an inhibition of a leak potassium conductance (Munakata and Akaike, 1994). Notably, $H_2$ receptor stimulation has been reported to depress firing in several brain regions. More specifically, when microelectrophoretically applied to cells in the cortex, midbrain central gray, or hippocampus, histamine depresses firing, and this depression is attenuated by metiamide, a $H_2$ receptor antagonist (Haas and Wolf, 1977). The nature of this depression remains unclear; it could reflect an indirect action mediated by $H_2$-induced excitation of nearby GABAergic neurons or axon terminals (Greene and Haas, 1990; Haas and Greene, 1986). In the hippocampus, histamine, via the $H_2$ receptor, excites interneurons and increases the frequency of spontaneous inhibitory postsynaptic potentials (IPSPs) (Brown et al., 2001).

Like the $H_1$ receptor, the $H_2$ receptor is involved in anxiety. Pre-administration (subcutaneous; s.c.) of ranitidine, a $H_2$ receptor antagonist, reverses the anxiogenic-like effects of histamine (i.p.) in rats tested in the EPM (Khodarahmi et al., 2011). Moreover, i.c.v. infusions of the $H_2$ receptor agonist impromidine dose-dependently reduces mice’ defensive behaviours in the light/dark box test (Malmberg-Aiello et al., 2002). Curiously, i.c.v. infusions of ranitidine into rats have been reported to reduce their defensive behaviours in a different paradigm, the EPM (Zarrindast et al., 2005). Local intracerebral infusion studies indicate that the effects of $H_2$ receptor antagonism, like that of $H_1$ receptor, differ between brain regions. For example, infusions of ranitidine into the dorsal or ventral hippocampus increase rats’ open-arm avoidance in the EPM, suggesting anxiogenesis (Zarrindast et al., 2006; Rostami et al., 2006). Conversely, injections of ranitidine into the nucleus basalis magnocellularis region increase rats’ open arm activity in the EPM, indicating anxiolysis (Privou et al., 1998). In contrast, ranitidine injected
into the central amygdala do not affect rats’ open arm exploration in the EPM (Zarrindast et al., 2005).

1.5.3 H$_3$ receptor

Unlike the H$_1$ and H$_2$ receptors, the H$_3$ receptor is exclusively presynaptic (Brown et al., 2001), and its gene produces numerous splice variants, resulting in a large number of H$_3$ receptor isoforms with distinctive distributions and pharmacological properties (Bakker et al., 2006; Drutel et al., 2001). The H$_3$ receptor is heterogeneously expressed throughout the brain, with high densities in regions receiving histaminergic projections (Pollard et al., 1993). H$_3$ receptors are found in the cerebral cortex, hippocampus, and amygdala (Pollard et al., 1993). They are also expressed in the striatum, globus pallidus, nucleus accumbens, and thalamus (Pollard et al., 1993). In the hypothalamus, where histaminergic fibres are at their densest, H$_3$ receptors are only moderately expressed (Pollard et al., 1993). They are also found in the tuberomammillary nucleus, where they are likely expressed on the cell bodies of the histaminergic neurons there (Brown et al., 2001; Pollard et al., 1993). In the LS, the H$_3$ receptor is expressed at low-moderate levels (Pollard et al., 1993).

The H$_3$ receptor is coupled to adenylyl cyclase via the G$_i$ G-protein (Brown et al., 2001). Consequently, stimulation of the H$_3$ receptor inhibits adenylyl cyclase activity, thereby reducing the conversion of ATP into cAMP (Brown et al., 2001). H$_3$ receptor activation also blocks high-voltage activated calcium channels in presynaptic terminals, leading to decreased transmitter release (Brown et al., 2001). This reduction in transmitter release can range from 20 to 60%, depending on the extracellular calcium concentration (Arrang et al., 1985; Hill et al., 1997; Schlicker et al., 1989) The H$_3$ receptor can also activate other signaling transduction cascades (Haas et al., 2008). For example, it can stimulate phospholipase A2, which releases arachidonic
acid from phospholipids (Bongers et al., 2007; Alberts et al., 2007). As well, H₃ receptor stimulation has been shown to activate protein kinase B (Akt) and one of its substrates, glycogen synthase kinase-3 (GSK-3); the Akt-GSK-3 axis is important for normal brain function (Bongers et al., 2007). Finally, the H₃ receptor can activate mitogen-activated kinase (MAP) pathways (Haas et al., 2008; Giovannini et al., 2003).

The H₃ receptor can function as an autoreceptor or heteroreceptor. As an autoreceptor, its activation decreases the release and synthesis of histamine (Arrang et al., 1983; Arrang et al., 1985). In the histaminergic neurons of the TM, the activation of H₃ receptors inhibits multiple high-threshold calcium channels (Takeshita et al., 1998) and inhibits firing (Brown et al., 2001). As a heteroreceptor, the H₃ receptor has been reported to decrease the release of various other neurotransmitters, including glutamate (Brown and Reymann, 1996), GABA (Garcia et al., 1997), dopamine (Schlicker et al., 1993), noradrenaline (Schlicker et al., 1989), serotonin (Schlicker et al., 1988), acetylcholine (Arrang et al., 1995), and peptides (Hill et al., 1997).

Similar to the H₁ and H₂ receptors, the H₃ receptor is implicated in anxiety; however, its role is more complex. For example, H₃ receptor knockout mice show reduced open arm avoidance (i.e. anxiolysis) in the EPM (Rizk et al., 2004). These same mice, however, show significantly higher startle amplitudes than their wildtype counterparts in the acoustic startle response paradigm (Rizk et al., 2004). The effects of thioperamide, a H₃ receptor antagonist, in the light/dark box test are mixed; thioperamide (s.c.) decreases the time mice spend in the light (Malmberg-Aiello et al., 2002), while other studies report no effect on mice’s defensive behaviours (Imaizumi and Onodera, 1993; Yuzurihara et al., 2000). The H₃ receptor may be more involved in rodents’ depression, rather than anxiety, -like behaviours. More specifically, injections (i.p.) of R-α-methylhistamine, a H₃ receptor agonist, or thioperamide do not affect rats’
defensive behaviours in the EPM (Pérez-García et al., 1999). Thioperamide (i.p.), however, does decrease immobility in mice tested in the forced swim test, a model of depression (Pérez-García et al., 1999). This idea is further expanded in another paper showing that injections (i.p.) of the selective H₃ receptor agonists immepip and R-α-methylhistamine have no effect on rats’ defensive behaviours in the EPM or Vogel type conflict test (Yokoyama et al., 2009). Both of these tests are classical, benzodiazepine-sensitive models of anxiety in which diazepam produces clear anxiolytic-like effects (Yokoyama et al., 2009). However, the same H₃ agonists do decrease anxiety-related behaviours in three atypical, antidepressant-sensitive models of anxiety (Yokoyama et al., 2009). More specifically, they reduce isolation-induced aggressive behaviour in the mouse resident-intruder test, freezing in the rat conditioned fear stress test, and isolation-induced vocalizations in guinea pig pups (Yokoyama et al., 2009). These findings suggest that H₃ receptor activation may result in anxiolytic-like effects akin to those of selective serotonin reuptake inhibitors rather than those of benzodiazepine anxiolytics (Yokoyama et al., 2009).

1.5.4. Histamine, the lateral septum and anxiety – Chapter 3

Given the link between histamine, the lateral septum, and anxiety, the histaminergic system of the lateral septum is likely involved in anxiety. In fact, the TM shares bi-directional connections with the lateral septum (Brown et al., 2001; Ericson et al., 1991). More importantly, the lateral septum expresses H₁, H₂, and H₃ receptors, albeit at low-moderate levels (Palacios et al., 1981; Karlstedt et al., 2001; Pollard et al., 1993). Indeed, histamine, when infused unilaterally into the lateral septum, produces anxiogenic-like effects in the EPM – histamine dose-dependently decreases open-arm activity (Zarrindast et al., 2008). The histamine-induced anxiogenesis is attenuated by pre-treatment with the H₁ receptor antagonist pyrilamine or the H₂ receptor antagonist ranitidine, indicating that the H₁ and H₂ receptors mediate rats’ open-arm
avoidance in the EPM (Zarrindast et al., 2008). As discussed above, one limitation of the EPM is that open arm exploration can be affected by appetitive motivation – food deprivation can increase rats’ open arm activity (Wall and Messier, 2001; Geen et al., 2003a; Geen et al., 2003b; Inoue et al., 2004). In Zarrindast’s study, only one model of anxiety, the EPM, was used (Zarrindast et al., 2008). The lateral septum, and histamine itself, have been implicated in reward and motivational processes (Sheehan et al., 2004; Zlomuciza et al., 2008; Sakata et al., 1990; Morimoto et al., 2001). Thus, whether the histamine-induced decrease in open arm exploration in fact reflects an increase in anxiety or instead reflects a decrease in appetitive motivation is unclear. In addition, Zarrindast et al. (2008) used unilateral infusions, and unilateral infusions of a drug (e.g. muscimol or morphine) into the LS have been reported to produce different behavioural outcomes in the EPM than when the same drug was bilaterally infused (Trent and Menard, 2011; Merrer et al., 2006). Finally, the authors did not investigate the contribution of lateral septal H₃ receptors in rats’ defensive behaviours. Thus, Chapter 3 first aimed to determine whether histamine, when bilaterally infused into the lateral septum, affected rats’ defensive behaviours in the EPM and NISF tests. Subsequent experiments investigated whether the effects of histamine in the LS were mediated by the H₁, H₂, or H₃ receptors.

1.6. Hippocampal theta activity

Hippocampal theta activity is a large amplitude (1-2mV), near-sinusoidal oscillatory pattern that can be recorded from electrodes placed almost anywhere in the hippocampal formation or entorhinal cortex (McNaughton et al., 2007; Vertes and Kocsis, 1997). Its frequency can range from 4 to as high as 14 Hz (Vanderwolf et al., 1976).

Hippocampal theta rhythm is associated with a variety of processes. For example, theta rhythm appears with voluntary movement, such as when rats rear, jump (Vanderwolf, 1969), or
run, whether freely (Skaggs et al., 1996), on a treadmill (Fox et al., 1986), or in a running wheel (Buzsáki et al., 1993; Hyman et al., 2003). As well, theta activity is linked to sensorimotor integration (Bland and Oddie, 2001), spatial navigation (Buzsáki, 2005), and learning and memory (Hasselmo, 2005). Interestingly, theta rhythm also appears when mice or rats show immobility during fear conditioning (Whishaw, 1972; Sainsbury et al., 1987a; Seidenbecher et al., 2003) or when a predator, such as a cat or ferret, is present (Sainsbury et al., 1987b). These latter reports suggest that hippocampal theta is linked to behaviours related to defense and affect; that is, fear and anxiety.

1.6.1. The neural circuitry generating hippocampal theta rhythm

The MS, together with the diagonal band of Broca (DBB), is the main rhythm generator of theta activity (Vertes and Koscis, 1997; Buzsáki 2002; Vertes et al., 2004; Hangya et al., 2009). Stimulation of the MS-DBB drives theta rhythm (James et al., 1977; Kramis and Vanderwolf, 1980; McNaughton et al., 1977), while lesions or temporary inactivation of the MS-DBB greatly disrupts theta activity in the hippocampus as well as in the entorhinal cortex (Donovick, 1968; Gray, 1971; Mizumori et al., 1989; Sainsbury and Bland, 1981; Yoder and Pang, 2005). The MS-DBB sends cholinergic and GABAergic projections to the hippocampus (Lewis et al., 1967; Köhler et al., 1984; Frotscher and Leranth, 1985; Gulyás et al., 1991), whereby the cholinergic neurons excite both the pyramidal cells and interneurons there (Frotscher and Leranth, 1985; Leranth and Frotscher, 1989) while the GABAergic cells inhibit GABAergic interneurons (Freund and Antal, 1988; Gulyas et al., 1991).

The brain stem reticular formation (RF) also plays a role in generating theta; its stimulation in rabbits elicits theta activity (Green and Arduini, 1954). Moreover, RF stimulation has been reported to increase the firing rates of MS-DBB pacemaking cells (Petsche et al., 1962;
Petsche et al., 1965). Later experiments isolated the nucleus pontis oralis (RPO) of the rostral pontine RF as the critical RF site in generating theta activity (Vertes et al., 2004), as electrical stimulation of the RPO (but not of surrounding brain stem sites) elicits theta (Vertes, 1980; Macadar et al., 1974; McNaughton et al., 1986), as well as increases the discharge rates of MS-DBB pacemaking cells (Bland et al., 1994). Curiously, when a retrograde tracer was injected into the MS-DBB, very few labelled cells were detected in the RPO or other RF nuclei (Vertes, 1988). Instead, the retrograde tracer labelled numerous cells in the supramammillary nucleus (SUM) (Vertes, 1988). Interestingly, the RPO projects to the SUM (Vertes and Martin, 1988), and in turn, the SUM projects to the MS-DBB and hippocampus (Haglund et al., 1984; Vertes, 1992). Cells in the SUM fire synchronously with theta (Kirk and McNaughton, 1991), and electrical stimulation of the SUM increases the discharge rates of MS-DBB cells and induces theta activity (Bland et al., 1990; Oddie et al., 1994). Conversely, temporary inactivation of the SUM disrupts RPO-elicited rhythmic discharge of MS-DBB pacemaker cells and abolishes theta activity in the hippocampus (Bland et al., 1994; Oddie et al., 1994). Thus, it is hypothesized that tonically firing cells of the RPO activate cells in the SUM, which then convert and relay the steady discharge into a rhythmic firing pattern to the pacemaker cells of the MS-DBB (Vertes et al., 2004).

On the flip side, the median raphe nucleus (MR) seems to modulate the desynchronization of hippocampal theta activity (Vertes et al., 2004). For example, its stimulation desynchronizes theta (Assaf and Miller, 1978; Vertes, 1981; Yamamoto et al., 1979), while its destruction leads to continuous theta activity, even under conditions where theta activity is normally not observed (i.e., during relaxed immobility) (Maru et al., 1979; Yamamoto et al., 1979). The inhibitory effects of the MR on hippocampal theta activity seem to be primarily
mediated by the MS (Vertes et al., 2004). In awake rabbits, low-amplitude stimulation of the MR reduces theta-burst activity of MS-DBB cells and disrupts theta activity in the hippocampus (Kitchigina et al., 1999), while conversely, temporary inactivation of the MR increases MS-DBB theta burst activity and evokes continuous hippocampal theta rhythm (Kitchigina et al., 1999; Vinogradova et al., 1999). The MR projects extensively to the MS (Vertes et al., 1999), where these projections form excitatory synaptic contacts with MS GABAergic cells (Leranth and Vertes, 1999). The excitation of these local MS-DBB GABAergic cells presumably inhibits septal pacemaking GABAergic and cholinergic septohippocampal cells, thereby desynchronizing hippocampal theta rhythm (Vertes et al., 2004). In addition to the MS, the MR also sends strong projections to the SUM and hippocampus, raising the possibility that it also acts on these structures to desynchronize theta (Vertes et al., 1999; Vertes et al., 2004).

1.6.2. Theta suppression model of anxiolysis

Early studies have shown that high frequency trains of stimulation to the midbrain reticular formation elicit hippocampal theta activity (Green and Arduini, 1954; Stumpf, 1965). In anesthetized and freely moving rats, the frequency of theta increases linearly with increasing levels of stimulation (McNaughton et al., 2007; McNaughton and Sedgwick, 1978). Interestingly, all known classes of clinically effective anxiolytics reduce the frequency of reticular-elicited theta activity (McNaughton et al., 2007). More specifically, drugs that act on the GABA_A receptor, such as the barbiturates pentobarbital (Kramis et al., 1975) and amylobarbital (McNaughton and Sedgwick, 1978; McNaughton et al., 1986; Coop et al., 1992), and hexobarbitone (Stumpf, 1965), or the benzodiazepines alprazolam (McNaughton et al., 1986), chlordiazepoxide (McNaughton et al., 1986; McNaughton and Coop, 1991; Zhu and McNaughton, 1991a), and diazepam (McNaughton et al., 1986) all suppress theta frequency.
Buspirone, an atypical anxiolytic and a 5-HT$_{1A}$ receptor agonist, also reduces theta frequency (McNaughton and Coop 1991; Zhu and McNaughton, 1991a), as do imipramine and fluoxetine, two antidepressants, the former a tricyclic and the latter a selective serotonin reuptake inhibitor, that are clinically effective in treating anxiety disorders (Zhu and McNaughton, 1991b; McNaughton et al., 2007). The fact that all these drugs have different pharmacological mechanisms of action (e.g., they act on different receptors, that is, GABA$_A$ or 5-HT$_{1A}$), and, aside from anxiolysis, have different physiological effects (i.e., the serotonergic drugs are not hypnotic, anticonvulsant, addictive, or muscle relaxant) strongly imply that reduced theta frequency and anxiolysis are related (McNaughton et al., 2007). Importantly, drugs with antipsychotic or sedative, but not anxiolytic, properties (e.g., haloperidol or chlorpromazine) do not suppress theta frequency (McNaughton et al., 1986). More recently, experimental compounds not yet used clinically in treating anxiety disorders reduce defensive behaviours in animal models of anxiety, as well as reduce hippocampal theta frequency. Examples of such compounds include: somatostatin, phenytoin, and the bradycardic agent ZD7288 (Engin et al., 2008; Yeung et al., 2012b; Yeung et al., 2012a). Thus, a common neurophysiological correlate of anxiolytic compounds, whether clinical or pre-clinical, is a reduction in the frequency of reticular-elicited hippocampal theta activity; this model has been referred to as the theta suppression model of anxiolysis (McNaughton et al., 2007; Yeung et al., 2012b).

1.6.3. Histamine, the lateral septum and hippocampal theta frequency – Chapter 4

As detailed in chapter three, bilateral infusions of histamine into the LS reduce defensive behaviours in the EPM and NISF, suggesting that histamine, acting in the LS, regulates rats’ behavioural defense. According to the theta suppression model of anxiolysis, the same infusions of histamine into the lateral septum should reduce the frequency of reticular-elicited
hippocampal theta. Thus, in chapter four, the findings reported in chapter three are extended by testing whether histamine, when bilaterally infused into the LS, decreases the frequency of reticular-elicited hippocampal theta in urethane-anesthetized rats.

1.7. Objectives of the thesis

To reiterate, the overarching goal of the thesis is to add to the pre-existing knowledge base regarding the lateral septum and its regulation of fear/anxiety/defensive behaviours. More specifically, the thesis has three objectives:

1. To determine whether the LS modulates rats’ anxiety-related behaviours in the NISF paradigm (Chapter 2)

2. To study the role of the histamine and its receptors (H$_1$, H$_2$, and H$_3$) in the LS in regulating rats’ anxiety-related behaviours in the EPM and NISF (Chapter 3)

3. To determine whether the effects of intra-LS infusions of histamine reduce hippocampal theta frequency, an alleged neurophysiological correlate of anxiolytic drug action (Chapter 4)
Chapter 2. Lesions of the dorsal lateral septum do not affect hyponeophagia in the novelty induced suppression of feeding paradigm but reduce defensive behaviours in the elevated plus maze and shock probe burying tests


2.1 Abstract

Past studies have shown that the lateral septum is involved in anxiety. Here, we tested whether the dorsal lateral septum contributes to hyponeophagia by using the novelty induced suppression of feeding (NISF) paradigm. We found that while lesions of the dorsal lateral septum did not affect home or novel cage responding in the NISF test, they did decrease open arm avoidance in the elevated plus maze and burying in the shock probe burying test. Our results suggest that the dorsal lateral septum does not regulate hyponeophagia in the NISF, but further experiments are needed to determine if the same is true for the intermediate and ventral lateral septum.

2.2 Introduction

The lateral septum (LS) is known for its role in regulating fear and defensive behaviours. Exposure to stressful or anxiety-provoking situations increases neural activity in the LS, as indicated by enhanced c-fos expression (Duncan et al., 1996). Accordingly, LS lesions decrease
anxiety-like behaviours in two commonly used animal models of anxiety, the elevated-plus maze (EPM) and shock probe burying test (SPBT) (Walf and Fyre, 2007; de Boer and Koolhaas, 2003). The EPM is an elevated cross-shaped maze with two opposing closed arms and two opposing open arms (Pellow et al., 1985). Rats typically avoid the open arms and prefer to explore the closed arms; increases in open-arm activity reflect reduced anxiety levels (Pellow et al., 1985). In the SPBT, rats attempt to bury an electrified probe by using their forepaws and head to push bedding material towards it; reductions in shock-probe burying suggest decreases in anxiety (Pinel and Treit, 1978). LS lesions increase open arm activity in the EPM and suppress burying behaviour in the SPBT (Menard and Treit, 1996). Similar results are found following infusions of midazolam, a benzodiazepine, into the LS (Pesold and Treit, 1996).

One shortcoming of the EPM is that changes in open-arm activity may reflect changes in appetitive motivation rather than anxiety, *per se* (Wall and Messier, 2001). Food deprivation, for instance, reliably increases open arm activity without affecting anxiety levels in other non-exploratory models of anxiety (Genn et al., 2003a). Moreover, the LS itself is implicated in reward and motivation (Sheehan et al., 2004). One test that could differentiate between the effects of anxiety and those of appetitive motivation is the novelty induced suppression of feeding (NISF) paradigm. In this paradigm, rodents take significantly longer to begin eating a palatable snack when it is offered in a novel cage compared to the home cage (Merali et al., 2003). Anxiolytic treatment attenuates the effects of the novel environment without affecting response latencies in the home cage, suggesting that the increased response latencies in the novel cage are due to increased anxiety (Merali et al., 2003). In the home cage, where the animals experience relatively little anxiety, the latency to begin feeding should reflect motivation levels. Consequently, anxiolytic effects in this test are indicated by a reduction in the latency to initiate
snack consumption in the novel cage without changing response latencies in the home cage, while treatment-induced changes in appetitive motivation would change the latency to initiate snack consumption in both the home and novel cages.

The role of the LS in NISF is currently unresolved. One study found that septal lesions reduced the latency to initiate snack consumption in the novel cage without changing home cage responding (Ross et al., 1975) while another study found no effects (Devenport and Balagura, 1971). However, the septal nucleus contains both the LS and medial septum (MS), and the MS's involvement in anxiety is equivocal (Menard and Treit, 1996; Pesold and Treit, 1996; Sheehan et al., 2004; Degroot et al., 2001). The purpose of the current study was to determine whether excitotoxic lesions of the LS affect motivation or anxiety-related behaviour in the NISF. We also included the EPM and SPBT as positive controls, as numerous studies have shown that lesions or chemical ablations of the LS reduce anxiety in the EPM and SPBT [see Sheehan et al., 2004 for a review].

2.3 Method

2.3.1 Subjects

Experimentally naïve, male Long-Evans rats (Charles River, Quebec) weighing 275–325 g at the time of surgery were used. They were maintained on a regular 12:12 light/dark cycle (lights on at 07:00) with food and water available ad libitum. Rats were doubly housed in polycarbonate cages (45.5 cm × 24 cm × 21 cm) and acclimatized to the colony for at least a week before surgery. Following surgery, rats were individually housed. All procedures met the regulations set by the Canadian Council on Animal Care and were approved by Queen's University Animal Care Committee.
2.3.2. Surgery

Rats were anaesthetised with isoflurane (4.5% for induction; 1–2% for maintenance) in oxygen and placed into a Kopf stereotaxic instrument. Buprenorphine (0.015 mg/kg, s.c.) was given preoperatively to reduce pain. Bupivacaine (Marcaine; 2.0 mg/kg), a local anaesthetic, was injected intradermally into the incision site. Ten minutes later, the scalp was incised along the midline and burr holes were drilled into the skull to allow the insertion of a microinjector (Hamilton 1 μL syringe) into the lateral septum (from bregma: 0.7 mm AP, ±1.2 mm ML; from dura: −4.8 mm at a 7° medial angle; or from bregma: −0.1 mm AP, ±1.2 mm ML; from dura: −4.4 mm at a 7° medial angle). Because we wanted to damage most of the anterior–posterior extent of the lateral septum while limiting damage to the medial septum, we used two sets of coordinates, with one set more anterior than the other. Excitotoxic lesions were created by infusing 0.15 μL of 1% ibotenic acid bilaterally into the target site at 0.02 μL/min. After the infusion, the microinjector was left in position for 3 min to minimize upflow of ibotenic acid up the cannula track. Post-operative injections (s.c.) included 3–5 mL lactated ringer and ketoprofen (Anafen; 5 mg/kg), an anti-inflammatory analgesic. Diazepam (0.5 mg/kg) was administered intra-rectally to prevent and treat (maximum of 3 injections) seizures. After waking from anaesthesia, rats were moved into a recovery room where for three days post-surgery, they received two daily injections of buprenorphine (0.015 mg/kg, s.c.) and a daily injection of ketoprofen (5 mg/kg, s.c.). The rats were returned to the colony 5 days post-surgery. Control (sham lesion) animals underwent the exact procedure as detailed above except that they were infused with 0.9% saline rather than ibotenic acid.
2.3.3. Behavioural testing

Rats were tested in the EPM at least 9 days post-surgery, followed 6 days later by the NISF, and ending 5 days later with the SPBT. All testing was conducted between 10:00 am and 2:00 pm. Subject testing order was counterbalanced according to experimental group. The testing procedures for the EPM and SPBT were identical to those outlined in Schwerdtfeger and Menard (2008). In the NISF, individual rats were given a dish containing 10 g of graham cracker pieces, in their home cage, for 15 min/day for six consecutive days. The first five exposures (days 1–5) to the dish were considered habituation days whereas the sixth exposure (day 6) was the home cage test. On the seventh day (the novel cage test), rats were moved to a new testing room and placed into an opaque cage lined with fresh bedding, where they were given the dish containing the graham crackers. The latency to begin consuming the graham crackers was recorded on all days.

2.3.4. Histology

At the end of behavioural testing, rats were anaesthetized with isoflurane (5%) mixed with oxygen. They received a lethal overdose of chloral hydrate (300 mg/kg, i.p.) and were transcardially perfused with 120 mL of 0.9% saline followed by 120 mL of 10% formalin. The brains were extracted, placed into 10% formalin for at least 2 weeks and kept in 30% sucrose until they sank. Adjacent series (1-in-4) of 40 μm coronal sections were collected and stored at −20 °C in a cyroprotective buffer containing 25% glycerol, 30% ethylene glycol, and 45% 0.1 M phosphate-buffered saline (PBS).

Lesions were evaluated using NeuN (neuronal nuclei) immunohistochemistry. We chose this method given its greater accuracy relative to conventional staining, e.g., Nissl staining labels both neurons and glial cells (including glia that move into and occupy lesion sites) making the
lesions difficult to delineate (Relo-Jongen and Feldon, 2002). Because NeuN is expressed in most neuronal cell types but not in glial cells, lesioned areas are obvious and clearly defined when NeuN is used as a marker (Relo-Jongen and Feldon, 2002). A 1-in-4 series was used for NeuN immunohistochemistry. Free-floating sections were rinsed in 0.1 M PBS and treated with 0.3% H₂O₂ in PBS for 30 min, rinsed again and placed in a blocking solution (10% normal horse serum, 2% bovine serum albumin, and 0.3% Triton X-100 in PBS) for 1 h. Then the tissue was incubated for 48 h at 4 °C in mouse monoclonal anti-NeuN serum (1:1000, Chemicon, Temecula, CA) diluted in 1% normal horse serum, 1% bovine serum albumin, and 0.3% Triton X-100 in PBS. Next, the tissue was rinsed and incubated for 1 h at room temperature with biotinylated secondary anti-mouse antibody (1:200) diluted in 1% bovine serum albumin and 0.3% Triton X-100 in PBS. Following another rinse, the sections were incubated for 1 h in avidin–biotin–peroxidase complex (1:200, ABC-Elite, Vector Laboratories). After a final rinse, the sections were placed in a chromagen solution containing 0.05% diaminobenzidine for 10 min, at which 0.03% H₂O₂ was added. The reaction was stopped in PBS. Sections were mounted onto gelatin-coated slides, dehydrated, cleared, and coverslipped.

Lesions were verified by reconstructing the damage onto stereotaxic atlas templates (Paxinos and Watson, 1998). Off-target or unilateral lesions were omitted from the statistical analysis. Lesions volumes were quantified using the Cavalieri volume estimator and Stereoinvestigator software (MicroBrightField, Colchester, VT).

2.3.5. Statistical analysis

Data from the NISF were analyzed with repeated measures ANOVA, with DAY as the within-subjects variable and LESION GROUP as the between-subjects variable. Data from the EPM and SPBT were analyzed using one-way analysis of variance (ANOVA). Correlation
coefficients were calculated using the Pearson moment-product correlation test. In all cases, alpha was set at \( p < 0.05 \).

2.4 Results

2.4.1 Histology

Fig. 2.1 shows sham \((n = 19)\) and LS lesions \((n = 23)\). Most of the damage was limited to the dorsal LS, with little damage to the intermediate and no damage to the ventral LS or medial septum. In the posterior aspects of the LS, we also noted loss of septofimbrial neurons at the level of the fimbria fornix \((-0.30 \text{ mm posterior from bregma})\) and posterior to the fornix \((-0.80 \text{ mm})\). While some lesions were more anterior relative to others, a great deal of overlap existed along the anterior–posterior axis. Despite the overlap, we sorted the lesions into two groups, one with relatively more anterior lesions and the other with relatively more posterior lesions. We found no significant groups differences in behaviour (i.e., home or novel cage response latencies, open arm activity, or duration of burying; all \( p \)-values > 0.1), so we collapsed the data into a single group.
Figure 2.1. Histological results. Row A depicts sham lesions, row B illustrates minimal lateral septal lesions, and row C shows maximal lateral septal lesions. Gray shading represents lesioned areas. Numbers refer to distance from bregma; adapted from (Paxinos and Watson, 1998).
2.4.2 Novelty-induced suppression of feeding

The latency to initiate consumption of a palatable snack over habituation days 1–5, the home cage test (day 6), and the novel cage test (day 7) between sham and LS lesioned rats is shown in Fig. 2.2. A repeated measure ANOVA found no DAY × LESION GROUP interaction \( (F_{2.74,18.7} = 1.18; p = 0.32) \), although a main effect of DAY was detected \( (F_{2.74,18.7} = 29.34; p < 0.01) \). Rats took significantly longer to begin eating the snack on Day 7 (novel cage test) than on Day 6 (home cage test) \( (t_{1.40} = -8.75; p = 0.03) \).

![Figure 2.2](image_url)

**Figure 2.2.** Mean (±SEM) latencies to initiate consumption of a palatable snack over habituation days 1–5, the home cage test (day 6) and the novel cage test (day 7) in the novelty-induced suppression of feeding paradigm by sham \( (n = 19) \) and lesioned \( (n = 23) \) rats.
2.4.3. Elevated plus maze

In the EPM, one of the sham lesioned rats was an outlier (with open arm activity more than 2.5 SD away from the mean) and his data was excluded from the EPM analysis. Rats with LS lesions showed significantly higher open arm activity than sham-lesioned rats. More specifically, LS-lesioned rats made more entries ($F_{1,39} = 5.83; p = 0.02$) and spent more time in the open arms ($F_{1,39} = 6.09; p = 0.02$) than sham-lesioned controls (refer to Fig. 2.3). The number of total arm entries did not vary between lesion groups ($F_{1,39} = 0.53; p = 0.47$).

![Graph showing open arm activity comparison between sham and LS lesioned rats in the elevated plus maze.](image)

**Figure 2.3.** Mean (±SEM) percentage of open arm entries and mean (±SEM) percentage of open arm time exhibited by sham ($n = 18$) and lesioned ($n = 23$) rats in the elevated plus maze. *p < 0.05 relative to sham controls.
2.4.4 Shock probe burying test

In the SPBT, burying levels were significantly reduced in LS-lesioned rats relative to sham lesioned rats ($F_{1,40} = 5.97; p = 0.02$; see Fig. 2.4). No group differences were found in the duration of time spent immobile ($F_{1,40} = 0.81; p = 0.37$), the number of shocks received ($F_{1,40} = 0.22; p = 0.64$), or mean shock reactivity scores ($F_{1,40} = 3.18; p = 0.08$).

![Figure 2.4](#)

**Figure 2.4.** Mean (±SEM) duration of time spent burying in the shock probe burying test by sham ($n = 19$) and lesioned ($n = 23$) rats. *$p < 0.05$ relative to sham controls.*

2.4.5 Lesion volume

Lesion volume did not correlate with home or novel cage response latencies, open arm activity, or the duration of shock probe burying (all p-values $> 0.1$).
2.5 Discussion

We found no significant differences in NISF response latencies between LS-lesioned rats and sham controls. More specifically, response latencies on day 6 and 7 did not differ between treatment groups, indicating that LS lesions do not affect motivation or anxiety levels in this test. However, LS lesions did substantially increase open arm activity in the EPM. These effects were not secondary to changes in general motor activity, since the number of total arm entries did not differ between groups. Likewise, LS lesions decreased the duration of shock probe burying. This decrease is unlikely due to non-specific changes in general motor activity or pain sensitivity because the duration of time spent immobile, the number of shock-probe contacts, and the mean shock reactivity score did not differ between groups. The EPM and SPBT results suggest that the null findings in the NISF are not due to technical or procedural errors in creating the lesions, but rather suggest that the LS is not involved in novelty induced suppression of feeding.

Prior studies have shown that the anxiolytic-like effects of septal lesions in the EPM and SPBT are only evident when the damage is posterior to the genu of the corpus collosum and anterior to the triangular septal nucleus (Menard and Treit, 1996; Treit and Pesold, 1990). Our current study extends these findings by demonstrating that excitotoxic lesions that are relatively restricted to the dorsal LS are sufficient for reducing rats’ open arm avoidance and shock probe burying. Other studies have implicated both the intermediate LS and the ventral LS in fear regulation (Duncan et al., 1996; Dielenberg et al., 2001; Cullinan et al., 1995), and it remains possible that these regions, which were relatively spared in the current study, could contribute to rats’ hyponeophagia in the NISF test.

While we did not find any lesion effects in the NISF, other regions of the brain have been implicated in hyponeophagia. Lesions of the medial septum decrease rats’ latency to initiate
consumption of familiar food (lab chow) in a novel environment, as well as novel food (canned sweet corn) in a familiar environment (Bannerman et al., 2004). The same pattern appears after either total lesions of the hippocampus or selective lesions of the ventral (but not dorsal) hippocampus; this is consistent with past studies showing that the ventral hippocampus is relatively more involved in anxiety than is the dorsal hippocampus (Bannerman et al., 2002; Trent and Menard, 2010; Engin and Treit, 2007). Interestingly, the medial septum sends cholinergic projections to the hippocampus; perhaps this innervation contributes to the hyponeophagia in the NISF. Accordingly, acetylcholine release in the hippocampus is enhanced after exposure to novelty (Giovannini et al., 2001). Future studies could examine how manipulating acetylcholine levels in the hippocampus affect NISF behaviour. The amygdala is also involved in hyponeophagia. Amygdala lesions did not affect consumption of novel food in a familiar environment, but increased the consumption of novel food in an unfamiliar environment (Dunn and Everitt, 1988). These results suggest that the amygdala is involved with the environmental or contextual aspects of the neophagic response (Dunn and Everitt, 1988).

Notably, the dorsal LS receives modest projections from the amygdala (Risold and Swanson, 1997b), raising the possibility that the latter structure, given its role in fear and anxiety (Davis, 1992), mediates, at least in part, the effects we found in the EPM and SPBT. Although early lesion studies suggested that the amygdala is not involved in rats’ open arm avoidance and burying behaviour (Treit et al., 1993b; Treit and Menard, 1997), subsequent studies using pharmacological manipulations of the amygdala have found effects on both open-arm avoidance and defensive burying (Zangrossi and Graeff, 1994; Legradi et al., 2007). Thus, the possibility remains that the amygdala and LS work together in regulating some aspects of defensive behaviour. Confirmation of this possibility requires further study.
In summary, our results suggest that the dorsal LS contributes to rats’ defensive response in the elevated plus-maze and shock-probe burying tests, but not to their neophagic responses in the NISF test. Further work is needed to determine whether the same is true for the intermediate and ventral LS.
Chapter 3. The histaminergic $H_1$, $H_2$, and $H_3$ receptors of the lateral septum differentially mediate the anxiolytic-like effects of histamine on rats' defensive behaviours in the elevated plus maze and novelty-induced suppression of feeding paradigm

As published in a slightly modified form in “The histaminergic $H_1$, $H_2$, and $H_3$ receptors of the lateral septum differentially mediate the anxiolytic-like effects of histamine on rats' defensive behaviours in the elevated plus maze and novelty-induced suppression of feeding paradigm.”


3.1 Abstract

The neural histaminergic system is involved in a wide range of physiological processes, including anxiety. Histaminergic neurons are localized in the tuberomammillary nucleus of the posterior hypothalamus and share bidirectional connections with the lateral septum, an area well implicated in anxiety. The current study examined whether the histaminergic system of the lateral septum regulates rats' defensive behaviours in two animal models of anxiety, the elevated plus maze (EPM) and novelty-induced suppression of feeding paradigm (NISF). We found that bilateral infusions of histamine (1.0µg and 5.0µg) into the lateral septum selectively decreased rats' defensive behaviours in the EPM (both doses) and NISF (1.0µg only). Follow-up studies found that pre-infusions of the $H_1$ and $H_2$ antagonists, pyrilamine (20µg) and ranitidine (20µg) respectively, reversed the anxiolytic-like effects of intra-LS histamine (1.0µg) in the NISF but not in the EPM, while pre-infusions of the $H_3$ antagonist ciproxifan (200pg) attenuated the anxiolytic-like effects of intra-LS histamine in the EPM but not in the NISF. This double
dissociation suggests that $H_1$ and $H_2$ receptors in the lateral septum, likely via a post-synaptic mechanism, mediate the anxiolytic-like effects of histamine in the NISF but not in the EPM. In contrast, lateral septal $H_3$ receptors mediate, likely pre-synaptically, the anxiolytic-like effects of histamine in the EPM but not in the NISF. Our findings indicate that these receptors differentially contribute to rats' specific defensive behaviours in the EPM and NISF, that is, avoidance of open spaces and hyponeophagia respectively.

3.2 Introduction

Histaminergic neurons are exclusively found in the tuberomammillary nucleus of the posterior hypothalamus (TM), where they project to almost all regions of the brain (Haas and Panula, 2003; Ito, 2000; Panula et al., 1984; Watanabe et al., 1984). The neural histaminergic system is implicated in a range of physiological processes including homeostasis, sleep, learning, memory, and more pertinently, anxiety (Brown et al., 2001). For example, exposure to stressful conditions increases histamine turnover rates in the rodent brain (Taylor and Snyder, 1971; Mazurkiewicz-Kwilecki, 1980; Mazurkiewicz-Kwilecki and Prell, 1986; Yoshitomi et al., 1986), while the opposite occurs following the administration of anxiolytic drugs such as diazepam or buspirone (Oishi et al., 1986; Oishi et al., 1992). Destruction of the rat TM reduces anxiety-related defensive behaviours in the elevated plus maze, a widely used animal model of anxiety (Frisch et al., 1998). In this model, rodents generally avoid the open arms and preferentially explore the closed arms; classical benzodiazepine-type anxiolytics reliably increase rats' open-arm exploration (Pellow et al., 1985). Rats with bilateral TM lesions spend significantly more time in the open arms relative to sham controls (Frisch et al., 1998). In contrast, open arm exploration is decreased following intraperitoneal (i.p.) injections of L-histidine, a histamine precursor that is converted to histamine in the central nervous system (Kumar et al., 2007).
Histamine acts via four distinct G-protein coupled receptors: H₁, H₂, H₃, and the recently discovered H₄ (Hill et al., 1997; Nakamura et al., 2000). H₁ and H₂ receptors are primarily post-synaptic (Brown et al., 2001; Hill et al., 1997). H₃ receptors are exclusively pre-synaptic and can act as autoreceptors (Hill et al., 1997; Arrang et al., 1983; Arrang et al., 1985; Garbarg et al., 1989) or as heteroreceptors (Hill et al., 1997; Molina-Hernández et al., 2001; García et al., 1997; Arrang et al., 1995; Schlicker et al., 1988; Schlicker et al., 1995; Schlicker et al., 1989). While closely related to the H₃ receptor, little is known about the H₄ receptor; indeed, only recently was it even found to be functionally expressed in the brain (Connelly et al., 2009). Past studies implicate the H₁, H₂, and H₃ receptors in anxiety. For instance, pre-treatment with the H₁ receptor antagonist pyrilamine (i.p.) blocked the anxiogenic-like increase in rats' open-arm avoidance in the EPM following i.p. injections of L-histidine (Kumar et al., 2007). Similarly, subcutaneous (s.c.) pre-treatment with the H₂ receptor antagonist ranitidine blocked the anxiogenic-like effects of intraperitoneally administered histamine (Khodarahmi et al., 2011).

The H₃ receptor's role in anxiety is more complex. Intraperitoneal injections of either a H₃ receptor agonist (R-α-methylhistamine) or antagonist (thioperamide) did not affect rats' defensive behaviours in the EPM (Pérez-García et al., 1999). However, thioperamide (i.p.) decreased immobility in mice in the forced swim test, a model of depression, suggesting that the H₃ receptor may be involved in rodents' depression (but not anxiety)-like behaviours (Pérez-García et al., 1999). Similarly, i.p. injections of the selective H₃ agonists R-α-methylhistamine or immepip did not affect rats' anxiety-related behaviours in the EPM or Vogel type conflict test, two classical, benzodiazepine-sensitive models of anxiety in which diazepam produced clear anxiolytic-like effects (Yokoyama et al., 2009). In contrast, these same H₃ agonists reduced anxiety-related behaviours in three atypical, antidepressant-sensitive models of anxiety.
(Yokoyama et al., 2009). More specifically, they decreased isolation-induced vocalizations in guinea pig pups, isolation-induced aggressive behaviour in the mouse resident–intruder test, and freezing in the rat conditioned fear stress test (Yokoyama et al., 2009). Thus, it appears that H₃ receptor activation produces anxiolytic-like effects resembling those of selective serotonin reuptake inhibitors but not those of benzodiazepine anxiolytics (Yokoyama et al., 2009).

The TM shares bi-directional connections with the lateral septum (LS), an area well known for its role in regulating fear and defensive behaviours (Brown et al., 2001; Ericson et al., 1991; Sheehan et al., 2004). Exposure to threat or potentially threatening contexts increases neural activity in the LS, as indicated by enhanced c-fos expression (Sheehan et al., 2004; Duncan et al., 1996; Nagahara and Handa, 1997; Canteras et al., 2001; Dielenberg et al., 2001; Cullinan et al., 1995). Accordingly, lesions of the LS reduce anxiety-like behaviours; more specifically, these lesions increase open arm activity in the EPM and decrease burying behaviour in the shock probe burying test, another animal model of anxiety (Menard and Treit, 1996). Infusions of midazolam, a benzodiazepine, or muscimol, a GABAₐ receptor agonist, into the lateral septum produce similar anxiolytic-like decreases in rats' open arm avoidance and shock probe burying (Pesold and Treit, 1996; Degroot et al., 2001).

Histamine, when unilaterally infused into the LS, produced anxiogenic-like decreases in open arm exploration in the EPM, and this decrease was attenuated by pre-treatment with either the H₁ antagonist pyrilamine or the H₂ antagonist ranitidine (Zarrindast et al., 2008). One limitation of the EPM, however, is that treatment-induced changes in open arm exploration can reflect changes in appetitive motivation rather than changes in anxiety levels (Wall and Messier, 2001). For example, food deprivation increases rats' open arm exploration without affecting their defensive behaviours in non-exploration based models of anxiety (Geen et al., 2003; Inoue et al.,
Moreover, the LS is known to be involved in reward and motivation (Sheehan et al., 2004), and histamine itself is implicated in reinforcement and feeding behaviours (Zlomuzica et al., 2008; Sakata et al., 1990; Morimoto et al., 2001). Given these potential confounds, it is important to determine whether the previously observed anxiogenic-like effects following intra-LS infusions of histamine (Zarrindast et al., 2008) reflect changes in anxiety or changes in appetitive motivation. One paradigm that can distinguish between the effects of anxiety and those of appetitive motivation is the novelty-induced suppression of feeding (NISF) paradigm. Here, rodents are offered a palatable snack in a familiar (home cage) and an unfamiliar (novel cage) environment (Merali et al., 2003). Rodents take markedly longer to begin eating the snack when it is offered in the unfamiliar, novel cage; this hyponeophobic effect is attenuated with anxiolytic drugs (Merali et al., 2003). Accordingly, anxiolytic-like effects in this test are indicated by a reduction in the latency to initiate snack consumption in the novel cage without changing response latencies in the home cage. In contrast, treatment-induced changes in appetitive motivation would change the latency to initiate snack consumption in both the home and novel cages. Thus, the initial purpose of the current study was to examine the effect of histamine, when locally infused into the lateral septum, on rats' defensive behaviours in the EPM and NISF tests (Experiment 1). As a follow-up, we wanted to determine if blocking the H₁ (Experiment 2), H₂ (Experiment 3), or H₃ (Experiment 4) receptor would attenuate any histamine-induced effects on behaviour. We chose pyrilamine, ranitidine, and ciproxifan as our H₁, H₂, and H₃ antagonists respectively due to their high affinity, selectivity, and efficacy (Hill et al., 1990; van der Goot and Timmerman, 2000; Fitzsimons et al., 2004; Daly et al., 1981; Cavanagh et al., 1983; Smit et al., 1996; Ligneau et al., 1998; Kathmann et al., 1998).
3.3 Materials and methods

3.3.1. Subjects

204 experimentally naïve, male Long–Evans rats (Charles River, Quebec) weighing 275–325 g at the time of surgery were used. Upon arrival, the rats were doubly housed in polycarbonate cages (45.5 × 24 × 21 cm) and allowed to acclimatize to the colony for at least 1 week prior to surgery. Following surgery, the rats were singly housed according to standard practice in our lab for surgically prepared animals. The colony was maintained on a regular 12:12 light/dark cycle (lights on at 0700 h) at approximately 21 °C with food and water available ad libitum. All procedures met the regulations established by the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee.

3.3.2. Surgery

The rats were anesthetized with isoflurane (4.5% for induction; 1–2% for maintenance) in oxygen and given buprenorphine (0.015 mg/kg, s.c.) preoperatively to reduce pain. Bupivacaine (Marcaine; 2.0 mg/kg), a local anesthetic, was injected subcutaneously into the incision site; afterwards, the rats were placed into a Kopf stereotaxic instrument. Ten minutes later, the scalp was incised along the midline and burr holes drilled into the skull. Two 23-guage, stainless-steel guide cannulae were bilaterally lowered to 1.5 mm above the lateral septum, according to the following coordinates (Paxinos and Watson, 1998): from bregma: 0.5 mm AP, ± 1.2 mm ML; from dura: − 3.4 mm at a 7° medial angle. The guide cannulae were secured to the skull using four small jeweller's screws and dental acrylic. To prevent clogging, a stylet was inserted into each cannula guide after surgery. Immediately following surgery, the rats were subcutaneously injected with the anti-inflammatory analgesic ketoprofen (Anafen; 5 mg/kg) and 5–10 mL lactated ringer. Body temperatures were maintained by placing the rats under a heat
lamp. After waking from anesthesia, the rats were transferred to a recovery room that was separated from the home colony. For three days post-surgery, the rats received two daily injections of buprenorphine (0.015 mg/kg, s.c.) and a daily injection of ketoprofen (5 mg/kg, s.c.). The rats were returned to the home colony four days post-surgery.

The surgical procedures for all four experiments were identical. 48 rats underwent surgery in Experiment 1, 53 in Experiment 2, 52 in Experiment 3, and 51 in Experiment 4.

3.3.3. Drugs and infusions

The drugs used in the current study were as follows: histamine dihydrochloride, the H₁ receptor antagonist pyrilamine maleate, the H₂ antagonist ranitidine hydrochloride, and the H₃ antagonist ciproxifan hydrochloride (Sigma-Aldrich, ON, Canada). All drugs were dissolved in 0.9% saline and stored in aliquots at −20 °C until use. Fresh aliquots were used on each testing day.

For three consecutive days before testing, the rats were habituated to the infusion procedures by moving them to the infusion room, gently restraining them using a towel, and briefly removing and replacing their cannula stylets. On testing days, the rats were restrained, and their cannula stylets removed. Two 30-gauge stainless-steel internal injectors were then lowered to 1.5 mm below the tip of the guide cannulae. The internal injectors were connected via polyethylene tubing (PE20) to one of two constant-rate 10 μL Hamilton microsyringes mounted onto a dual micro-syringe infusion pump (KD Scientific, MA). The infusion rate was set at 1 μL/min with a volume of 0.50 μL/side, for a total infusion volume of 1.0 μL. At the end of the infusion, the injectors were left in place for an additional 60 s to allow diffusion away from the tips and to limit the possibility of reflux. The movement of a bubble inside the polyethylene tubing was monitored to confirm drug flow. In addition, prior to replacing the cannula stylets, the
tops of each cannula guide and the tips of the internal injectors were examined for fluid efflux. Data from rats that displayed infusion problems (i.e., post-infusion efflux of fluid or blocked cannula guides) were excluded from analysis.

The rats were randomly assigned to their respective treatment groups, with different rats used in each experiment. The sample sizes listed below are from animals with verified cannula placements. The doses and infusion–test-intervals for Experiments 1–3 were based on prior literature (Zarrindast et al., 2008) and pilot work, whereas the dose and infusion–test-interval in Experiment 4 were based solely on pilot work. All drug doses are given as the total dose equally distributed across the two sides of the lateral septum.

In Experiment 1, the rats were bilaterally infused with either 1.0 μL of 0.9% saline or histamine (1.0 or 5.0 μg/rat) into the LS five minutes before behavioural testing. The three treatment groups in Experiment 1 were as follows: saline, 1.0 μg histamine, and 5.0 μg histamine.

In Experiment 2, the rats were bilaterally infused with 1.0 μL of 0.9% saline or the H₁ antagonist, pyrilamine (20 μg/rat). Five minutes later, the same rats were bilaterally infused with either 1.0 μL of 0.9% saline or histamine (1.0 μg/rat). Testing commenced five minutes after the second infusion. The four treatment groups in Experiment 2 were as follows: saline/saline (SAL/SAL), saline/histamine (SAL/HIS), pyrilamine/saline (PYR/SAL), and pyrilamine/histamine (PYR/HIS).

In Experiment 3, the rats were bilaterally infused with 1.0 μL of 0.9% saline or the H₂ antagonist ranitidine (20 μg/rat). Five minutes later, the same rats were bilaterally infused with either 1.0 μL of 0.9% saline or histamine (1.0 μg/rat). Testing took place five minutes following the second infusion. The four treatment groups in Experiment 3 were as follows: saline/saline...
In Experiment 4, the rats were bilaterally infused with 1.0 μL of 0.9% saline or the H₃ antagonist ciproxifan (200 pg/rat). Five minutes later, the same rats were bilaterally infused with either 1.0 μL of 0.9% saline or histamine (1.0 μg/rat). Testing began five minutes after the second infusion. The four treatment groups in Experiment 4 were as follows: saline/saline (SAL/SAL), saline/histamine (SAL/HIS), ciproxifan/saline (CIP/SAL), and ciproxifan/histamine (CIP/HIS).

3.3.4. Behavioural testing

After at least 9 days of post-surgical recovery, the rats were tested in the EPM followed 6 days later in the NISF. All testing was conducted between 1000 h and 1400 h. Testing order, on any given test day, was counterbalanced according to treatment group.

3.3.4.1. The elevated plus maze

The elevated plus maze was a wooden cross-shaped maze consisting of two opposing open arms (50 × 10 cm) and two opposing closed arms (50 × 10 × 50 cm). All arms had open roofs and were elevated 50 cm above the floor. The testing room was lit with a red light such that the center of the maze was at 3.5 lx. In our experience, testing under such conditions typically yields baseline levels of open arm activity (~ 30%) sufficient for detecting treatment effects in either direction. The rats were individually placed into the center of the maze, facing a closed arm, for a 5 min free exploration test. An experimenter sat quietly in one corner of the room and recorded the number of entries made into any arm (open or closed); an entry was defined as having all four paws in the arm. Testing sessions in the EPM were also taped using a digital camcorder. These tapes were later coded, using Observer VideoPro (Noldus, MA), for the
following behaviours: (a) the total time spent in the open arms and (b) the total time spent in the closed arms. The maze was cleaned with a 5% alcohol solution after each animal to minimize odor cues.

The percentage of open arm entries (open/open + closed) and the percentage of time spent in the open arms (open/open + closed) were used as the indices of anxiety in this test (Pellow et al., 1985). The number of total arm entries and the number of closed arm entries were used as indices of general locomotor activity (Pellow et al., 1985, Rodgers and Johnson, 1995).

3.3.4.2. Novelty induced suppression of feeding paradigm

For six consecutive days, individual rats were given a dish containing 10 g of graham cracker pieces in their home cage for 10 min/day. The first five days were considered habituation days whereas the sixth day was the home cage test. On the seventh day (the novel cage test), the rats were moved to a new testing room and placed into an opaque cage lined with fresh bedding, in which they were given the dish containing graham cracker pieces. The latency to begin consuming the graham crackers was recorded on all days. The rats received infusions only on days 6 and 7 — the home and novel cage test days. Data from rats that failed to habituate; that is, if their response latencies were 600 s throughout habituation days 1–5, were excluded from testing on days 6 and 7.

Anxiety reduction in this test is indicated by a reduced latency to begin consumption of the palatable snack in the novel cage (day 7) in the absence of changes in the latency to begin snack consumption in the home cage (day 6). An increase in appetitive motivation, however, is indicated by a decreased latency to initiate consumption of the palatable snack in both the home and novel cages (i.e., on days 6 and 7) (Merali et al., 2003).
3.3.5. Histology

At the end of behavioural testing, the rats were anesthetized with isoflurane (5%) mixed with oxygen. They received a lethal overdose of chloral hydrate (300 mg/kg, i.p.) and were transcardially perfused with 120 mL of 0.9% saline followed by 120 mL of 10% phosphate buffered formalin. The brains were extracted and submerged in formalin for at least 48 h, after which they were sliced into 40 μm coronal sections using a freezing cryostat. The sections were dry mounted onto gelatin-coated glass sides, stained with cresyl violet, and coverslipped. An observer who was unaware of the corresponding behavioural data determined the locations of the cannula injector tips and transcribed them onto atlas sheets (Paxinos and Watson, 1998). Data from rats that did not have both cannula tips in the lateral septum were excluded from the data analysis.

3.3.6. Statistics

Behavioural data from the EPM were analyzed using one-way analysis of variance (ANOVA) followed by pair-wise comparisons (Least Significant Difference Test, LSD) where appropriate. Data from the NISF was analyzed using a mixed repeated measures ANOVA, with DAY as the within-subjects variable and TREATMENT as the between-subjects variable. If the NISF data violated the assumption of sphericity, the mixed measures ANOVA statistics were reported as the Greenhouse–Geisser corrected values. If the corrected interaction variable (DAY * TREATMENT) showed significance, then data from habituation days 1–5, the home cage test (day 6), and the novel cage test (day 7) were first analyzed with a one-way ANOVA, then followed by LSD tests where appropriate.

In all cases, the alpha value was set at $p < 0.05$. 
3.4. Results

3.4.1. Experiment 1

3.4.1.1. Histology

Figure 3.1 is a photomicrograph of a coronal brain section showing the typical infusion site for bilateral cannulae targeting the lateral septum. Forty-one rats had cannula tips situated in the lateral septum, yielding the following group numbers: saline \((n = 12)\), 1.0μg histamine \((n = 15)\), and 5.0μg histamine \((n = 14)\). Seven rats had cannula tips situated outside one or both sides of the lateral septum and were not included in the data analysis.

**Figure 3.1.** Photomicrograph of a coronal brain section showing the typical infusion site (indicated by arrows) for bilateral cannulae correctly targeting the lateral septum.
3.4.1.2. The elevated plus maze

Three rats received incomplete infusions (i.e., at least one of the bubbles did not move during the infusion), and their data was not included in the data analysis. This resulted in the following n’s: saline (n = 12), 1.0μg histamine (n = 13), and 5.0μg histamine (n = 13) for the plus-maze data.

As shown in Figure 3.2, infusions of histamine into the LS substantially increased rats’ open-arm activity relative to saline animals. This pattern was confirmed significant by a one-way ANOVA for both the percentage of open arm entries ($F_{2,35} = 12.45; p < 0.01$) and percentage of open arm time ($F_{2,35} = 7.26; p < 0.01$). Post-hoc tests revealed that rats infused with histamine (1.0μg, or 5.0μg/rat) had a greater percentage of open arm entries and spent a greater percentage of time on the open arms than control rats ($p < 0.01$ for all relevant comparisons). While the number of total arm entries did not differ between treatment groups ($F_{2,35} = 0.71; p = 0.50$), the number of closed arm entries did ($F_{2,35} = 9.75; p < 0.01$). More specifically, histamine-treated rats (at all doses) made fewer closed arm entries than saline animals (all $p$’s < 0.01).
Figure 3.2. Mean (± SEM) percentage of open arm entries (white bars) and mean (± SEM) percentage of open arm time (gray bars) exhibited by rats in the elevated plus maze following infusions of saline (n = 12), 1.0μg histamine (n = 13), or 5.0μg histamine (n = 13) into the lateral septum. * p < 0.05 relative to saline (percentage open arm entries); † p < 0.05 relative to saline (percentage open arm time).

3.4.1.3. The novelty-induced suppression of feeding paradigm

The latency to initiate consumption of a palatable snack over habituation days 1–5, the home cage test (day 6), and the novel cage test (day 7) between the three infusion groups is shown in Figure 3.3. A mixed repeated measures ANOVA found a main effect of DAY (F_{3.52,133.80} = 27.72; p < 0.01) but failed to find a significant DAY × TREATMENT interaction (F_{7.04,133.80} = 0.88; p = 0.52). However, since our main variables of interest in the NISF were the home and novel cage latency scores, we decided to separately analyze, using one-way ANOVAs,
the data from each day of Experiment 1. Response latencies did not vary between infusion
groups on habituation days 1–5 (all p’s > 0.1) nor on day 6, the home cage test \( (F_{2,38} = 2.36; \ p = 0.11) \). On day 7, the novel cage test, response latencies significantly differed between the
infusion groups \( (F_{2,38} = 3.42; \ p < 0.05) \). More specifically, rats infused with 1.0μg histamine took
substantially less time to initiate snack consumption compared to the other two infusion groups
\( (p < 0.05) \). Response latencies in the novel cage did not differ between rats infused with saline or
5.0μg histamine \( (p = 0.71) \).

![Figure 3.3](image)

**Figure 3.3.** Mean (± SEM) latencies to initiate snack consumption over habituation days 1–5, the
home cage test (day 6), and the novel cage test (day 7) in the novelty-induced suppression of
feeding paradigm displayed by rats receiving infusions of saline \( (n = 12) \), 1.0μg histamine
\( (n = 15) \), or 5.0μg histamine \( (n = 14) \) into the lateral septum. 
\* \( p < 0.05 \) relative to all other
infusion groups (saline, 5.0μg histamine).
3.4.2. Experiment 2

3.4.2.1. Histology

Forty-four rats had cannula tips located in the lateral septum, yielding the following
group numbers: SAL/SAL \((n = 12)\), SAL/HIS \((n = 11)\), PYR/SAL \((n = 10)\), and PYR/HIS
\((n = 11)\). Nine rats had cannula tips located outside one or both sides of the lateral septum; their
data were not included in the data analysis.

3.4.2.2. The elevated plus maze

Five rats received incomplete infusions; their data were removed from data analysis,
yielding the following \(n\)’s: SAL/SAL \((n = 11)\), SAL/HIS \((n = 11)\), PYR/SAL \((n = 8)\), and
PYR/HIS \((n = 9)\).

The percentages of open arm entries \((F_{3,35} = 4.45; p < 0.01)\) and open arm time
\((F_{3,35} = 4.18; p < 0.01)\) significantly differed between treatment groups (Fig. 3.4). Subsequent
pair-wise comparisons revealed that rats in the SAL/HIS, PYR/SAL, and PYR/HIS groups made
more entries and spent more time in the open arms than SAL/SAL rats \(\text{all } p\’s < 0.05\). The
number of total arm entries did not differ between infusion groups \((F_{3,35} = 1.33; p = 0.28)\).
However, the number of closed arm entries significantly differed between infusion groups
\((F_{3,35} = 3.96; p < 0.05)\); SAL/HIS and PYR/HIS rats made fewer closed arm entries than
SAL/SAL rats \(\text{all } p\’s < 0.05\).
3.4.2.3. The novelty-induced suppression of feeding paradigm

One rat failed to habituate and was not tested. Thus, the group n’s were as follows:

SAL/SAL (n = 12), SAL/HIS (n = 10), PYR/SAL (n = 10), and PYR/HIS (n = 11).

The latency to initiate consumption of a palatable snack over habituation days 1–5, the home cage test (day 6), and the novel cage test (day 7) between the four drug groups is shown in Figure 3.5. A mixed repeated measures ANOVA found a main effect of DAY ($F_{4,41,172.08} = 20.29; p < 0.01$) and a DAY × TREATMENT interaction ($F_{13,24,172.08} = 2.47; p < 0.01$). Response latencies did not differ between infusion groups on habituation days 1–5 (all...
p’s > 0.1) or in the home cage test ($F_{3,39} = 1.94; p > 0.1$). In the novel cage test, however, latency scores significantly differed between treatment groups ($F_{3,39} = 2.90; p < 0.05$). Post-hoc tests revealed that SAL/HIS rats took much less time to initiate snack consumption in the novel cage relative to SAL/SAL rats ($p < 0.05$). The effect of histamine on the latency to initiate consumption in the novel cage was blocked by co-infusions of pyrilamine, as latency scores in PYR/HIS rats were significantly higher than those of SAL/HIS rats ($p < 0.05$) and did not differ from those of SAL/SAL rats. Importantly, pyrilamine was ineffective when administered alone as latency scores from SAL/SAL and PYR/SAL rats did not differ from each other ($p > 0.1$).

**Figure 3.5.** Mean (± SEM) latencies to initiate snack consumption over habituation days 1–5, the home cage test (day 6), and the novel cage test (day 7) in the novelty-induced suppression of feeding paradigm displayed by rats receiving infusions of saline/saline (SAL/SAL; $n = 12$), saline/1.0μg histamine (SAL/HIS; $n = 10$), 20μg pyrilamine/saline (PYR/SAL; $n = 10$), or 20μg pyrilamine/1.0μg histamine (PYR/HIS; $n = 11$) into the lateral septum. *p < 0.05 relative to SAL/SAL and PYR/HIS rats.*
3.4.3. Experiment 3

3.4.3.1. Histology

Forty-six rats had cannula tips located in the lateral septum, yielding the following group numbers: SAL/SAL ($n = 13$), SAL/HIS ($n = 10$), RAN/SAL ($n = 10$), and RAN/HIS ($n = 13$). Six rats had cannula tips situated outside one or both sides of the lateral septum and were not included in the data analysis.

3.4.3.2. The elevated plus maze

The percentages of open arm entries ($F_{3,42} = 9.42; p < 0.01$) and open arm time ($F_{3,42} = 6.17; p < 0.01$) greatly differed between infusion groups (Fig. 3.6). Post-hoc comparisons revealed that rats in the SAL/HIS, RAN/SAL, and RAN/HIS groups made more entries and spent more time in the open arms than SAL/SAL rats (all $p$’s $< 0.01$). While the number of total arm entries did not differ between infusion groups ($F_{3,42} = 1.49; p = 0.23$), the number of closed arm entries was significantly different ($F_{3,42} = 3.41; p < 0.05$). SAL/HIS and RAN/HIS rats made fewer closed arm entries than SAL/SAL rats (all $p$’s $< 0.05$).
Figure 3.6. Mean (± SEM) percentage of open arm entries (white bars) and mean (± SEM) percentage of open arm time (gray bars) exhibited by rats in the elevated plus maze following infusions of saline/saline (SAL/SAL; \( n = 13 \)), saline/1.0μg histamine (SAL/HIS; \( n = 10 \)), 20μg ranitidine/saline (RAN/SAL; \( n = 10 \)), or 20μg ranitidine/1.0μg histamine (RAN/HIS; \( n = 13 \)) into the lateral septum. *\( p < 0.05 \) relative to saline (percentage open arm entries); *\( p < 0.05 \) relative to saline (percentage open arm time).
3.4.3.3. The novelty-induced suppression of feeding paradigm

Two rats failed to habituate and were not tested while three were failing to thrive and were euthanized. Three rats received incomplete infusions and did not contribute data to the analysis. Thus, the resulting group numbers were as follows: SAL/SAL (n = 12), SAL/HIS (n = 8), RAN/SAL (n = 9), and RAN/HIS (n = 9).

NISF response latencies over habituation days 1–5, the home cage test (day 6), and the novel cage test (day 7) from the four infusion groups are shown in Figure 3.7. A mixed repeated measures ANOVA detected a main effect of DAY (F(4,43,150.57) = 12.54; p < 0.01) and a DAY × TREATMENT interaction (F(13.29,150.57) = 2.02; p < 0.01). Response latencies did not differ between infusion groups on habituation days 1–5 (all p’s > 0.1) or in the home cage test (F(3,34) = 0.26; p > 0.5). However, in the novel cage test, latency scores differed between infusion groups (F(3,34) = 3.57; p < 0.05). More specifically, SAL/HIS rats took less time to initiate snack consumption in the novel cage relative to SAL/SAL rats (p < 0.05). The effect of histamine on latency scores in the novel cage was reversed by co-infusions of ranitidine, as response latencies in RAN/HIS rats were significantly higher than those of SAL/HIS rats (p < 0.05). By itself, ranitidine was ineffective since latency scores from SAL/SAL and RAN/SAL rats were similar (p > 0.1). Response latencies between SAL/SAL, RAN/SAL, and RAN/HIS rats in the novel cage test did not differ (all p’s > 0.1).
Figure 3.7. Mean (± SEM) latencies to initiate snack consumption over habituation days 1–5, the home cage test (day 6), and the novel cage test (day 7) in the novelty-induced suppression of feeding paradigm displayed by rats receiving infusions of saline/saline (SAL/SAL; \( n = 12 \)), saline/1.0μg histamine (SAL/HIS; \( n = 8 \)), 20μg ranitidine/saline (RAN/SAL; \( n = 9 \)), or 20μg ranitidine/1.0μg histamine (RAN/HIS; \( n = 9 \)) into the lateral septum. *\( p < 0.05 \) relative to all other infusion groups (SAL/SAL, RAN/SAL, RAN/HIS).
3.4.4. Experiment 4

3.4.4.1. Histology

Forty-two rats had cannula tips situated in the lateral septum, yielding the following group numbers: SAL/SAL \((n = 9)\), SAL/HIS \((n = 11)\), CIP/SAL \((n = 10)\), and CIP/HIS \((n = 12)\). Nine rats had cannulae tips located outside one or both sides of the lateral septum; their data were not included in the data analysis.

3.4.4.2. The elevated plus maze

Two rats received incomplete infusions and their data were excluded from the analysis, yielding the following \(n\)’s: SAL/SAL \((n = 9)\), SAL/HIS \((n = 10)\), CIP/SAL \((n = 10)\), and CIP/HIS \((n = 11)\).

The percentages of open arm entries \((F_{3,36} = 5.67; p < 0.01)\) and open arm time \((F_{3,36} = 6.07; p < 0.01)\) significantly differed between infusion groups (Fig. 3.8). Post-hoc comparisons found that SAL/HIS rats made more entries and spent more time in the open arms than rats from the SAL/SAL, CIP/SAL, and CIP/HIS groups (all \(p\)’s < 0.01), whereas the latter three groups did not differ from each other (all \(p\)’s > 0.1). Neither the number of total arm entries \((F_{3,36} = 0.43; p = 0.73)\) nor the number of closed arm entries \((F_{3,36} = 2.22; p = 0.10)\) differed between infusion groups.
Figure 3.8. Mean (± SEM) percentage of open arm entries (white bars) and mean (± SEM) percentage of open arm time (gray bars) exhibited by rats in the elevated plus maze following infusions of saline/saline (SAL/SAL; *n* = 9), saline/1.0μg histamine (SAL/HIS; *n* = 10), 200ng ciproxifan/saline (CIP/SAL; *n* = 10), or 200ng ciproxifan/1.0μg histamine (CIP/HIS; *n* = 11) into the lateral septum. *p* < 0.05 relative to all other infusion groups (percentage open arm entries); †*p* < 0.05 relative to all other infusion groups (percentage open arm time).
3.4.4.3. The novelty-induced suppression of feeding paradigm

Four rats failed to habituate and were not tested, while one rat lost its skull cap before testing. An additional rat had an incomplete infusion and did not contribute data to the analysis, yielding the following group numbers: SAL/SAL ($n = 8$), SAL/HIS ($n = 9$), CIP/SAL ($n = 9$), and CIP/HIS ($n = 10$).

NISF response latencies over habituation days 1–5, the home cage test (day 6), and the novel cage test (day 7) from the four infusion groups are shown in Figure 3.9. A mixed repeated measures ANOVA detected a main effect of DAY ($F_{3.87,123.88} = 11.18; p < 0.01$), but failed to detect a DAY × INFUSION GROUP interaction ($F_{11.61,123.88} = 1.30; p = 0.23$). Given the consistently positive results observed in the NISF test in Experiments 1–3, we analyzed the data from each day of Experiment 4 separately using one-way ANOVAs. Response latencies did not vary between infusion groups on habituation days 1–5 (all $p$’s > 0.1) or on day 6, the home cage test ($F_{3,36} = 0.62; p = 0.6$). However, response latencies significantly differed on day 7, the novel cage test ($F_{3,36} = 3.61; p < 0.05$). Subsequent post-hoc tests indicated that SAL/HIS and CIP/HIS rats took much less time to initiate snack consumption in the novel cage test relative to SAL/SAL rats ($p < 0.05$). Ciproxifan by itself did not affect latency scores in the novel cage, as response latencies between CIP/SAL and SAL/SAL rats did not differ.
Figure 3.9. Mean (± SEM) latencies to initiate snack consumption over habituation days 1–5, the home cage test (day 6), and the novel cage test (day 7) in the novelty-induced suppression of feeding paradigm displayed by rats receiving infusions of saline/saline (SAL/SAL; n = 8), saline/1.0μg histamine (SAL/HIS; n = 9), 200ng ciproxifan/saline (CIP/SAL; n = 9), or 200ng ciproxifan/1.0μg histamine (CIP/HIS; n = 10) into the lateral septum. *p < 0.05 relative to SAL/SAL rats.
3.4.5. Data from animals with misplaced and/or unilateral infusions across Experiments 1–4

The number of animals with a misplaced cannula guide or that received an incomplete infusion on one side of the lateral septum in any given experiment was too small for any meaningful data analysis. However, it is instructive to visually examine this data to get a sense of whether bilateral infusions into the lateral septum are necessary for histamine-induced anxiolytic-like effects in either the EPM or NISF. To this end, the behavioural data from rats that either had one of their cannula guides placed outside of the lateral septum or that received an incomplete infusion on one side of the lateral septum were pooled across experiments (see Table 3.1). Visual inspection of this data suggests that unilateral infusions of histamine are not sufficient to evoke anxiolytic-like effects in the EPM and NISF. Further, it appears that when histamine is infused into one side of the lateral septum it might lead to the opposite; i.e., anxiogenic-like effects. The latter point needs to be considered with some caution, given the unusually high levels of open-arm activity displayed by saline-treated rats with misplaced and/or incomplete infusions on one side of the lateral septum.
Table 3.1. Mean (± SEM) of behaviours displayed by rats in the EPM and NISF with one cannula inside and one cannula outside the lateral septum or that had an incomplete infusion on one side (pooled across all experiments)

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Saline</th>
<th>1.0µg Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 9)</td>
<td>(n = 14)</td>
</tr>
<tr>
<td>% Open arm entries</td>
<td>43.43 ± 6.25</td>
<td>14.32 ± 4.94</td>
</tr>
<tr>
<td>% Open arm time</td>
<td>52.99 ± 8.98</td>
<td>14.94 ± 5.55 (n = 13)(^a)</td>
</tr>
<tr>
<td>Total number of arm entries</td>
<td>17.22 ± 1.98</td>
<td>14.00 ± 1.70</td>
</tr>
<tr>
<td>Closed arm entries</td>
<td>9.89 ± 1.62</td>
<td>11.36 ± 1.33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Saline</th>
<th>1.0µg Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n =6)</td>
<td>(n = 14)</td>
</tr>
<tr>
<td>Home cage (sec)</td>
<td>125.83 ± 28.22</td>
<td>129.93 ± 27.20</td>
</tr>
<tr>
<td>Novel cage (sec)</td>
<td>432.00 ± 80.44</td>
<td>351.64 ± 53.06</td>
</tr>
</tbody>
</table>

Note: variations in sample sizes between the EPM and NISF are due to differences in the number of rats that had infusion problems between the two tests. \(^a\) video for one rat was lost, so its % open arm time data was unavailable.
3.5. Discussion

Here, we report for the first time, that bilateral infusions of histamine into the lateral septum reduced rats' anxiety-related behaviours in the EPM and NISF tests. The anxiolytic-like effect of intra-lateral septal histamine in the EPM was selectively reversed by the H$_3$ receptor antagonist ciproxifan but not by the H$_1$ and H$_2$ receptor antagonists, pyrilamine and ranitidine, respectively. In contrast, the anxiolytic-like effects of histamine in the NISF were reversed by pyrilamine and ranitidine, but not by ciproxifan.

We consistently observed, across all experiments, increased open arm exploration in the EPM in rats infused with histamine. Moreover, we found no evidence suggesting that this histamine-induced increase in open arm exploration was secondary to treatment-related increases in locomotor activity (e.g., hyperactivity), as the total number of arm entries did not differ between infusion groups across Experiments 1–4. In fact, in Experiments 1–3, intra-LS infusions of histamine reduced the number of entries into the closed arms of the maze, suggesting a shifting of the rats' natural preference for enclosed spaces over open spaces. Prior reports indicate that rats' open-arm exploration is sensitive to changes in appetitive motivation (Wall and Messier, 2001). However, we did not find any evidence suggesting that histamine affects appetitive motivation in the NISF. More specifically, in all experiments, histamine (at 1.0 μg/rat) did not affect latency scores in the home cage test, but clearly decreased rats' hyponeophagic responses in the novel cage test. That is, their novel cage latency scores were greatly reduced compared to control rats. Thus, the most parsimonious interpretation of our data is that intra-lateral septal infusions of histamine decrease anxiety-like behaviours in the EPM and NISF.

Although we did not directly address the issue of neuroanatomical specificity, it seems unlikely that our findings reflect actions of the drugs outside of the LS. The lateral ventricles
border the LS, raising the possibility that our observed drug effects reflect drug diffusion from the lateral septum into the lateral ventricles and then downstream to drug-sensitive sites. However, the effective doses of histamine from the current study (1.0 and 5.0μg/rat) were at least 4–20 times lower than an effective dose following i.c.v. administration (Zarrindast et al., 2005). More specifically, histamine i.c.v. only elicited an effect in the EPM when administered at 20μg/rat and not at 10μg or 5μg (Zarrindast et al., 2005). Furthermore, some of our misplaced cannulae were situated in the lateral ventricles, and the behavioural data (Table 1) for these animals did not resemble that of rats infused bilaterally with histamine. A similar concern is whether the effects of our intra-LS infusions reflect drug diffusion from the LS into the medial septum (MS). However, in all experiments, the cannula tips were situated in the dorsal lateral septum, roughly 2 mm dorsal to the tip of the MS. Prior work has shown that when a radiolabelled tracer (1.0μL) is injected, 90% of the radioactivity is restricted to within 1 mm of the injection site (Meyers, 1974). In the current paper, the drugs were infused at 0.5μL/side, such that the total volume infused per side was 0.5μL in Experiment 1 and 1.0μL in Experiments 2 to 4. Although the likelihood that the current findings reflect drug diffusion to the MS is low, future studies should explore the potential involvement of the medial septal histaminergic system in behavioural defense regulation.

Our findings that intra-LS infusions of histamine increase rats’ open arm exploration in the EPM contrast with previous work reporting that intra-LS infusions of histamine decreased rats' open arm exploration (Zarrindast et al., 2008). The discrepancies between our findings and those of the previous study could reflect the procedural differences between our experimental protocols, such as rat strain (Long–Evans versus Wistar) or experimenter effects (Crabbe et al., 1999). The most likely explanation, however, is that those authors used unilateral infusions
whereas we infused the drug bilaterally. In support, when we visually examined data from rats with one misplaced cannula, we found that rats with histamine infused into one side of the lateral septum showed substantively lower levels of open arm activity relative to saline controls. Moreover, these histamine-infused rats did not differ from saline controls in feeding latencies in the NISF. Notably, others have reported different behavioural outcomes in the EPM following bilateral infusions of muscimol or morphine into the lateral septum versus unilateral infusions of the same drug (Trent and Menard, 2010; Merrer et al., 2006). We are unclear as to why unilateral infusions of histamine into the LS increase anxiety-like behaviours in the EPM whereas bilateral infusions have the opposite effect. The LS sends GABA-ergic projections to various hypothalamic nuclei and midbrain regions (Sheehan et al., 2004). Decreasing the activity of these output neurons, whether by electrolytic or pharmacological lesions, reduces anxiety-like behaviours in the EPM (Menard and Treit, 1996; Pesold and Treit, 1996; Degroot et al., 2001).

Perhaps histamine, when unilaterally infused into the LS, somehow increases the net activity of these neurons, while bilaterally infused histamine decreases their net activity. Future studies are needed to delineate the mechanisms underlying the differential effects of unilateral versus bilateral infusions of histamine into the LS.

The effects of intra-cerebral infusions of histamine on anxiety-related behaviours depend on the site of administration. For example, bilateral infusions of histamine at 10μg/rat into the dorsal hippocampus increased open arm activity in the plus-maze (Zarrindast et al., 2006). In contrast, histamine (2.5μg, 5.0μg, and 7.5μg/rat) injected unilaterally into the ventral hippocampus decreased open arm activity in the EPM (Rostami et al., 2006). Similarly, open arm activity in the EPM is decreased following bilateral infusions of histamine (0.01μg, 0.1μg, and 0.5μg/rat) into the central amygdala (Zarrindast et al., 2005). Infusions of histamine (at 5nmol or
40nmol/rat) into the periaqueductal gray or inferior colliculus did not affect rats' anxiety-like behaviours in the EPM (Santos et al., 2003).

In the current study, the anxiolytic-like actions of histamine in the NISF were completely blocked by pre-administration of either pyrilamine, an H₁ antagonist, or ranitidine, an H₂ antagonist, but not by pre-treatment with ciproxifan, an H₃ antagonist. This finding suggests a post-synaptic mechanism of action for the anxiolytic-like effects of intra-lateral septal histamine on rats' hyponeophagic behaviour. It remains unknown if and how the activation of H₁ and/or H₂ receptors affects the firing of lateral septal neurons. Activation of either the H₁ or H₂ receptors has been observed to either hyperpolarize or depress firing in hippocampal and cortical neurons (see Brown et al., 2001 for a review). Destruction or pharmacological inactivation of the LS produces anxiolytic effects (Menard and Treit, 1996; Pesold and Treit, 1996; Degroot et al., 2001), similar to those reported in the current study. For example, lateral septal lesions decrease anxiety-like behaviours in the plus-maze and shock probe burying tests (Menard and Treit, 1996), as do intra-lateral septal injections of indirect and direct agonists of GABA, i.e., midazolam or muscimol, respectively (Pesold and Treit, 1996; Degroot et al., 2001; Trent and Menard, 2010). Although it seems likely that histamine exerts its anxiolytic-like effects in the NISF by directly decreasing the activity of lateral septal projection neurons via the H₁ and/or H₂ receptors, electrophysiological studies are needed to either confirm or refute this possibility.

In contrast to what we observed in the NISF, the anxiolytic-like effects of intra-LS histamine in the EPM appear to be mediated at the H₃ (but not the H₁ or H₂) receptors. Prior studies found that although peripheral (i.p.) administration of the H₃ agonists, R-α-methylhistamine and immepip reduced anxiety in atypical (antidepressant-sensitive) models of anxiety, such treatments were without effect in classical benzodiazepine-sensitive models of
anxiety, including the EPM (Pérez-García et al., 1999; Yokoyama et al., 2009). Our contradictory findings in the EPM could reflect procedural differences, including the specific agonist used, dose, route of administration and/or rat strain. Interestingly, as a presynaptic heteroreceptor, the H₃ receptor has been reported to decrease glutamate release from neurons (Molina-Hernández et al., 2001). The LS receives massive glutamatergic input from the hippocampus (Risold and Swanson, 1997b). Blocking this input reduces rats’ anxiety-like behaviours; for example, infusions of CNQX, a non-NMDA receptor antagonist, into the septum increased open arm activity in the EPM and suppressed burying in the shock probe burying test (Menard and Treit, 2000). In a further experiment, infusions of the benzodiazepine receptor agonist, midazolam into the hippocampus increased rats’ open-arm exploration in the plus-maze, and this anxiolytic-like effect was reversed by co-infusions of l-glutamate into the lateral septum (Menard and Treit, 2001). Finally, tetrodotoxin lesions of the fimbria fornix, which contains glutamatergic projection fibers from the hippocampus to the lateral septum, increased open-arm exploration in the EPM (Degroot and Treit, 2004). Thus, to the extent that activation of H₃ receptors reduces glutamate release at hippocamposeptal terminals, this could lead to anxiolytic-like effects, at least in the EPM. Notably, the LS also receives projections from a variety of monoaminergic (e.g. serotonin and dopamine) and cholinergic cell groups (Sheehan et al., 2004). Whether the histaminergic system of the LS interacts with these other neurotransmitter systems to regulate open arm avoidance is unknown; however, it seems clear that the mechanism underlying the anxiolytic-like effects of intra-lateral septal histamine in the EPM is pre-synaptic.

We observed unexpected anxiolytic-like effects in the EPM, but not in the NISF, when either pyrilamine, an H₁ antagonist, or ranitidine, an H₂ antagonist, were administered alone. Similar effects occurred in the plus-maze after infusions of chlorpheniramine, an H₁ receptor
antagonist, or ranitidine, an H₂ antagonist, into the nucleus basalis magnocellularis region (Privou et al., 1998). Infusions of pyrilamine into the dorsal hippocampus also increased open-arm exploration, whereas infusions of ranitidine at that site had the opposite effect, producing selective decreases in open-arm exploration (Zarrindast et al., 2006). In the ventral hippocampus, infusions of either pyrilamine or ranitidine produced anxiogenic-like effects in the EPM (Rostami et al., 2006), while local injections of pyrilamine or ranitidine into the central amygdala did not affect defensive behaviours in the EPM (Zarrindast et al., 2005). These findings suggest that the effects of H₁ or H₂ receptor antagonists on anxiety-like behaviours are highly site-specific.

The mechanism(s) underlying the anxiolytic-like effects of intra-LS pyrilamine and ranitidine in the EPM are unclear. Pyrilamine, when injected i.p. into rats, decreases vasopressin content in the hippocampus (Taga et al., 2001). Notably, the vasopressin V₁ₐ receptor is highly expressed in the lateral septum (Sheehan et al., 2004), and infusions of the V₁ₐ receptor antagonist d(CH₂)₅Try(ME)AVP into the septal area increased open arm activity in the plus-maze (Liebsch et al., 1996). It is possible, then, that pyrilamine decreases anxiety-like behaviours in the EPM by decreasing vasopressin levels in the lateral septum and subsequent V₁ₐ activation there. Likewise, ranitidine has been shown to decrease serotonin turnover in the cerebral cortex (Otsuka et al., 2003). The serotonergic 5-HT₁ₐ receptor is highly expressed in the LS, and infusions of the 5-HT₁ₐ receptor agonist, 8-OH-DPAT into the lateral septum have been shown to decrease open arm exploration (Cheeta et al., 2000). Thus, the anxiolytic effects of intra-LS ranitidine might reflect ranitidine-induced decreases in serotonin turnover at that site leading to decreases in 5-HT₁ₐ receptor activity. Whether intra-LS pyrilamine and ranitidine exert their anxiolytic effects in the EPM by acting on the vasopressin and serotonin systems respectively
remains unknown and needs to be verified with future experiments. Notably, in the NISF, infusions of pyrilamine or ranitidine alone did not affect rats' hyponeophagic behaviour. The lateral septum is a heterogeneous structure (Risold and Swanson, 1997a), and as such, different subregions of the LS could differentially contribute to the specific anxiety-like behaviours involved in the EPM and the NISF. For example, past work in our laboratory has shown that the dorsal LS regulates anxiety-like behaviours in the EPM, but not in the NISF (Chee and Menard, 2011). Thus, H₃ receptors in the dorsal LS might be mediating rats' defensive behaviours in the EPM, while H₁ and H₂ receptors in other areas of the LS, i.e., the intermediate and/or ventral parts, might be mediating rats' hyponeophagic responses in the NISF.

In summary, we report that intra-LS infusions of histamine decrease rats' anxiety-like behaviours in the EPM and NISF. Moreover, we show that the H₁ and H₂ receptors mediate the anxiolytic-like effects of histamine in the NISF, but not in the EPM, most likely via a post-synaptic mechanism. In contrast, the H₃ receptor, via a pre-synaptic mechanism, regulates the anxiolytic-like effects of histamine on behaviours in the EPM but not in the NISF. Future work should be directed at elucidating the mechanisms underlying the anxiolytic-like effects of intra-lateral septal histamine, more specifically, how activation of H₁, H₂, and H₃ receptors influence cellular activity in the LS and thus alter rats' anxiety-like behaviours.
Chapter 4. **Behavioural anxiolysis without reduction of hippocampal theta frequency after histamine application in the lateral septum of rats**

*Manuscript is under review “Behavioural anxiolysis without reduction of hippocampal theta frequency after histamine application in the lateral septum of rats.” Chee SSA, Menard JL, Dringenberg HC. 2013. Hippocampus*

4.1 Abstract

Hippocampal theta activity is linked to various processes, including locomotion, learning and memory, and defense and affect (i.e., fear and anxiety). Interestingly, all classes of clinically effective anxiolytics, as well as experimental compounds that decrease anxiety in pre-clinical animal models of anxiety, reduce the frequency of hippocampal theta activity elicited by stimulation of the reticular formation in freely behaving or anesthetized animals. In the present experiments, we found that bilateral histamine infusions (0.5 µg/hemisphere) into the lateral septum (LS) of rats decreased anxiety-like responses in two models of anxiety, the elevated plus maze and novelty-induced suppression of feeding test. Surprisingly, these same infusions significantly increased hippocampal theta frequency elicited by reticular stimulation in urethane-anesthetized rats. In contrast to these findings, additional experiments showed that the clinically effective anxiolytic buspirone (40 mg/kg, i.p.) reduced theta frequency, confirming previous observations. Taken together, the dissociation of behavioural anxiolysis and theta frequency reduction noted here suggest that hippocampal theta frequency is not a direct index of anxiety levels in rodents. Further, the mechanisms underlying the behavioural and physiological effects elicited by histamine in the lateral septum require further study.
4.2. Introduction

Hippocampal theta activity is a near-sinusoidal, large amplitude oscillatory pattern that can be recorded from electrodes placed virtually anywhere in or around the hippocampus or entorhinal cortex (McNaughton et al., 2007). The frequency of hippocampal theta can range from 4 to 14 Hz (Vanderwolf et al., 1975). Theta activity has been associated with a number of functions, including voluntary movement (Vanderwolf, 1969; Whishaw and Vanderwolf, 1973), sensorimotor integration (Bland and Oddie, 2001), spatial navigation (Buzsáki, 2005), and learning and memory (Winson, 1978; Berry and Seager, 2001; Hasselmo, 2005). Notably, theta rhythm also appears when rats or mice exhibit immobility during fear conditioning (Whishaw, 1972; Sainsbury et al., 1987a; Seidenbecher et al., 2003) or in the presence of a predator such as a cat or ferret (Sainsbury et al., 1987b). These latter findings suggest a link between hippocampal theta and behaviours related to defense and affect (i.e., fear and anxiety).

Hippocampal theta can be elicited with high frequency trains of stimulation to the midbrain reticular formation (Green and Arduini, 1954; Stumpf, 1965). In freely moving and anesthetized rats, theta frequency increases linearly with increasing levels of stimulation (McNaughton et al., 2007). Interestingly, all classes of clinically effective anxiolytics (barbiturates, benzodiazepines, 5-HT$_{1A}$ receptor agonists, selective serotonin reuptake inhibitors [SSRIs]) reduce the frequency of reticular-elicited theta (McNaughton et al., 2007). Importantly, antipsychotics or drugs that have sedative, but not anxiolytic, effects (e.g., haloperidol, chlorpromazine) do not suppress hippocampal theta frequency (McNaughton et al., 2007). Recently, experimental compounds that are not yet used clinically to treat various anxiety disorders but reduce defensive behaviours in animal models of anxiety (e.g., somatostatin, phenytoin, the bradycardic agent ZD7288) have also been reported to decrease hippocampal theta frequency (Engin et al., 2008; Yeung et al., 2012b; Yeung et al., 2012a). Thus, a reduction
of reticularly-evoked theta frequency may be a common neurophysiological effect of all clinical and pre-clinical pharmacological anxiolytic agents examined to date; this model has recently been referred to as the theta suppression model of anxiolysis (McNaughton et al., 2007; Yeung et al., 2012b).

Recent work in our laboratory has shown that bilateral infusions of histamine into the lateral septum (LS), an area implicated in anxiety (Sheehan et al., 2004), reduces defensive behaviours in two animal models of anxiety, the elevated plus maze (EPM) and novelty induced suppression of feeding paradigm (NISF) (Chee and Menard, 2013). Anxiolytic-like effects in the EPM are inferred when rats show an increased exploration of the open arms at the expense of closed arm exploration (Pellow et al., 1985). In the NISF, rodents are offered a palatable snack in a familiar (home cage) and unfamiliar environment (novel cage), with animals taking significantly more time to initiate snack consumption in the novel cage (Merali et al., 2003). Anxiolytic effects in the NISF are indicated by a reduction in the latency to initiate snack consumption in the novel cage without affecting feeding latencies in the home cage. We have shown that, following bilateral infusions of histamine into the LS, rats show typical, anxiolytic-like behavioural profiles in both the EPM and NISF paradigms relative to saline-infusion control animals (Chee and Menard, 2013), indicative of an important role of histamine, acting in the LS, in the regulation of behavioural defense in rodents. Interestingly, the histaminergic system has also been implicated in the regulation of the hippocampal theta rhythm (Johnson et al., 2012; Hajós et al., 2007; Masuoka and Kamei, 2007; Xu et al., 2004), suggesting a link between histamine, anxiety, and hippocampal theta activity.

Thus, the purpose of the present study was to extend our prior findings by testing whether histamine, infused into the LS, would decrease the frequency of hippocampal theta, as predicted
by the theta suppression model of anxiolysis (McNaughton et al., 2007; Yeung et al., 2012a). We initially confirmed the anxiolytic-like, behavioural effects of intra-LS histamine infusions (Experiment I; see Chee and Menard, 2013). Subsequently, the effects of intra-LS infusions of histamine on reticular-elicited hippocampal theta activity were examined in urethane-anesthetized rats (Experiment II).

4.3. Materials and Methods

4.3.1. Subjects

In total, 42 experimentally naïve male Long-Evans rats (Charles River, Québec) were used, with 20 and 22 rats allocated to Experiment I (n = 20; weight of 275-325 g at the time of surgery) and II (n = 22; weight 300-600 g at the time of electrophysiological procedure), respectively. For Experiment I, rats were initially housed as pairs in polycarbonate cages (45.5 x 24 x 21 cm). Following cannulae implantation, rats were transferred to single housing, in accordance with standard operating procedures for surgically prepared animals at Queen’s University. For Experiment II, rats were housed in groups of four in polycarbonate cages (51 x 40 x 21 cm). The colony was maintained on a 12:12 dark/light cycle and at a temperature of approximately 21°C, with food and water available ad libitum. The rats in both experiments were allowed to acclimatize to the colony for at least 1 week before surgery. All procedures were in compliance with the regulations established by the Canadian Council on Animal Care and were approved by the Queen’s University Animal Care Committee.

4.3.2. Drugs

The drugs used were histamine dihydrochloride (Sigma-Aldrich, ON, Canada) and buspirone hydrochloride (Tocris Bioscience, R&D Systems, Inc., Minneapolis, MN, USA). Both histamine and buspirone were dissolved in 0.9% saline. Histamine was stored in aliquots at -
20°C until use, and a fresh aliquot was used on each test day. Buspirone was freshly prepared on the day of the experiment.

4.3.3. Experiment I: Behavioural procedures

4.3.3.1. Surgery

Rats were anesthetized with isoflurane (4.5% for induction; 1–2% for maintenance) in oxygen and given Tramadol (20 mg/kg, s.c.) preoperatively to reduce pain. Bupivacaine (Marcaine; 2 mg/kg, s.c.) was injected into the incision site, after which the rats were placed into a stereotaxic instrument. Ten minutes later, the scalp was disinfected and incised along the midline, exposing the skull. Burr holes were drilled into the skull to allow two 23-gauge, stainless-steel guide cannulae to be lowered 1.5 mm above the right and left lateral septum (from bregma: +0.5 mm AP, ± 1.2 mm ML; from dura: −3.4 mm at a 7° medial angle; coordinates from Paxinos and Watson, 1998). The guide cannulae were secured to the skull with four small jeweller's screws and dental acrylic. To maintain cannulae patency, a stylet was inserted into each cannula after surgery. Immediately following surgery, the rats were given the analgesic meloxicam (Metacam; 2 mg/kg, s.c.) and lactated ringer solution (5 mL, s.c.) and placed under a heat lamp to maintain body temperature. After waking from anesthesia, the rats were moved to a recovery room separate from the home colony. For three days post-surgery, rats received a daily injection of Tramadol (20 mg/kg, s.c.) and meloxicam (1 mg/kg, s.c.). The recovery room temperature was set to 25°C. The rats were returned to the home colony four days after undergoing surgery and were given at least five additional recovery days prior to the onset of behavioural procedures (see below).
4.3.3.2. Infusions

Initially, all rats were habituated to the infusion procedures by bringing them to the infusion room for three consecutive days. Rats were gently restrained using a towel and the cannula stylets were briefly removed and replaced. On test days (see below), rats were restrained to remove the cannula stylets and lower two 30-gauge stainless steel internal injectors to a depth of 1.5 mm below the tip of the guide cannulae. Each internal injector was connected via polyethylene tubing (PE20) to a constant-rate 10 μL Hamilton microsyringe mounted onto a dual micro-syringe infusion pump (KD Scientific, MA). Bilateral infusions of histamine (n = 10; volume of 0.5 μL per hemisphere, concentration of 0.5 μg/μL) or 0.9% saline (n = 10; same volume) were carried out using an infusion rate of 1 μL/min. At the end of the infusion, the injectors were left in place for an additional minute to allow diffusion of fluid away from the cannula tips and to reduce the possibility of reflux. The movement of a bubble inside the polyethylene tubing was monitored to verify drug flow. Prior to replacing the cannulae stylets, the tips of the internal injectors and tops of each cannula guide were examined for fluid efflux. Data from rats that showed infusion problems (e.g., cannulae guide blockage or post-infusion efflux of fluid) were excluded from the data analysis. A five-minute period was allowed between the completion of the infusion and the behavioural testing. The histamine dose and infusion-test interval were derived from prior work in our laboratory (Chee and Menard, 2013).

4.3.3.3. Behavioural testing

Following complete recovery, rats were first tested in the EPM, followed 6 days later by testing in the NISF (all tests conducted between 1000h and 1400h).
4.3.3.3.1. The elevated plus maze

The elevated plus maze was a wooden cross-shaped maze with two opposing open arms (50 x 10 cm) and two opposing closed arms (50 x 10 x 50 cm). All arms had open roofs and were elevated 50 cm above the floor. The testing room was lit with a red light so that the center of the maze was at 3.5 lx. Five minutes after completion of the infusion procedure, rats were individually placed into the center of the maze, facing a closed arm, for a 5 minute free exploration test. An experimenter sat quietly in one corner of the testing room and recorded the number of entries made into open and closed arms, with an entry defined as all four paws placed in the arm. Testing sessions in the EPM were also recorded with a digital camcorder and later coded by an experimenter blind to the subject’s drug condition using Observer VideoPro (Noldus, MA) for the following behaviours: (a) the total time spent in the open arms and (b) the total time spent in the closed arms. The maze was cleaned with a 5% alcohol solution after each test session to minimize odour cues. The percentage of open arm entries (open/open + closed) and the percentage of time spent in the open arms (open/open + closed) were used as measures of anxiety in the EPM (Pellow et al., 1985). The number of total arm entries and closed arm entries were used as measures of general locomotor activity (Pellow et al., 1985; Rodgers and Johnson, 1995).

4.3.3.3.2. The novelty induced suppression of feeding paradigm

Testing occurred over seven consecutive days. Individual rats were given a dish containing 10 g of graham cracker pieces in their home cage for 10 min/day for six consecutive days, with the first five days serving as habituation days, while the sixth day was the home cage test. On the seventh day (the novel cage test), the rats were brought to a new testing room, placed into an opaque cage lined with fresh bedding, and given a dish containing 10 g of cracker pieces.
The latency to initiate food consumption was recorded on all seven days. The rats received infusions (5 min before testing) only on days 6 and 7, the home and novel cage test days, respectively. Difference scores were calculated by subtracting home cage latency scores from novel cage latency scores. Anxiety reduction in the NISF is indicated by a reduced latency to initiate consumption of the graham cracker in the novel cage (day 7) in the absence of changes in the latency to initiate snack consumption in the home cage (day 6). An increase in appetitive motivation is indicated by a reduction in the latency to initiate snack consumption in both the home and novel cage tests (days 6 and 7) (Merali et al., 2003).

4.3.3.4. Histology

At the end of behavioural testing, rats were deeply anesthetized with isoflurane, followed by rapid decapitation. Brains were extracted and submerged in formalin for at least 48 h before they were sliced (40 μm coronal sections) using a freezing cryostat. Sections were dry mounted onto gelatin-coated glass slides. An observer who was unaware of the corresponding behavioural data determined the location of the guide cannulae tips, microinjector tips, and/or electrode tracks, and transcribed them onto brain atlas sheets (Paxinos and Watson, 1998). Data obtained in rats with inaccurate microinjector or cannula tips were excluded from data analysis.

4.3.3.5. Statistics for behavioural data

Data from the EPM were first analyzed using one-way analysis of variance (ANOVA). An analysis of covariance (ANCOVA) was performed for the percentage of open arm entries and percentage of open arm time using the number of total arm entries as the covariate. Data from the NISF was analyzed with a two-factor mixed ANOVA, with DAY as the within-subjects variable and DRUG as the between-subjects variable. Further, the difference scores for each rat (obtained
by subtracting the home cage latency from the novel cage latency) were analyzed using a one-
way ANOVA. For all analyses, significance was set at \( p < 0.05 \).

4.3.4. Experiment II: Electrophysiology procedures

4.3.4.1. Surgical preparation

Rats were deeply anesthetized with urethane (1.5 g/kg total; administered as 0.5 g/kg x 3
every 15 minutes, i.p., with supplements given as necessary). After the third injection of
urethane, the local anesthetic Bupivacaine (Marcaine; 2 mg/kg) was injected subcutaneously into
the skin near the incision site, according to the guidelines set by the Queen’s University Animal
Care Committee. Following complete anesthesia induction, the rat was placed into a stereotaxic
instrument. Throughout the experiment, body temperature was monitored using a rectal
thermometer and kept at approximately 37°C using an electrical heating pad and fleece
insulating blankets.

The scalp was incised along the midline, exposing the skull. Burr holes were drilled into
the skull above the following areas: hippocampus (from bregma: -4.5 mm AP, + 2.0 mm ML),
reticular formation (from bregma: -7.0 mm AP, + 1.6 mm ML; same as McNaughton and Coop,
1991), and lateral septum (from bregma: -0.1 mm AP, ± 0.4 mm ML). An additional hole was
drilled over the cerebellum to allow the insertion of a ground connection (jeweller’s screws
attached to miniature connectors). A bipolar recording electrode (two 125-µm diameter Teflon-
insulated stainless steel wires with a vertical tip separation of about 1 mm) was lowered into the
hippocampus (from dura: -3.0 to -3.5 mm DV), and a stimulation electrode (Series 100
concentric bipolar electrode; Rhodes Medical Instruments, David Kopf Instruments) was
lowered into the reticular formation (from dura: -7.2 mm; McNaughton and Coop, 1991). A
microinjector (Hamilton 10 µL syringe) was inserted into the lateral septum of one hemisphere
(from dura: - 4.4 mm DV) to infuse either 0.9% saline (0.5 μL) or histamine (0.5 μg/0.5 μL). The microinjector was left in position for 1 minute after the infusion to minimize fluid reflux up the injector track. The same procedure was then repeated on the opposite hemisphere, with the infusion order (left versus right LS) counterbalanced between rats. An additional group of rats was prepared in the same manner as that described above, with the exception that they did not receive LS infusions. Instead, these rats were given a systemic injection of buspirone hydrochloride (40 mg/kg, i.p.)

4.3.4.2. Electrophysiology and data collection

The bipolar hippocampal recording electrode was connected to an amplifier and A/D converter (PowerLab 4/s system running Chart software v.5.4; AD Instruments). The raw signal was digitally filtered between 4 Hz and 10 Hz and stored for subsequent offline analysis using Chart software. Stimulation of the reticular formation consisted of 2 s trains of 0.1 ms negative-going pulses delivered at 100 Hz, supplied by a stimulation isolation unit providing a constant current output (ML 180 Stimulus Isolator; AD Instruments, Toronto, ON, Canada); these stimulation parameters are identical to those used in previous work (McNaughton and Coop, 1991).

An input-output curve for hippocampal theta activity prior to the delivery of infusions or drug treatment was established by stimulating the reticular formation at sequentially increasing intensities (0.01-0.20 mA in 0.01 mA increments; one stimulation episode for each intensity; inter-stimulus interval of about 6 s), followed by sequentially decreasing intensities (0.20-0.01 mA in 0.01 mA decrements; one stimulation per intensity, 6 s inter-stimulus interval). Following this, infusions (histamine or saline) or drug (buspirone) injections were delivered (see above). For rats receiving infusions, a second input-output curve was established 5 minutes after
the end of the infusion. Rats injected with buspirone had a second input-output curve established 40 minutes after the drug administration. At the end of the experiment, all rats were transcardially perfused with 120 mL of 0.9% saline followed by 120mL of 10% phosphate buffered formalin, followed by the same histological procedures as those described above.

4.3.4.3. Data analysis and statistics

The intensity of reticular stimulation required to elicit theta activity differs between subjects (McNaughton and Sedgwick, 1978). Thus, the initial threshold value to elicit theta activity was determined for each animal. Subsequently, theta activity during the 2 s stimulation epoch elicited by four stimulation intensities (1.25 x threshold, 1.50 x threshold, 1.75 x threshold, and 2.00 x threshold) was analyzed for peak frequency using power spectral analysis (Fast Fourier Transform (FFT), windowed using Cosine-Bell function; FFTs computed by Chart software).

The threshold intensities required to elicit theta activity for rats receiving intra-LS infusions were analyzed with a two-factor mixed analysis of variance (ANOVA), with TIME (pre- versus post-infusion) and DRUG (saline versus histamine) as the within- and between-subjects variables, respectively. For rats injected with buspirone, the threshold intensities were analyzed with a one-way repeated measures ANOVA with TIME as the within-subjects variable.

Theta frequency data from rats receiving intra-LS infusions were initially analyzed with a four-way omnibus mixed ANOVA, with TIME, INTENSITY (1.25 x threshold, 1.50 x threshold, 1.75 x threshold, and 2.00 x threshold), and ORDER (ascending versus descending stimulation) as the three within-subjects variables and DRUG as the between subjects variable. The data was then separated for increasing and decreasing stimulation intensities and separately analyzed with three-factor mixed ANOVAs, with TIME and INTENSITY as the two within-subjects variables.
and DRUG as the between-subjects variable. Subsequent pair-wise comparisons were computed with the Least Significant Difference (LSD) test. When necessary, the effect of stimulation intensity on theta frequency at each control condition (i.e., saline rats; or histamine rats, pre-infusion) was first analyzed with a two-factor repeated measures ANOVA with TIME and INTENSITY as the two variables, followed (if needed) by a one-way repeated measures ANOVA with INTENSITY as the within-subjects variable.

Theta frequency data from rats given buspirone was also separated for increasing and decreasing stimulation intensities and then analyzed with two-factor repeated measures ANOVAs, with TIME and INTENSITY as the two within-subjects variables, followed by LSD tests.

For all analyses, the alpha value was set at $p < 0.05$.

4.4. Results

4.4.1. Experiment I: Behaviour

4.4.1.1. Histology

Figure 4.1 shows the location of the cannulae tips. 20 rats had cannula tips located in the lateral septum, yielding the following group numbers: saline ($n = 10$) and histamine ($n = 10$).

![Figure 4.1](image)

**Figure 4.1.** Histological results for Experiment I. Circles denote the locations of cannula tips for bilateral infusions of saline (white circles) or histamine (black circles) into the lateral septum. Numbers refer to the distance (in mm) from bregma. Atlas plates are adapted from Paxinos and Watson (1998).
4.4.1.2. Elevated plus maze

Rats receiving bilateral intra-LS infusions of histamine showed significantly higher percentages of open arm entries ($F_{1,19} = 18.7; p < 0.001$) and percentages of open arm time ($F_{1,19} = 14.9; p = 0.001$) than saline-infused rats (Figure 4.2). While the number of closed arm entries did not differ significantly between saline and histamine rats ($F_{1,19} = 0.07; p = 0.797$), the number of total arm entries did ($F_{1,19} = 6.4; p = 0.021$). Therefore, we ran separate ANCOVAs, with the number of total arm entries as the covariate, for the percentages of open arm entries and open arm time. When the total number of arm entries is accounted for, the percentages of open arm entries and open arm time were still significant (both $p$’s < 0.05).

![Graph showing mean percentage of open arm entries and open arm time for rats in the elevated plus maze following bilateral infusions of saline ($n = 10$) or 1.0 μg histamine ($n = 10$) into the lateral septum. * denotes a significant ($p < 0.05$) difference.]

Figure 4.2. Mean (± S.E.M.) percentage of open arm entries and open arm time shown by rats in the elevated plus maze following bilateral infusions of saline ($n = 10$) or 1.0 μg histamine ($n = 10$) into the lateral septum. * denotes a significant ($p < 0.05$) difference.
4.4.1.3. Novelty induced suppression of feeding

One rat failed to thrive and was euthanized, yielding the following group sizes for this experiment: saline \((n = 9)\) and histamine \((n = 10)\).

The latency to initiate consumption of a palatable snack over the initial five habituation days, the home cage test (day 6), and the novel cage test (day 7) in the two experimental conditions is shown in Figure 4.3. When all days are included, the mixed ANOVA revealed a significant effect of DAY \((F_{2,126,36,146} = 8.3; p < 0.001)\), but failed to detect a DAY x DRUG interaction \((F_{2,126,36,146} = 0.96; p = 0.397)\). However, if the mixed ANOVA was restricted to only the home and novel cage test days (i.e., days 6 and 7 respectively), the main effect of DAY \((F_{1,17} = 43.1; p < 0.001)\) as well as the DAY x DRUG interaction \((F_{1,17} = 19.2; p < 0.001)\) are significant. This significant interaction is mirrored in the analysis of the difference scores (novel cage latency minus home cage latency for each rat), which revealed a significant difference \((t_{18} = 4.48; p < 0.001)\) between the two groups, with saline rats displaying a greater increase in the latency to feed in the novel environment than rats infused with histamine (Figure 4.4).
Figure 4.3. Mean (± S.E.M.) feeding latencies over habituation days 1-5, the home cage test (day 6), and the novel cage test (day 7) in the novelty induced suppression of feeding paradigm exhibited by rats receiving bilateral infusions of saline ($n = 9$) or 1.0 μg histamine ($n = 10$) into the lateral septum.
Figure 4.4. Mean (± S.E.M.) difference scores (novel cage latency – home cage latency) for rats bilaterally infused with saline (n = 9) or 1.0 µg histamine (n = 10) into the lateral septum. * denotes a significant (p < 0.05) difference between the two groups.
4.4.2. Experiment I: Electrophysiology

4.4.2.1. Histology

The placement sites of the microinjector used to infuse saline or histamine into the lateral septum are shown in Figure 4.5. A total of 16 rats had microinjector tips located in the lateral septum of both hemispheres, yielding the following group numbers: saline \((n = 8)\) and histamine \((n = 8)\).

![Figure 4.5. Histological results for Experiment II. Circles denote the locations of microinjector tips for bilateral injections of saline (white circles) or histamine (black circles) into the lateral septum. Numbers refer to the distance (in mm) from bregma. Atlas plates are adapted from Paxinos and Watson (1998).](image)

4.4.2.2. Intra-LS infusions of histamine or saline

Spontaneous hippocampal activity in urethane-anesthetized rats consisted of irregular, mixed frequency activity. We never observed an instance of spontaneous theta activity in the hippocampal recordings outside the periods of reticular stimulation under the present, experimental conditions.

In all rats, reticular stimulation resulted in the appearance of pronounced hippocampal theta activity (Fig. 4.6A), with a peak frequencies between 4.5 to 5.5 Hz, as revealed by power spectral analysis (Fig. 4.6B). Theta activity rapidly ceased \((< 0.5 \text{ seconds})\) once stimulation ended.
Under control conditions (pre- and post-saline infusion, pre-histamine infusion), theta frequency increased in relation to increasing stimulation intensities (1.25 to 2 x threshold), an effect that was consistent regardless of whether stimulation currents were applied incrementally or decrementally (Fig. 4.7; see below for statistical analyses). As shown in Fig. 4.6 and Fig. 4.7, saline infusions into the LS did not result in any obvious changes in theta activity and frequency elicited during reticular stimulation. Surprisingly, following histamine infusions, theta frequency increased during all stimulation intensities and in both the ascending and descending stimulation conditions (Fig. 4.6 and Fig. 4.7).

The observations summarized above were confirmed by the statistical analyses. Initial, exploratory tests revealed that there was a main effect of stimulation ORDER (ascending vs. descending intensities) on theta frequency ($F_{1, 21.65} = 32.68; p < 0.01$), which prevented us from collapsing data across these conditions. Thus, separate omnibus analyses was computed for the ascending and descending stimulation condition to examine the factors of TIME, INTENSITY, and DRUG on theta frequency.
Figure 4.6. A. Representative examples of unfiltered (black) and filtered (4-10 Hz; grey) hippocampal theta activity elicited by electrical stimulation (black bar; 2 s duration, 100 Hz, 2 x threshold intensity) of the reticular formation before and after the infusion of saline (left) or histamine (1.0 μg; right) into the lateral septum. B. Corresponding power spectra for the filtered activity shown in (A).
**Figure 4.7.** A. Mean (± S.E.M.) peak hippocampal theta frequency elicited by reticular formation stimulation (at four intensities) given in an ascending order in rats receiving lateral septum infusions of either saline ($n = 8$; top panels) or histamine (1.0 μg; $n = 8$; middle panels), or a systemic injection of buspirone (40 mg/kg; $n = 6$; bottom panels). B. Corresponding data for the same experimental groups when stimulation intensities were applied in a descending order. * denotes a significant ($p < 0.05$) difference between pre- and post-drug values at that particular stimulation intensity, while # denotes values approaching statistical difference ($p = 0.06$).
For the data collected with ascending stimulation intensities, an omnibus three-factor mixed ANOVA detected a significant TIME * INTENSITY * DRUG interaction ($F_{2.05, 28.67} = 5.44; p = 0.01$), as well as significant main effects of TIME ($F_{1, 28.67} = 49.35; p < 0.01$) and DRUG ($F_{1, 14} = 8.28; p = 0.01$). The main effect of INTENSITY was not significant ($F_{1.70, 28.67} = 1.75; p = 0.20$). However, the INTENSITY * DRUG interaction was significant ($F_{1.70, 28.67} = 3.78; p = 0.04$), as were the TIME * DRUG ($F_{1, 28.67} = 57.22; p < 0.01$) and INTENSITY * TIME ($F_{2.05, 28.67} = 4.03; p = 0.02$) interactions. Subsequent pair-wise comparisons found that, in saline-infused rats, theta frequencies across all stimulation intensities did not change pre- or post-drug application (all $p$’s > 0.7). In contrast, theta frequencies across all stimulation intensities were significantly higher following intra-LS application of histamine (all $p$’s < 0.04).

Because the main effect of INTENSITY did not reach significance in the omnibus analysis, while the INTENSITY * DRUG interaction was significant, we separately analyzed the effect of stimulation intensity on elicited theta frequency in saline- and in histamine-infused rats using a two-factor ANOVA, with TIME and INTENSITY as the two within-subjects variables. In saline controls, the main effect of INTENSITY was significant ($F_{2.70, 14.69} = 29.23; p < 0.01$). In contrast, in histamine-infused rats, the main effect of INTENSITY failed to reach significance ($F_{1.62, 13.75} = 1.07; p = 0.38$), although the interaction between TIME and INTENSITY was significant ($F_{1.96, 13.75} = 4.33; p = 0.04$). Further analysis of only the pre-infusion data in these rats detected a significant main effect of INTENSITY ($F_{1.83, 12.81} = 3.92; p = 0.05$).

For data collected with decreasing stimulation intensities, an omnibus three-factor mixed ANOVA found significant main effects of TIME ($F_{1.40, 10} = 5.96; p = 0.03$) and INTENSITY ($F_{1.70, 40.10} = 48.77; p < 0.01$), as well as a significant TIME * DRUG ($F_{1.40, 10} = 15.29; p < 0.01$) interaction. All other main effects or interactions (i.e., DRUG, TIME * INTENSITY, DRUG *
INTENSITY, and TIME * INTENSITY * DRUG) were non-significant (all $p$’s > 0.1).

Subsequent pair-wise comparisons indicated that intra-LS saline infusions did not significantly change theta frequencies at any intensity level (all $p$’s > 0.3). In contrast, intra-LS infusions of histamine significantly increased theta frequencies at the 1.25, 1.50, and 2.00 x threshold intensities (all $p$’s < 0.02), while the increase seen at the 1.75 x threshold intensity approached significance ($p = 0.059$).

We also examined whether saline or histamine LS infusions changed the threshold stimulation intensity required to elicit hippocampal theta activity. Mean ($\pm$ standard error of the mean) threshold currents were 0.069 ($\pm$ 0.008 mA) and 0.068 ($\pm$ 0.006 mA) before and after saline infusions, respectively, while histamine animals required 0.069 ($\pm$ 0.006 mA) and 0.074 ($\pm$ 0.006 mA) pre- and post-infusion, respectively. Statistical analyses indicated that the threshold current required to elicit theta activity did not differ post intra-LS infusions of either saline or histamine (all $p$’s > 0.1 for main effects of TIME, DRUG and the TIME * DRUG interaction).

**4.4.2.3. Buspirone**

Given the surprising increase in theta frequency following intra-LS application of histamine, changes in theta frequency were examined in an additional group of animals administered the clinically used anxiolytic agent buspirone (40 mg/kg, i.p.). As shown in Fig. 4.7, buspirone reliably decreased theta frequency at almost all stimulation intensities (see below).

For the data collected with sequentially increasing stimulation intensities, a two-way repeated measures ANOVA found significant main effects of TIME ($F_{1,15} = 17.29; p < 0.01$) and INTENSITY ($F_{3,15} = 11.08; p < 0.01$) although the TIME by INTENSITY interaction ($F_{3,15} = 1.60; p = 0.23$) was non-significant. Subsequent post hoc tests found that for all stimulation intensities, buspirone significantly reduced theta frequencies (all $p$’s < 0.02). Similarly, for
sequentially decreasing stimulation intensities, a two-way repeated measures ANOVA detected significant main effects of TIME ($F_{1, 15} = 18.19; p < 0.01$) and INTENSITY ($F_{3, 15} = 15.68; p < 0.01$), but failed to detect a significant TIME by INTENSITY interaction ($F_{3, 15} = 1.37; p = 0.29$). Follow-up pair-wise comparisons found that buspirone significantly decreased theta frequencies at 1.50, 1.75, and 2.00 x threshold intensities (all $p$’s < 0.01), while the decrease at 1.25 x threshold approached significance ($p = 0.057$).

The mean threshold intensities required to elicit hippocampal theta activity did not change significantly following buspirone treatment, with threshold intensities of 0.087 ± 0.01 mA and 0.095 ± 0.02 mA before and after drug administration, respectively ($F_{1, 5} = 3.05; p = 0.14$).

4.5. Discussion

The present set of experiments showed that infusions of histamine into the LS exerted anxiolytic-like effects in two established animal models of anxiety. Relative to saline-infused animals, rats that received histamine showed increased open arm activity in the EPM and exhibited comparable feeding latencies between familiar and unfamiliar (presumably “fear inducing”) environments in the NISF test. These results are consistent with previous data demonstrating, in these two models of rodent anxiety, clear anxiolytic effects of intra-LS histamine application (Chee and Menard, 2013). Subsequently, we examined whether histamine infusions into the LS modulated hippocampal theta activity elicited by electrical stimulation of the brainstem reticular formation. Surprisingly, while saline infusions had no effect, histamine consistently increased theta frequency across a range of stimulation intensities (1.25-2 x threshold). In contrast, consistent with previous results (McNaughton and Coop, 1991), systemic administration of the clinically used anxiolytic agent buspirone reduced theta frequency. These
data show, for the first time, that a pharmacological manipulation producing behavioural anxiolysis can increase the frequency of hippocampal theta activity, results that are inconsistent with the influential theta suppression model of anxiolysis (McNaughton et al., 2007; Yeung et al., 2012b).

We have previously shown that histamine, when infused into the LS, increased open arm activity in the EPM, indicating an anxiolytic-like effect (Chee and Menard, 2013), which was replicated in the present investigation. The increased open arm activity following histamine application did not result from changes in locomotor activity (e.g., hyperactivity), since the number of closed arm entries (a measure of general activity) did not differ between the histamine and saline groups. Moreover, the increased open arm activity persisted after adjusting for the total number of arm entries. In the NISF test, saline-infused rats showed a significant increase in feeding latencies in a novel cage relative to the home cage, which is thought to reflect greater anxiety levels in the unfamiliar test environment (Merali et al., 2003). This increase in feeding latency was less pronounced in histamine-infused animals, indicating an anxiolytic effect. Importantly, feeding latencies in the familiar home cage did not differ between the two infusion groups, suggesting that changes in appetitive motivation (e.g., hunger-satiety levels) were unlikely to account for the behavioural effects of histamine in the NISF paradigm. While previous studies have reported conflicting behavioural results following intra-LS infusions of histamine (e.g., Zarrindast et al., 2008; discrepancies discussed in detail in Chee and Menard, 2013), the current behavioural experiments clearly replicate and confirm our initial work showing behavioural anxiolysis following intra-LS infusions of histamine.

Given that histamine, acting in the LS, consistently produced anxiolytic effects, we were surprised that the same infusions increased the frequency of reticular-elicited hippocampal theta
activity in urethane anesthetized preparations. A large body of work has demonstrated that anxiolytic drugs consistently reduce reticular-evoked theta frequency in both conscious, awake, and anesthetized rodents (McNaughton et al., 1986; McNaughton et al., 2007), a phenomenon sometimes referred to as the theta suppression model of anxiolysis (Yeung et al., 2012b). Pharmacological agents that have been confirmed to induce this effect include the clinically used agents diazepam and buspirone (McNaughton and Gray, 2000; McNaughton and Coop, 1991), as well as experimental compounds that exert anxiolytic effects in pre-clinical, animal models of anxiety (Engin et al., 2008; Yeung et al., 2012a; Yeung et al., 2012b). It is important to acknowledge that, in previous work on theta slowing by anxiolytics, drugs were administered either systemically (e.g., McNaughton and Coop, 1991; McNaughton et al., 2007), intracerebroventricularly (e.g., Engin et al., 2008), or directly into the hippocampus (Yeung et al., 2012a; Yeung et al., 2012b); in all of these administration techniques, the drugs exert direct actions in the hippocampal formation. In contrast, we administered histamine into extra-hippocampal tissue (see below for a discussion of potential drug diffusion) and an area that may have an indirect, modulatory function in the regulation of hippocampal theta activity (Risold and Swanson, 1997). Thus, it is possible that the theta suppression model of anxiolysis applies only to conditions when drugs can directly affect hippocampal circuitry. However, the data presented here, as well as recent observations that anxiogenic compounds (given systemically) do not result in an increase in theta frequency (Yeung et al., 2013), highlight the notion that hippocampal theta frequency is not a direct index of anxiety levels in rodents. Thus, the neurophysiological correlates of, and mechanisms mediating anxiety/fear clearly extend beyond changes in frequency of hippocampal theta activity.
The effect of LS histamine to increase hippocampal theta frequency was most pronounced for the lowest (1.25 x threshold) stimulation intensity, delivered during the initial, ascending (from 1.25 to 2 x threshold) stimulation sequence. This effect may be been due to the fact that it was the first stimulation delivered after the infusion of histamine was completed. However, it is clear that the histaminergic theta modulation was also apparent during the descending stimulation sequence, including the final stimulation episode, which again employed the 1.25 x threshold intensity and demonstrated a statistically significant increase in the theta frequency.

Urethane is the most commonly used anesthetic in studies examining the effects of pharmacological manipulations on elicited theta activity (McNaughton et al., 2007). Compared to non-anesthetized animals, urethane generally reduces the frequency of reticular-elicited hippocampal theta activity (Kramis et al., 1975). Also, the theta present under urethane anesthesia is largely comprised of Type 2 theta activity, which is critically dependent on the release of acetylcholine (Kramis et al., 1975; Vanderwolf, 1988). Given these effects, it is conceivable that pharmacologically induced changes in theta frequency might vary between freely moving and urethane-anesthetized animals (McNaughton et al., 2007). However, previous work using urethane-anesthetized rats (Yeung et al., 2012a, Yeung et al., 2012b), as well as our buspirone experiment, were able to detect theta frequency decreases following anxiolytic treatments. Thus, it appears unlikely that the use of urethane accounts for the failure to observe theta frequency reductions following histamine infusions into the LS.

In view of the surprising effects of intra-LS histamine on theta frequency, we decided to include an additional experimental group treated with the clinically effective anxiolytic buspirone, a drug previously shown to reduce hippocampal theta frequency (McNaughton and
Coop, 1991). These experiments revealed a clear reduction of theta frequency following buspirone treatment, in agreement with previous work (McNaughton and Coop, 1991). These results confirm that the methodology used here (anesthesia type and dose, electrode placements, etc.) allowed for the successful detection of pharmacologically induced theta slowing. In addition, using a statistical approach, we also verified the adequacy of our stimulation parameters by demonstrating significant main effects of stimulation intensity on theta frequency, as well as linear relationships between intensity and frequency (linear contrasts, data not shown) in the various control conditions (e.g., pre- and post saline infusion, pre-histamine infusion). Thus, even though previous work has used somewhat different stimulation protocols (e.g., a greater range of intensities; see Yeung et al., 2012a, Yeung et al., 2012b), it appears that the failure to observe theta slowing after intra-LS histamine cannot be attributed to specific aspects of the electrophysiological or other, experimental procedures employed in the current investigation.

Our observations that pharmacological manipulations of the LS reduce anxiety in two common used animal models are consistent with the proposed role of the LS in the regulation of fear and defensive behaviours (Sheehan et al., 2004). For example, C-fos expression in the rodent LS increases following exposure to stressful and potentially threatening conditions (Cullinan et al., 1995; Duncan et al., 1996; Nagahara and Handa, 1997; Canteras et al., 2001; Dielenberg et al., 2001), while destruction or pharmacological inactivation of the LS reduce anxiety-related behaviours in the EPM and shock probe burying test (Menard and Treit, 1996; Pesold and Treit, 1996; Degroot et al., 2001). Given this pattern of results, we hypothesize that histamine acts to inhibit neural activity in the LS, resulting in the behavioural anxiolysis observed in the present study. The LS sends GABAergic projections to various hypothalamic nuclei, including the lateral
hypothalamus (LH) (Risold and Swanson, 1997). It is possible that, by deactivating the LS, these 
downstream hypothalamic sites become disinhibited. In support, infusions of muscimol, a 
GABA_A receptor agonist, into the LH decreases open arm activity in the EPM (Schwerdtfeger 
and Menard, 2008). Thus, histamine, when infused into the LS, could exert its anxiolytic-like 
effects by inhibiting LS neural activity, consequently leading to a disinhibition of hypothalamic 
nuclei, such as the LH. The histaminergic H_1, H_2, and H_3 receptors are expressed throughout the 
LS, albeit in low-moderate levels (Palacios et al., 1981; Karlsteadt et al., 2001; Pollard et al., 
1992). Previously, we have shown that the behavioural effects of intra-LS histamine in the NISF 
test are mediated by both H_1 and H_2 receptors, while effects in the EPM appear to be due to 
activation of H_3 receptors (Chee and Menard, 2013). To the best of our knowledge, the cellular 
effects of histamine receptor activation on LS neurons have not been investigated. There are 
examples of inhibitory effects mediated by all of these receptors on membrane potentials and 
discharge rates of various forebrain neurons (Brown et al., 2001; Molina-Hernández et al., 2001), 
but whether such effects are also present in the LS remains to be determined.

The LS is interconnected with a number of brain regions implicated in the generation or 
modulation of the hippocampal theta rhythm. For example, the LS projects to the medial septum 
(MS) and supramammillary nucleus (SUM), both of which play important roles in generating 
theta activity (Thinschmidt et al., 1995; Vertes and Kocsis, 1997; Dragoi et al., 1999; Buzsáki 
2002). While some reports noted relatively sparse projections from the LS to the MS (Leranth et 
al., 1992; Witter et al., 1992), other work has indicated these fibers to be more substantial 
(Risold and Swanson, 1997). The exact neurochemical nature of these LS projections to the MS 
is unclear, but it is likely that they are GABAergic, as the large majority of LS projection 
neurons contain this neurotransmitter (Risold and Swanson, 1997; Sheehan et al., 2004).
Stimulation of the MS or SUM is highly effective in driving hippocampal theta activity (Kramis and Vanderwolf, 1980; Bland et al., 1990; Bland et al., 1994) and the SUM, in particular, has been implicated in the control of the frequency of theta activity, as temporary inactivation of the SUM significantly reduces theta frequency (Kirk and McNaughton, 1993). Application of histamine in the LS may therefore result in the disinhibition of theta generators in the SUM or MS, resulting in the enhancement of theta frequency seen in our experiments.

An alternative region that could mediate the effects of intra-LS infusions of histamine is the LH, which, as mentioned above, receives dense GABAergic input from the LS (Risold and Swanson, 1997). Previous work has shown that, in freely moving rats, electrolytic lesions of the LH decreased theta frequency (de Ryck and Teitelbaum, 1978; Jurkowlaniec et al., 1989), while electrical stimulation resulted in a current-related increase in theta frequency (Whishaw et al., 1972). These effects are likely indirect and could be mediated by the extensive projections from the LH to the MS (Saper et al., 1979; Swanson and Cowan, 1979). Overall, the anatomical evidence summarized above is consistent with the notion that the LS is in a position to modulate theta by targeting several important theta generators, although the precise contributions of these different generators to the effects reported here are currently unknown.

An important concern with intracerebral drug application lies in the diffusion area and neuroanatomical specificity of the infused substance. For example, as the lateral ventricles border the LS, drug effects may actually reflect diffusion into the ventricles and other, remote target sites. However, the histamine dose used here (1.0 μg/rat) was at least 20 times lower than the effective dose (20 μg/rat) required to produce effects in the EPM with intracerebroventricular application (Zarrindast et al., 2005). It also appears unlikely that diffusion from the LS to the medial septum (MS; an important regulator of theta activity; Vertes and Kocsis, 1997) accounts
for the effects seen here. Prior work has demonstrated that, for intracerebral infusions of 1.0 μL of a radiolabelled tracer, 90% of the radioactivity is restricted to an area of 1 mm surrounding the injection site (Meyers, 1974). Given that the volume used here was smaller (0.5 μL/hemisphere), and the fact that all injector tips were located in the dorsal LS (approximately 2 mm dorsal to the MS), it appears unlikely that our findings reflect drug actions outside of the LS, such as the MS.

In summary, we report that bilateral infusions of histamine into the LS reduce anxiety-like behaviours of rats in two behavioural models, the EPM and NISF, likely via inhibition of that site. Surprisingly, the same infusions resulted in an increase in hippocampal theta frequency elicited by reticular formation stimulation in anesthetized rats. Collectively, these observations provide a challenge to the theta suppression model of anxiolysis, which proposes that a reduction in theta frequency serves as a physiological hallmark of anxiety-reducing, pharmacological treatments (McNaughton et al., 2007; Yeung et al., 2012b). The effect of intra-LS histamine to increase theta frequency could be mediated by an inhibition of GABAergic projection neurons in the LS, resulting in a subsequent disinhibition of important theta generators in the fore- and midbrain (e.g., MS, SUM, LH). However, additional experiments are required to confirm this hypothesis.
Chapter 5. **General Discussion**

5.1. **Summary of main findings, limitations, and future directions**

As reviewed in Chapter 1, the LS is implicated in fear and anxiety (Sheehan et al., 2004). The overarching goal of this thesis was to further investigate the LS’s role in the regulation of rats’ fear, anxiety, and defensive behaviours. To that end, the thesis had three objectives, each one addressed by an empirical chapter (i.e., Chapters 2-4).

5.1.1. **Objective 1/Chapter 2**

The first objective was to determine whether the LS is involved in rats’ hyponeophagia. As detailed in Chapter 2, lesions of the dorsal LS did not affect rats’ appetitive motivation or anxiety-related behaviours in the NISF. However, these same lesions reduced their anxiety-related behaviours in the EPM and SPBT, suggesting that the null findings in the NISF were not due to experimental (e.g., technical or procedural) factors, but rather, that the dorsal LS is simply not involved in novelty induced suppression of feeding. Clearly, the LS does play a role in rats’ hyponeophagia: as reported in Chapter 3, intra-LS infusions of histamine reduce rats’ hyponeophagic responses, as do intra-LS infusions of NPY (Trent and Menard, 2011). Note that in the current lesion study, the intermediate and ventral LS received little or no damage respectively, and so therefore these sites could mediate rats’ hyponeophagic responses. To confirm this possibility, future studies should target the intermediate and/or ventral LS to determine whether they contribute to rats’ novelty induced suppression of feeding.

Past studies have reported that the anxiolytic-like effects of septal lesions in the EPM and SPBT only resulted if the damage was posterior to the genu of the corpus callosum and anterior to the triangular septal nucleus (Menard and Treit, 1996; Treit and Pesold, 1990). The data presented in Chapter 2 is the first to demonstrate that these same anxiolytic-like effects could be
produced with excitotoxic lesions that are relatively localized to the dorsal LS. An extension of this finding would be to determine whether lesions of the intermediate or ventral LS are also sufficient in producing anxiolytic-like effects in the EPM and SPBT. Another avenue of research could be to examine the effects of lesions of the LS along its anterior-posterior axis on rats’ defensive behaviours. Posterior (but not anterior) lesions of the entire septum (medial and lateral, combined) have been reported to reduce defensive behaviours in the EPM and SPBT (Treit and Pesold, 1990), but whether the same is true for the LS remains unknown. The LS is not a homogeneous structure; it can be subdivided into different subregions based on anatomical connections and neurochemistry (see Risold and Swanson, 1997a). Whether these subregions also serve different functions has yet to be explored, but one technique could be to lesion these specific subregions and observe the resulting effects, if any, on defensive behaviour.

5.1.2. Objective 2/Chapter 3

The second objective of the thesis was to determine whether histamine and the $H_1$, $H_2$, and $H_3$ receptors in the LS contribute to rats’ defensive behaviours in the EPM and NISF. Indeed, we found, for the first time, that bilateral infusions of histamine into the LS selectively reduced rats’ defensive behaviours in these two models of anxiety. Most of the past research on histamine and anxiety exclusively used the EPM as the sole model of anxiety (see Zarrindast et al., 2006; Frisch et al., 1998; Zarrindast et al., 2008; Rostami et al., 2006; Zarrindast et al., 2005; Santos et al., 2003; Kumar et al., 2007). Here, we demonstrate for the first time that intra-LS application of histamine reduced hyponeophagia, and importantly, that intra-LS infusions of histamine did not affect rats’ appetitive motivation. Follow-up experiments found that blocking the $H_1$ or $H_2$ receptor reversed the anxiolytic-like effects of intra-LS histamine in the NISF but not in the EPM. Conversely, blocking the $H_3$ receptor reversed the anxiolytic-like effects of
intra-LS histamine in the EPM but not in the NISF. Notably, i.p. injections of H3 receptor agonists or antagonists have been reported to affect defensive behaviours in other models of anxiety or depression but not in the EPM (Pérez-García, 1999; Yokoyama et al., 2009). As such, our study is the first to date showing that the H3 receptor actually contributes to rats’ anxiety-related behaviours in the EPM. The double dissociation between the H1 and H2, and the H3 receptors, which we are the first to report, suggests that the H1 and H2 receptors in the LS, probably via a post-synaptic mechanism, mediate the anxiolytic-like effects of histamine in the NISF but not the EPM, while in contrast, the H3 receptors of the LS, likely via pre-synaptic binding sites, regulate the anxiolytic-like effects of histamine in the EPM but not in the NISF.

These findings suggest that these receptors differentially regulate rats’ specific defensive behaviours; that is, the H1 and H2 receptors mediate rats’ hyponeophagia, while the H3 receptor mediates their avoidance of open spaces. While speculative, this segregation of function, like that reported in Chapter 2 regarding the dorsal LS’s contribution to rats’ open arm exploration and defensive burying but not their hyponeophagia, may bestow an adaptive advantage in that it allows for the selection of the optimal response to a specific type of threat.

We had previously reported (see Chapter 2) that the dorsal LS is not involved in the NISF, but as reported here, intra-LS infusions of histamine clearly reduce rats’ hyponeophagic responses in the NISF. An explanation for the latter effect could be, as the diffusion area of the infused liquid was unlikely to be restricted to only the dorsal LS, that the infused histamine was activating H1 and H2 receptors in the intermediate or ventral LS. To confirm this possibility, receptor densities for the H1, H2, and H3 receptors for the dorsal, intermediate, and ventral LS should be mapped. Little is currently known regarding the H4 receptor and its function in the central nervous system. It is expressed in the rat central nervous system (Strakhova et al., 2009),
although whether it is specifically expressed in the rat LS is unclear. The H₄ receptor has been implicated in pain and itch (Coruzzi et al., 2007; Dunford et al., 2007), but its role in anxiety remains to be determined. As such, future studies could investigate the H₄ receptor’s contribution to rats’ anxiety-related responses.

5.1.3. Objective 3/Chapter 4

The final objective of the thesis was to determine whether local application of histamine into the LS would reduce hippocampal theta frequency, a hypothesized neurophysiological correlate of anxiolytic drug action. First, we successfully replicated the behavioural (i.e., anxiolytic-like) effects of intra-LS bilateral infusions of histamine in the EPM and NISF tests. Subsequently, we found that, in urethane-anesthetized rats, the same intra-LS histamine infusions increased, rather than decreased, the frequency of reticular-elicited hippocampal theta. This increased theta was not related to the electrophysiology or other experimental procedures, as rats systemically injected with buspirone, a clinically effective anxiolytic previously reported to slow hippocampal theta frequency (McNaughton and Coop, 1991), showed reduced hippocampal theta frequency. To the best of our knowledge, the data in Chapter 4 is the first to show that a pharmacological compound with anxiolytic-like properties increases hippocampal theta frequency. All prior studies have reported a slowing of hippocampal theta frequency following the administration of clinically effective anxiolytics (see McNaughton et al., 2007 for a review) or experimental, pre-clinical compounds that reduce anxiety in animals (Engin et al., 2008; Yeung et al., 2012b; Yeung et al., 2012a). Altogether, our data indicates that hippocampal theta frequency is not a direct measure of anxiety levels in rodents.

One aspect of the study not extensively discussed in the empirical paper (i.e., Chapter 4) was that the behavioural data, from both Chapter 3 and Chapter 4, was collected from freely...
moving animals while in contrast, the electrophysiology data was collected from urethane-anesthetized animals. Urethane is the most commonly used anesthetic in studies examining the effects of pharmacological agents or manipulations on elicited theta (McNaughton et al., 2007). Compared to non-anesthetized animals, urethane seems to generally reduce the frequency of reticular-elicited hippocampal theta (Kramis et al., 1975). Therefore, a drug’s (e.g. histamine) effect on theta frequency could potentially differ between awake and anesthetized animals (McNaughton et al., 2007). To truly elucidate the link between hippocampal theta rhythm and behavioural anxiolysis, future studies should examine the effects of anxiolytic (or anxiogenic) compounds on hippocampal theta activity and behavioural anxiety in freely moving animals. A challenge with such an experiment would be that hippocampal theta correlates with motor movement – the more vigorous the movement, the higher the frequency of theta (Vanderwolf, 1969; Vanderwolf, 1975). Thus, such experiments would require very strict behavioural monitoring in order to determine whether treatment-induced changes in theta frequency truly reflect changes in anxiety rather than simply changes in locomotion.

5.2. Integration: LS disinhibition hypothesis

As reported in Chapter 2 and reviewed in section 1.4, lesions or pharmacological inactivation of the LS generally reduce rats’ anxiety-like behaviours (for an exception, see Yadin et al., 1993). Given that the projections from the LS to its targets are predominantly GABAergic (Risold and Swanson, 1997b), a decrease in LS activity would likely disinhibit its target structures. This disinhibition could be what produces the behavioural anxiolysis seen following inactivation of the LS. In support, infusions of the GABA\(_A\) agonist muscimol into the LH, a structure that the LS densely projects to, decrease open arm exploration in the EPM (Schwerdtfeger and Menard, 2008). However, this evidence is circumstantial in that the data is
behavioural and does not directly show that LS inactivation increases LH neural activity. Therefore, to test the LS disinhibition hypothesis, future studies could examine the effects of LS inactivation on the neural activity of its downstream target sites. For example, muscimol could be applied to the LS while multicellular recordings are taken from the LH, or any other site that the LS projects to. It would also be interesting to observe the effects of LH excitation (via electrical stimulation or intra-LH infusions of glutamate or other excitatory amino acid agonist) on rats’ behaviours in the EPM or NISF. If the LS disinhibition hypothesis holds, such a manipulation ought to reduce rats’ anxiety-related behaviours in those tests.

As extensively discussed in Chapter 3, and to an extent, in Chapter 4, to explain the anxiolytic-like effects of intra-LS histamine application, we speculated that histamine reduces the neural activity of LS cells. Histamine H₁ and H₂ receptors have been reported, in certain brain regions, to hyperpolarize cells (Haas, 1981; Brown et al., 2001; Haas and Wolf, 1977). Thus, it is possible that the activation of these receptors in the LS hyperpolarizes the cells there. The H₃ receptor is presynaptic and can reduce glutamate release from neurons (Molina-Hernández et al., 2001). Blocking glutamatergic input, presumably from the hippocampus, reduces rats’ defensive behaviours in the EPM and SPBT (Menard and Treit, 2000). As such, the activation of H₃ receptors on hippocampal terminals in the LS could reduce the release of glutamate from these terminals, thereby decreasing excitatory input to LS cells. To the best of our knowledge, the effects of H₁, H₂, or H₃ receptor activation in the LS have not yet been studied. To test the hypothesis that intra-LS histamine reduces LS neural activity, future studies are needed to test whether H₁ or H₂ receptor activation in the LS hyperpolarizes or depresses the firing of LS neurons; and, whether the activation of lateral septal H₃ receptors reduces glutamate levels there and/or reduces the firing of LS neurons. The idea that histamine application in the
LS decreases LS neural activity fits with the findings reported in Chapter 4, where infusions of histamine into the LS were found to increase reticular-elicited hippocampal theta frequency. More specifically, the LS is interconnected with brain regions implicated in the generation of hippocampal theta activity, such as the MS, SUM, and interestingly, the LH (Vertes and Koscis, 1997). All three of these areas, especially the MS and SUM, have been shown to drive theta activity. The large majority of LS projection neurons are GABAergic (Risold and Swanson, 1997b), although whether the LS to MS connections are also GABAergic is unclear. Regardless, reducing lateral septal GABAergic output, for example, via intra-LS application of histamine, could result in the disinhibition of LS targets (i.e., the MS, SUM, and LH) and lead to an increase in hippocampal theta frequency. To test this hypothesis, an experiment could be designed in which hippocampal theta is recorded following local inactivation of the LS, such as with intra-LS infusions of muscimol. If decreased LS activity truly results in the disinhibition of theta generator sites, then the intra-LS muscimol infusions should also increase the frequency of hippocampal theta. Altogether, the mechanisms presented here to explain the anxiolytic-like effects of LS inactivation as well as the effects (i.e., behavioural anxiolysis and increased hippocampal theta frequency) of intra-LS application of histamine are purely speculative and warrant additional study.

5.3. The LS – future directions

5.3.1. Other neurochemical systems

The LS contains axon terminals and receptors for numerous neurochemicals involved in fear and anxiety, of which histamine is only one (Sheehan et al., 2004). The contributions of these other neurochemicals in the LS to rats’ defensive behaviours have yet been fully explored
and represent potential directions for future research. Two examples of such neurochemicals, galanin and morphine, are described below:

Galanin is a 29 amino-acid neuropeptide abundantly expressed in the central nervous system (Karlsson and Holmes, 2006). Notably, $^{125}$I-galanin shows dense binding in the LS, especially in its dorsal aspects (Skofitsch et al., 1986), and the galanin receptor subtype GALR1 is expressed in the LS (Gustafson et al., 1996). Galanin contributes to rats’ defensive behaviours, although its effects are variable. For example, it produces anxiolytic-like effects in the rat Vogel conflict test when administered i.c.v. (Bing et al., 1993), but if it is locally injected into the amygdala, it produces the opposite (i.e., anxiogenic-like) effects in the same test and does not affect open arm exploration in the EPM (Moller et al., 1999). To the best of our knowledge, only one study has examined the role of galanin in the LS in rats’ defensive behaviours to date, in which local application of the galanin antagonist M40 into the LS was found to suppress rats’ defensive burying in the SPBT (Echevaria et al., 2005). Thus, future experiments could determine whether galanin and its specific receptor subtypes modulates rats’ anxiety-related behaviours in the SPBT and other models of anxiety, such as the EPM and NISF.

Peripheral or i.c.v. administration of morphine or $\mu$ (µ) opiate receptor agonists reduce rodents’ anxiety-like behaviours (Zarrindast et al., 2005; Asakawa et al., 1998; Köks et al., 1999). Notably, the LS expresses $\mu$-opioid receptors (Mansour et al., 1995). Unilateral infusions of morphine into the mouse LS produce anxiogenic-like effects; that is, the infusions reduce both open-arm exploration in the EPM and head-dipping frequencies in the hole-board test (Merrer et al., 2006). Surprisingly, bilateral infusions of morphine into the LS do not affect behaviours in either of these tests (Merrer et al., 2006). Future experiments could examine if a similar dissociation also occurs in rats, as well as whether morphine alters rats’ defensive behaviours in
other models of anxiety such as the NISF or SPBT. In addition to µ-opioid receptors, the LS expresses kappa (κ) and delta (δ) opioid receptors (Mansour et al., 1995). Moreover, cells in the LS produce enkephalins (Risold and Swanson, 1997a), and the LS contains dense methionine-enkephalin immunoreactive fibres (Onténiente et al., 1989). As such, the contribution of these other opioid receptors (especially the δ receptors) to rats’ anxiety-related behaviours could also be studied.

5.3.2. Other animal models of anxiety

Although we obtained significant findings in the SPBT in Chapter 2, we were unable to acquire usable data from the SPBT in Chapter 3. More specifically, we had extremely low burying baselines; for example, in Experiment 1 of Chapter 3, 9 out of 12 saline-infused rats showed burying durations of either zero (n = 7) or less than 7 seconds (n = 2). Similarly low burying levels were present in Experiments 2-4. Such low baselines precluded the detection of any histamine effects on burying. The SPBT is susceptible to procedural variables such as experimenter effects (de Boer and Koolhaas, 2003). That said, future studies could examine whether intra-LS infusions of histamine are also anxiolytic in this paradigm (i.e., whether intra-LS histamine decreases rats’ defensive burying). As reviewed in section 1.2.5, the EPM, NISF, and SPBT all use different anxiogenic stimuli (e.g. open space versus novel environments versus a discrete painful stimulus), and so it would be interesting to determine whether the anxiolytic-like effects of intra-LS histamine generalize to other defensive behaviours such as defensive burying.

In addition to the EPM, NISF, and SPBT, a myriad of other animal models of anxiety exist (Haller and Alicki, 2012). One example in the social interaction test, where pairs of unfamiliar male rats are placed into test boxes, and the time they spend interacting socially is
measured (File and Seth, 2003). Increases in social interaction indicate an anxiolytic-like effect, while decreases indicate an anxiogenic-like effect (File and Seth, 2003). The test conditions (i.e., light levels and familiarity of the test box) can be manipulated to produce different levels of anxiety, depending if the experimenter is searching for anxiolytic or anxiogenic-like effects (File and Seth, 2003). The highest amounts of social interaction and therefore lowest levels of anxiety occur in a dimly lit, familiar test box, whereas the lowest amounts of social interaction and therefore highest levels of anxiety occur in a brightly light, unfamiliar test box (File and Seth, 2003). Anxiolytic drugs such as chlordiazepoxide increase social interaction, while anxiogenic drugs such as yohimbine decrease it (for a review see File and Seth, 2003). Other models of anxiety include the open-field or distress vocalization tests (see Haller and Alicki, 2012 for a list). Given that the dorsal LS contributes to rats’ anxiety-related responses in a test-specific manner (i.e. it modulates open arm exploration and shock probe burying but not hyponeophagia), as well as the differential contributions of the H₁, H₂, and H₃ receptors in the EPM and NISF tests, it would be interesting to determine if the dorsal LS or the histaminergic system affects anxiety-like behaviours in these other models.

5.4. Fear, anxiety, and defense: beyond the LS

5.4.1. The hypothalamus

The LS is strongly interconnected with the hypothalamus; two of its major targets are the LH and anterior hypothalamic nucleus (AHN) (Risold and Swanson, 1997b). These two hypothalamic sites have been shown to differentially regulate rats’ defensive behaviours (Schwerdtfeger and Menard, 2008). More specifically, bilateral infusions of muscimol into the LH decrease open arm exploration in the EPM without affecting shock-probe burying in the SPBT, while the same infusions into the AHN suppress shock probe burying without affecting
open arm exploration (Schwerdtfeger and Menard, 2008). These results suggest that the LH and AHN differentially regulate rats’ defensive behaviours; the LH mediates exploratory behaviours in potentially threatening environments, whereas the AHN modulates defensive responding to proximal discrete threats (Schwerdtfeger and Menard, 2008). Whether the LS acts in tandem with the LH or AHN to regulate rats’ anxiety-related behaviours is unknown. The LS sends predominantly ipsilateral projections to these two structures (Risold and Swanson, 1997b). If communication between the LS and the LH or AHN is necessary in mediating defensive behaviours, these behaviours should be suppressed if the LS of one hemisphere and contralateral LH or AHN are disabled. Given the double dissociation between the LH and AHN’s roles in the EPM and SPBT respectively, we predict that disabling the LS-LH circuit would affect rats’ behaviour in the EPM not in the SPBT, and that disabling the LH-AHN circuit would affect rats’ behaviour in the SPBT but not in the EPM.

5.4.2. The medial septum

The MS’s role in anxiety is not clearly defined. Electrolytic lesions of the MS reduce rats’ defensive behaviours in the EPM and SPBT (Menard and Treit, 1996). In contrast, infusions of the benzodiazepine midazolam into the MS do not affect open arm exploration or shock probe burying (Pesold and Treit, 1996), while intra-MS infusions of the GABA_A agonist muscimol produce anxiolytic-like effects similar to those of electrolytic MS lesions (Degroot and Treit, 2001). Notably, electrolytic lesions of the MS reduce rats’ latencies to initiate snack consumption of a familiar food in a novel environment, as well as their latencies to consume new food in a familiar environment (Bannerman et al., 2004). Given the MS’s role in regulating hippocampal theta activity (Vertes and Koscis, 1997), and the connection between hippocampal theta activity and anxiety (McNaughton et al., 2007), it is likely that the MS contributes to rats’
anxiety-related behaviours. To more clearly delineate the MS’s role in anxiety, it should be lesioned using excitotoxins, which unlike electrolytic lesions, spare fibres of passage while destroying cell bodies, and have the effects of such lesions evaluated in the EPM, NISF, and SPBT. Moreover, as discussed in Chapter 3, while the likelihood of drug diffusion from the LS to the MS is low, the contribution of the histaminergic system of the MS to rodents’ behavioural defense should also be studied.

5.5. Concluding remarks

To summarize, in this thesis, we demonstrate for the first time that while the dorsal LS does not mediate rats’ hyponeophagic responses in the NISF, it does modulate their defensive behaviours in the EPM and SPBT. Moreover, we are the first to show that bilateral infusions of histamine into the LS reduce rats’ anxiety-related behaviours in the EPM as well as the NISF. In addition, we report a novel double dissociation between the H₁, H₂, and H₃ receptors in regulating rats’ anxiety-related behaviours. More specifically, the H₁ and H₂ receptors modulate defensive responding in the NISF but not in the EPM, whereas the H₃ receptors mediate anxiety-like behaviours the EPM but not in the NISF. This latter finding, that the H₃ receptor is involved in rats’ defensive behaviours in the EPM is also novel, as prior studies have reported that H₃ receptor agonists or antagonists affected behaviours in other models of anxiety or depression, but not in the EPM (Pérez-García, 1999; Yokoyama et al., 2009). Finally, we report for the first time that intra-LS infusions of histamine, which produces behavioural anxiolysis, increases rather than decreases the frequency of reticular-elicited hippocampal theta activity. Altogether, while the LS’s exact role in anxiety regulation is far from being fully understood, and certainly, more work is needed to elucidate the mechanisms underlying its regulation of anxiety, the empirical work from this thesis adds to the existing literature regarding the neurobiology of fear/anxiety.
More importantly, the data presented in this thesis (in particular, the data from Chapter 3) while preliminary, could ultimately aid in the development of more effective, novel drugs to treat anxiety disorders in humans.
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