INVESTIGATING THE ROLE OF NEUROPEPTIDE Y IN THE VENTRAL HIPPOCAMPUS IN REGULATING RATS’ ELEVATED PLUS-MAZE AND SHOCK-PROBE BURYING BEHAVIOURS

by

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Abstract

Neuropeptide Y (NPY) has recently gained interest as a potential regulator of anxiety-like behaviours. However, its anxiety-associated effects in the ventral hippocampus are not well known, even though this structure is also heavily implicated in anxiety regulation. The goal of this thesis was to investigate whether neuropeptide Y and its Y₁ and Y₂ receptors are associated with anxiety regulation in the ventral hippocampus. To determine whether NPY has a role in anxiety regulation in the ventral hippocampus, I infused rats with either NPY or vehicle control. Next, to determine whether the effects of NPY on anxiety-related behaviour are associated with the Y₁ receptor, I infused rats with either the Y₁ antagonist, BIBO 3304 or saline, followed by infusions of either NPY or saline just prior to testing (Experiment 2). In Experiment 3, I infused the Y₂ antagonist, BIIE 0246 prior to infusions of either NPY or saline. In all experiments, the rats were tested in the elevated plus-maze (EPM) and the shock-probe burying test (SPBT). Infusions of NPY alone into the ventral hippocampus decreased rats’ shock-probe burying duration ($p = .007$), but did not affect rats’ open-arm exploration (Experiment 1). This pattern of findings was replicated in Experiment 2 and 3. The results demonstrate that infusions of NPY into the ventral hippocampus powerfully suppress defensive burying in rats, while not affecting their open-arm activity. This suppression of burying was attenuated by infusions of BIBO 3304 but not BIIE 0246, suggesting that this anxiolytic-like effect is associated with the Y₁, but not Y₂, receptor. Thus, NPY seems to have an anxiolytic-like effect in the ventral hippocampus, in a test-specific fashion, that is associated with the Y₁ receptor.
Co-Authorship

I, Samuel Yoon, participated in the study design, data collection, data analysis, and the preparation of this thesis manuscript. Janet L. Menard also contributed to the above, and provided editorial guidance throughout the preparation of the manuscript.
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<tr>
<td>aCSF</td>
<td>Artificial Cerebrospinal Fluid</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BIBO 3304</td>
<td>Y₁ receptor antagonist</td>
</tr>
<tr>
<td>BIBP 3226</td>
<td>Y₁ receptor antagonist</td>
</tr>
<tr>
<td>BIIE 0246</td>
<td>Y₂ receptor antagonist</td>
</tr>
<tr>
<td>EPM</td>
<td>Elevated Plus Maze</td>
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<tr>
<td>GABA</td>
<td>Gamma-Aminobutyric Acid</td>
</tr>
<tr>
<td>i.c.v.</td>
<td>intracerebroventricular</td>
</tr>
<tr>
<td>[Leu³¹, Pro³⁴]NPY</td>
<td>Y₁, Y₄, Y₅ receptor agonist</td>
</tr>
<tr>
<td>PP</td>
<td>Pancreatic Polypeptide</td>
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<td>PYY</td>
<td>Peptide YY</td>
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<td>NPY</td>
<td>Neuropeptide Y</td>
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<tr>
<td>NPY₁₃-₃₆</td>
<td>Y₂ receptor agonist</td>
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<tr>
<td>SAL</td>
<td>Saline</td>
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<tr>
<td>s.c.</td>
<td>Subcutaneous</td>
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<td>SEM</td>
<td>Standard Error of the Mean</td>
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Chapter 1

Introduction

Anxiety can be defined as the feeling of an unpleasant, pervasive uneasiness in anticipation of a negative event (Rachman, 2004a). While anxiety is evolutionarily adaptive for avoiding potentially dangerous scenarios (Bateson, Brilot, & Nettle, 2011), it can become maladaptive in either excess or deficiency. The harmful effects of excessive anxiety can be seen in cases of generalized anxiety disorder (GAD). Those who suffer from GAD are afflicted by a pervasive, excessive, uncontrollable and irrational apprehension for events that significantly impact daily functioning (American Psychiatric Association, 2013). Due the importance of anxiety, many psychological and biological theories have been proposed attempting to explain this phenomenon (Rachman, 2004b) but the exact neurological mechanisms of anxiety are not fully known. Past investigations have shed light into certain brain areas and neurotransmitters involved in anxiety, but additional research is required to further map its neurological underpinnings.

1.1 Animal Models of Anxiety

A useful method of studying the neurological basis of anxiety is through the use of animal models of anxiety. Using animals in research allows for precise control of environmental and experimental conditions for procedures that may not be possible in human research. For example, direct infusions of various psychopharmacological compounds to specific areas of the brain are not possible in humans, but can alter stereotyped anxiety-like behaviours in animals. Data from these experiments are invaluable in characterizing the efficacy and side effects of psychopharmaceutical compounds, especially prior to the use of unproven pharmaceuticals in
clinical trials with humans. While it is not possible to determine whether an animal is subjectively feeling the emotion of anxiety, ethologically relevant anxiety-like behaviours can be used as indices of anxiety-like states.

There are many models of animal anxiety that are in use and have been extensively validated. Several of these models use physiological measures (e.g., stress-induced hyperthermia; Olivier et al., 2003), while others use anxiety-like behaviours. An ethological approach to anxiety studies unconditioned, innate defensive responses generated by features in the experimental environment (Blanchard & Blanchard, 1988). Many tests have been developed using these ethologically relevant anxiety-related behaviours. However, to study how anxiety is affected, it is important to consider multiple behavioural tests, as defensive behaviours may have different neural mechanisms. For example, many early models of animal anxiety such as the EPM were validated by benzodiazepine-associated anxiolysis. With the advent of other anxiolytic pharmaceuticals, it was found that some clinically effective anxiolytics did not produce a previously defined ‘anxiolytic-like effect’ in some models of anxiety. For example, while benzodiazepines reduce anxiety-like behaviours such as thigmotaxis in the elevated plus-maze, this is not the case for other anxiolytic drugs, such as buspirone, a 5-HT_{1A} partial agonist (Griebel, 1995). However, other tests such as the SPBT is sensitive to non-benzodiazepine anxiolytic compounds such as buspirone (Treit & Fundytus, 1988). Thus, the use of multiple models of anxiety is useful because it allows for better validation of a compound affecting anxiety. In consideration of these factors, I used both the elevated plus-maze test and the shock probe burying tests.

1.1.1 Elevated Plus Maze Test
The elevated plus maze (EPM) test, initially developed to test novel anxiolytic drugs using rats, is one of the most (popular) widely used models of anxiety for rats and mice, due to its affordability, simplicity, bidirectional drug sensitivity, and rapidity (Blanchard & Blanchard, 1988; Carobrez & Bertoglio, 2005; Lister, 1987). Furthermore, because the EPM does not require the use of appetitive or painful stimuli as part of its protocol, it is unlikely that these factors affect the sensitivity of the EPM to detect changes in anxiety-like behaviours (Bourin, Petit-Demoulière, Dhonnchadha, & Hascöet, 2007). The EPM consists of an elevated, “+” shaped maze with two walled arms and two open arms (Pellow, Chopin, File, & Briley, 1985). Naïve rats placed in the center of the maze are faced with an ethologically relevant conflict between exploration and protection. Prey species such as rats display a tendency to stay near vertical surfaces to avoid potential dangers such as predation (i.e., thigmotaxis). This tendency results in an approach/avoidance conflict, where the rats’ preference for proximity to vertical surfaces conflicts with the drive to explore the novel environment that may result in potential rewards. Generally, rats placed into the EPM without any drug treatment prefer to remain in the closed arms more than the central space between arms and the open arms (Bourin et al., 2007). When forced to stay in the open arms, rats display physiological (i.e., increased plasma corticosterone) and behavioural signs of stress (i.e., time spent freezing and immobile; Pellow et al., 1985). The EPM has been extensively pharmacologically validated for its sensitivity to compounds that affect anxiety in humans. Only clinically effective anxiolytic compounds, such as diazepam and phenobarbitone, selectively increased exploration of the open arms (Pellow et al., 1985). In contrast, anxiogenic compounds (i.e., yohimbine, and to a lesser extent caffeine and amphetamines) selectively decreased the exploration of the open arms (Pellow et al., 1985).
Importantly, behaviours recorded in the EPM can be used to distinguish between anxiolytic-like effects and locomotive effects – both of which can affect exploration of the open arms. A sedative effect can result in a general decrease in activity, which detrimentally affects exploratory behaviours. Initial work with the EPM used total arm entries as a global indicator of locomotion (Lister, 1987), but subsequent factor analysis suggested that total entries indicates mixed anxiety/locomotor activity, and that the total number of closed arm entries provides a more valid index of general exploratory activity (Rodgers & Johnson, 1995). In this study, I used both the total number of entries made and the total closed-arm entries as indices of locomotion to lend greater certainty that a selective, anxiolytic-like effect occurred in the EPM separate from any sedative effects.

1.1.2 Shock Probe Burying Test

The shock-probe burying test (SPBT) was first introduced by Treit and colleagues in 1981. The SPBT is a measure of rats’ untrained defensive responses to potentially dangerous stimuli. When exposed to an acute, noxious stimulus, a rat’s response is to “bury” it, using stereotyped rapid movement of the forepaws and/or shoveling motions with the head, to push the bedding material towards and over an electrified shock probe (Treit, Pinel, & Fibiger, 1981). This response was suppressed following a single dose of anxiolytic drugs (i.e., diazepam), and reflected the relative clinical potencies of other anxiolytic compounds (Treit et al., 1981). Ethologically, defensive burying may have been used to drive off approaching predators by spraying sand (Owings & Coss, 1977). In the laboratory, the burying response has been elicited in rats by certain unconditioned stimuli (i.e., a mouse trap and a light bulb) and conditioned stimuli that emitted a secondary noxious stimulus following habituation (i.e., air puff out of
polyethylene tubing, shock from wire-wrapped probe, flash from a light bulb; Terlecki, Pinel, & Treit, 1979).

The SPBT consists of a test chamber that contains an electrified shock probe attached to one wall (Treit et al., 1981). A rat is placed in the center of the chamber, and whenever the rat makes contact with the probe with either its’ paws or snout, it receives a brief contact-induced electric shock. After the shock, the rat moves away from the probe and eventually uses its’ front limbs and head to push bedding material, from the floor of the test chamber, towards and over the shock probe. The duration of burying is used as the primary index of anxiety. The duration of time rats spend burying the electrified probe is decreased by anxiolytic (i.e., anxiety reducing) drugs, such as Valium™ and increased by anxiogenic (i.e., anxiety generating) drugs, such as yohimbine (Treit et al., 1981; Tsuda et al., 1988). Furthermore, drugs that are ineffective in treating clinical anxiety, such as the antipsychotic haloperidol, do not selectively affect defensive burying (Treit et al., 1981).

One advantage of the SPBT is that it allows for the examination of potential confounding variables, including drug-induced sedation (as indexed by the time spent immobile throughout the test), drug-induced changes in pain sensitivity (as indexed by the mean shock reactivity scores), and drug-induced changes in associative learning and memory (as indexed by total number of contact-induced probe shocks received; Treit et al., 1981). The inclusion of indices to rule out potential confounds has made the SPBT a very useful model of animal anxiety.

1.2 Neuropeptide Y and Anxiety

Recent research into the neural mechanisms of anxiety has focused on the potential involvement of neuropeptide Y in anxiety regulation. Neuropeptide Y (NPY) is a 36-amino acid neurotransmitter found abundantly throughout the central nervous system (Allen et al., 1983;
Along with peptide YY (PYY), NPY is part of the pancreatic polypeptide (PP) family of peptide transmitters made up of 36-amino acids. NPY is evolutionarily well-conserved: it is found in most mammals, some bony and cartilaginous fish, several species of amphibians, and peptides analogous to NPY have been found in a large number of invertebrate species (Larhammar, Blomqvist, & Söderberg, 1993).

NPY is found abundantly in the mammalian brain, including areas such as the hypothalamic areas, nucleus accumbens, the septum, the periaqueductal gray, the hippocampus, amygdala, the thalamus and the basal ganglia (Thorsell & Heilig, 2002). NPY has a functional role in several regulatory systems including the regulation of feeding, circadian rhythms, vasoconstriction, alcohol intake, and serves an endogenous neuro-protective response to seizures (Kalra, Dube, Sahu, Phelps, & Kalra, 1991; Lall & Harrington, 2006; Qureshi, Dayao, Shirali, Zukowska-Grojec, & Hauser, 1998; Thiele, Koh, & Pedrazzini, 2002; Vezzani & Sperk, 2004). NPY is also involved in the regulation of various hypothalamic peptides. NPY stimulates the corticotrophic axis (Krysiak, Obuchowicz, & Herman, 1999) and inhibits the gonadotropic and somatotropic systems (Catzefflis et al., 1993).

Importantly, NPY appears to have a role in the regulation of affective behaviours, including anxiety (Heilig, Söderpalm, Engel, & Widerlöv, 1989). This was first established by evidence that intracerebroventricular (i.c.v.) administration of NPY has anxiolytic-like effects. Intracerebroventricular infusions of NPY increased rats’ open-arm entries in the elevated plus-maze test (EPM; Heilig et al., 1989), reduced their fear-potentiated startle responses (Broqua, Wettstein, Rocher, Gauthier-Martin, & Junien, 1995), and stress-induced gastric erosions (Heilig & Murison, 1987). Furthermore, NPY (i.c.v.) potently mimicked the anxiolytic-like actions of
indirect GABA_A agonists (i.e., benzodiazepines) in the Vogel punished-drinking and Geller-Seifter punished-feeding tests (Heilig et al., 1989).

Studies using infusions of NPY i.c.v. have yielded valuable information regarding the general anxiolytic-like actions of NPY, but are not informative about the specific structures that are involved in anxiety regulation. Whether infusions of NPY produce an anxiolytic-like or other regulatory effect depends on both the structure in which NPY is active and the NPY receptors that are involved in that region. To examine this issue, local infusions of NPY can be used to identify structures that are associated with the anxiolytic-like actions of NPY. Several brain structures have been implicated in the anxiolytic-like actions of NPY, including the amygdala, periaqueductal gray, lateral septum, locus coeruleus and dorsal hippocampus (Heilig et al., 1993b; Kask, Rägo, & Harro, 1998; Smiałowska, Wierońska, Domin, & Zieba, 2007; Tasan et al., 2010; Trent & Menard, 2011). The anxiolytic-like effects of local infusions of NPY in these regions are associated with specific NPY Y receptors. For example, the anxiolytic-like actions of NPY in the amygdala are specifically associated with activity at the Y_1 receptor (Heilig et al., 1993a). Thus, it is important to distinguish both the NPY receptors that may be selectively associated with its anxiety-regulatory function, in addition to areas contributing to NPY-associated anxiety regulation.

1.2.1 Neuropeptide Y Y-receptors

NPY Y-receptors are G-protein coupled receptors that inhibit cAMP synthesis (Bockaert & Pin, 1999; Dan Larhammar, Söderberg, & Lundell, 1998). To date, five receptors have been cloned and identified: the Y_1, Y_2, Y_4, Y_5 and y_6 receptors (Wraith et al., 2000). Although the Y_3 receptor was initially identified by its unique binding profile in vivo to NPY (Lee & Miller, 1998), it was later found to be a chemokine receptor and renamed CXCR4 (Wraith et al., 2000).
1.1.1.1 The $Y_1$ Receptor and Anxiety

The $Y_1$ receptor can be found throughout various organs in the body, including the brain. Regarding the latter, the $Y_1$ receptor is found in high densities in the frontal cortex and the nucleus of the solitary tract, and moderate concentrations in the hippocampus, amygdala, and the lateral dorsal septum (for review: Kask et al., 2002). Low concentrations of the $Y_1$ receptor can also be found in the nucleus accumbens, paraventricular nucleus, lateral hypothalamus, the arcuate nucleus, ventral tegmental area, periaqueductal gray, and the locus coeruleus (Kask et al., 2002). Another autoradiographical study found $Y_1$ receptor located in the dentate gyrus of the hippocampus, amygdala, and some thalamic and hypothalamic nuclei (Dumont, St-Pierre, & Quirion, 1996).

The evidence for the $Y_1$ receptor’s role in anxiety regulation is well established. The anxiety-regulatory role of the $Y_1$ receptor was first hypothesized following the anxiolytic-like effects of infusions of [Leu$^{31}$, Pro$^{34}$]NPY, a selective $Y_1$, $Y_4$ and $Y_5$ receptor agonist into the central amygdala without changes in feeding. In contrast, the $Y_2$ receptor agonist (i.e., NPY$_{13-36}$) did not affect anxiety-like behaviours (Heilig et al., 1993). Another study found that decreasing the density of $Y_1$ receptors via a $Y_1$ receptor-specific anti-sense oligonucleotide resulted in an anxiogenic-like response in the EPM (Wahlestedt, Pich, Koob, Yee, & Heilig, 1993). The development of selective $Y_1$ receptor antagonists such as BIBP 3226 and BIBO 3304 further provided evidence for its role in anxiolytic-like actions when activated. Infusions of BIBP 3226 i.c.v. resulted in increased thigmotaxis in the open-field test (von Hörsten et al., 1998) and was able to produce conditioned place avoidance, signifying an anxiogenic-like effect (A. Kask, Kivastik, Lembit, & Harro, 1999). Infusions of NPY into the basolateral nucleus of the amygdala was found to facilitate the extinction of fear-potentiated startle response, while infusions of
BIBO 3304 were found to increase the time taken to extinguish this response (Gutman, Yang, Ressler, & Davis, 2008). More recently, infusions of BIBO 3304 into the lateral septum were found to attenuate the anxiolytic-like actions of NPY in the SPBT (Trent & Menard, 2011). Thus, the Y\textsubscript{1} receptor seems to be strongly associated with the regulation of anxiety-like behaviours.

1.1.1.2 The Y\textsubscript{2} Receptor and Anxiety

The NPY Y\textsubscript{2} receptor comprises the majority of Y-receptors in the CNS (Aicher, Springston, Berger, Reis, & Wahlestedt, 1991), and is found in varying concentrations in almost every structure in the brain (for review: A. Kask et al., 2002). High levels of NPY Y\textsubscript{2} binding can be found in the hippocampal formation, the lateral septum, and the area postrema (A. Kask et al., 2002). The Y\textsubscript{2} receptor is expressed predominantly pre-synaptically in the CNS (Chen & van den Pol, 1996; Colmers & Bleakman, 1994), and can act as an autoreceptor whose activation decreases the release of NPY into the synaptic cleft (King, Williams, Doods, & Widdowson, 2000). The pre-synaptic Y\textsubscript{2} receptor also acts as a heteroreceptor, in that its activation can inhibit the release of glutamate and/or GABA from the pre-synaptic neuron (Bacci, Huguenard, & Prince, 2002; Greber, Schwarzzer, & Sperk, 1994; Silva, Carvalho, Carvalho, & Malva, 2001).

The NPY Y\textsubscript{2} receptor has also been implicated in anxiety regulation. One study using NPY Y\textsubscript{2} receptor knockout mice found increased open-arm entries, indicating an anxiolytic-like effect (Redrobe, Dumont, Herzog, & Quirion, 2003). Another study using i.c.v. infusions of the Y\textsubscript{2} receptor-specific antagonist (i.e., BIIE 0246) resulted in an anxiolytic-like effect in the EPM (Bacchi et al., 2006). Similarly, infusions of BIIE 0246 into the central amygdala resulted in an anxiolytic-like effect in the EPM without changes in alcohol consumption (Kallupi et al., 2013). However, a different study using lateral septal infusions of the Y\textsubscript{2} receptor agonist (i.e., NPY\textsubscript{13-}}
obtained increased open-arm exploration and increased shock-probe burying latency. In the same study, the pre-infusion of the Y2 receptor antagonist (i.e., BIIE 0246) blocked the anxiolytic-like effects of NPY13-36 in the EPM but not the SPBT (Trent & Menard, 2013). Thus it appears that the Y2 receptor role in NPY-associated anxiety regulation is test-specific.

1.1.1.3 The Y4, Y5, and Y6 receptors

The NPY Y4 receptor is unique in its higher affinity to the pancreatic polypeptide rather than NPY (Bard, Walker, Branchek, & Weinshank, 1995), and is found in the hypothalamus, limbic system and the medullary brainstem (Fetissov, Kopp, & Hökfelt, 2004; Parker & Herzog, 1999). There is some evidence for the Y4 receptor’s involvement in anxiety- and depression-related behaviours in Y4 receptor knockout mice (Painsipp et al., 2008; Tasan et al., 2009). However, it is unlikely that the Y4 receptor contributes significantly to anxiety regulation (Asakawa et al., 1999; Kask et al., 2002). Rather, the Y4 receptor has been found to synergistically regulate circadian energy expenditure and physical activity along with the Y2 receptor (Edelsbrunner, Painsipp, Herzog, & Holzer, 2009; Wultsch et al., 2006; Zhang et al., 2010), and contribute to the regulation of the gonadotropic system (Jain, Pu, Kalra, & Kalra, 1999; Raposinho et al., 2000; Sainsbury et al., 2002).

The Y5 receptor was initially identified in the paraventricular nucleus as the ‘feeding receptor’ (Gerald et al., 1996) and later found to be widely distributed throughout the brain. The Y5 receptor has been identified in the paraventricular nucleus, lateral septum, the hippocampus, arcuate nucleus, basolateral amygdala, and the supraoptic nucleus (Dumont, Fournier, & Quirion, 1998; Gerald et al., 1996; Jacques, Tong, Shen, & Quirion, 1998; Wolak et al., 2003). The activation of the Y5 receptor using Y5-selective agonists seems to have a potent stimulatory effect on appetite (Cabrele et al., 2000). Many investigations have similarly posited that the Y5
receptor has an important role in peripheral energy availability and appetite regulation (Blumenthal et al., 2002; Campbell, Ffrench-Mullen, Cowley, Smith, & Grove, 2001). While anxiolytic-like effects following infusions of the Y5 receptor-selective ligand i.c.v. have been observed, the Y5 receptor has also been implicated in NPY-induced sedation (Sørensen, Lindberg, Wörtwein, Bolwig, & Woldbye, 2004) and evidence for the Y5 receptor’s contribution to NPY-related anxiety regulation is mixed (Kask, Vasar, Heidmets, Allikmets, & Wikberg, 2001).

The Y6 receptor is a recent addition to the NPY Y-receptor family. While functional in mice and rabbits (Gregor, Feng, DeCarr, Cornfield, & McCaleb, 1996; Weinberg et al., 1996), the Y6 receptor is nonfunctional in humans, primates, and rats due to a shift mutation in the coding gene (Burkhoff, Linemeyer, & Salon, 1998; Gregor et al., 1996; Wraith et al., 2000). The function of Y6 receptors has yet to be identified.

1.2 The Hippocampus and Anxiety

One potential site of NPY’s anxiety regulatory activity is the hippocampus. The hippocampus is implicated in several important functions, such as memory formation (Izquierdo & Medina, 1997), spatial memory and navigation (Buzsaki & Moser, 2013), and anxiety (McHugh et al., 2004). Several types of cells have been identified in the hippocampus, including large hippocampal pyramidal cells and a heterogenous GABAergic interneuron population (Somogyi & Klausberger, 2005). Among these, bistratified cells are immunoreactive to NPY (Szilagyi, 2011). When hippocampal slices were placed in a bath application of NPY, the excitatory pre-synaptic potentials of pyramidal neurons in the CA1 and CA3 were reversibly reduced (Klapstein & Colmers, 1993). A similar inhibitory action was observed in a dose-dependent manner in another study (Bijak & Smialowska, 1995). The inhibitory action of NPY
on hippocampal cells has been attributed to its direct action at the synaptic terminal reducing Ca2+ influx (Colmers, Lukowiak & Pittman, 1988).

The hippocampus can be anatomically and functionally divided into two domains: the dorsal and ventral hippocampus. Anatomically, the dorsal and ventral hippocampi have different afferent and efferent projections suggesting that they might serve separate functions (Moser & Moser, 1998; Risold & Swanson, 1997). For example, the dorsal hippocampus receives input from visuo-spatial processing areas which would support its role in functions related to spatial learning (Amaral & Witter, 1989; Burwell & Amaral, 1998; Dolorfo & Amaral, 1998; Dolorfo & Amaral, 1998). In fact, investigations into the functional role of these structures revealed a dissociation of memory-related and anxiety-related functions, whereby the dorsal hippocampus is predominately responsible for spatial learning and memory and the ventral hippocampus for anxiety-related behaviours. Excitotoxic lesions to the dorsal hippocampus that spared the ventral hippocampus impaired performance in spatial learning tests such as the water maze, T-maze, and the 4/8 radial maze tasks (McHugh, Deacon, Rawlins, & Bannerman, 2004; Moser & Moser, 1998; Pothuizen, Zhang, Jongen-Rêlo, Feldon, & Yee, 2004). Local infusions of GABA_A agonists that temporarily disrupt activity (i.e., muscimol) into the dorsal hippocampus have yielded similar disruptions in spatial learning tasks such as the water maze, while leaving anxiety-related physiological and behavioural responses relatively unaffected (Moser & Moser, 1998; Tuvnes, Steffenach, Murison, Moser, & Moser, 2003).

In contrast to the role of the dorsal hippocampus in spatial learning, investigations of ventral hippocampal lesions revealed a wealth of support for its role in anxiety-related behaviours. Anatomically, the ventral hippocampus is well-situated to regulate anxiety-like states. The ventral hippocampus projects to the prefrontal cortex (Barbas & Blatt, 1995; Jay &
Witter, 1991), is closely connected to the amygdala (Petrovich, Caneras, & Swanson, 2001; Pitkänen, Pikkainen, Nurminen, & Ylinen, 2000; Swanson & Cowan, 1977), the lateral septum (Risold & Swanson, 1997), and subcortical structures associated with the hypothalamic-pituitary-adrenal (HPA) axis (Amaral & Witter, 1989; Jacobson & Sapolsky, 1991; Swanson & Cowan, 1977). Lesions of the ventral hippocampus resulted in attenuation of defensive behaviours in both conditioned (i.e., conditioned foot shock) and unconditioned (i.e., cat odour and live cat exposure) tasks of anxiety (Pentkowski, Blanchard, Lever, Litvin, & Blanchard, 2006). Temporary inactivation of the ventral hippocampus, using muscimol, attenuated rats’ unconditioned fear behaviours following exposure to an electrified probe (McEown & Treit, 2010).

Although early studies suggested that the functions of the dorsal and ventral hippocampi were separate, it is now known that either the dorsal or ventral hippocampus do not exclusively contribute to spatial memory and anxiety, respectively. For example, the dorsal hippocampus also seems to contribute to anxiety-related behaviours that may be associated with spatial exploration, such as the EPM (Solati, Zarrindast, & Salari, 2010). Likewise, the ventral hippocampus also harbors functions related to spatial memory. Rats that had their dorsal hippocampi ablated were still able to learn the water maze task (de Hoz, Knox, & Morris, 2003). Given these findings, it is more likely that the dorsal and ventral hippocampi have strongly preferential roles in spatial learning and anxiety, respectively, rather than discrete, separate functions. While the dorsal hippocampus predominantly regulates spatial memory and learning with some anxiety-related functions, the ventral hippocampus is associated with a much wider range of anxiety-related behaviours and their regulation.

1.2.1 The Ventral Hippocampus and NPY
Despite its’ known involvement in anxiety and behavioural defense regulation, surprisingly little information is available regarding the involvement of the hippocampus in NPY’s anxiolytic-like actions. To the best of my knowledge, only a single infusion study has examined the potential involvement of NPY at that site in anxiety regulation (Smiałowska, Wierońska, Domin, & Zieba, 2007). Those authors report that local infusions of NPY into the CA1 subfield of the dorsal hippocampus produced an anxiolytic-like effect in the elevated plus-maze test. This anxiolytic-like effect was attenuated by BIBO 3304, a Y₁ receptor antagonist, as well as BIIE 0246, a Y₂ receptor antagonist, suggesting that the NPY-associated anxiolytic-like effect is mediated by both of these receptors. However, to date, no investigations have focused on the potential role of the ventral hippocampus in the regulation of anxiety by NPY. This is surprising in light of the extensive evidence (reviewed above) that the ventral hippocampus plays a relatively broader role in anxiety regulation than does its dorsal counterpart (Bannerman et al., 2004; McHugh et al., 2004; Menard & Treit, 2001), as well as evidence that the effects of disrupting hippocampal activity often differ, depending on whether the dorsal or ventral aspects are targeted (Bannerman et al., 1999, 2004; Kristopher McEown & Treit, 2010). Regarding the latter, local infusions of the direct GABA$_A$ agonist muscimol into the ventral hippocampus are known to produce anxiolytic-like effects in a variety of models, including the EPM and shock-probe burying tests (for review: Engin & Treit, 2007; McEown & Treit, 2010). Given the correspondence between the effects of GABA-ergic agonists and NPY (Colmers, Lukowiak, & Pittman, 1988), it seems likely that local application of NPY into the ventral hippocampus would produce anxiolysis. The moderate to high densities of Y₁ and Y₂ receptors found in the ventral hippocampus (Parker & Herzog, 1999) and their extensive involvement in NPY’s anxiolytic properties (Kask et al., 2002) give further evidence for the potential contributions of NPY in the
ventral hippocampus to anxiety regulation. Due to the well-established link between the Y₁ and Y₂ receptors and anxiety regulation, it is probable that these receptors would have some role in mediating any anxiolytic-like effects of NPY at this site.

The current thesis will investigate whether NPY in the ventral hippocampus plays a role in regulating anxiety-like behaviours, and if so, whether the Y₁ and/or the Y₂ receptors are associated with this function. Both the ventral hippocampus (which expresses both Y₁ and Y₂ receptors) and NPY are extensively implicated in the regulation of anxiety. To test ventral hippocampal NPY’s potential involvement in anxiety regulation, I will conduct three experiments. In the first experiment, I will locally infuse NPY into the ventral hippocampus of rats and observe its effects on their behaviour in the EPM and SPBT. If NPY significantly affects anxiety-like behaviours in these tests, I will conduct two additional experiments: one will test the effects of the co-infusion of an Y₁ receptor antagonist, and the other an Y₂ receptor antagonist to determine their roles in NPY-associated anxiety regulation in the ventral hippocampus.

1.4 Objectives

1. The first objective of the current thesis is to determine whether NPY in the ventral hippocampus is associated with anxiolytic-like effects.

2. I will also determine whether the NPY Y₁ receptor is associated with the anxiolytic-like effects of ventral hippocampal infusions of NPY.

3. In addition to the second objective, I will determine whether the NPY Y₂ receptor has a similar role in anxiety regulation in the ventral hippocampus.
Chapter 2: Methods

2.1 Experiment 1: Infusions of NPY into the ventral hippocampus

2.1.1 Subjects

The subjects were 16 naïve Long-Evans rats, obtained from Charles River, QC, weighing 300-350g at the time of surgery. When the rats arrived from Charles River, they were pair-housed in a temperature and humidity controlled colony room (21°C ± 2°C), and kept on a 12:12 light/dark cycle (lights on at 0700h). Food and water was provided ad libitum. The rats were habituated to the colony for at least 1 week prior to surgery. All procedures and protocols for this project are in accordance with the guidelines of the Canadian Council on Animal Care and were approved by Queen’s University Animal Care Committee, prior to commencement of the experiments.

2.1.2 Drugs

Porcine NPY was obtained from Polypeptide Laboratories in San Diego, CA. NPY was dissolved in sterile water rather than aCSF due to poor solubility.

2.1.3 Surgery

Each rat was weighed and anesthetized with Isoflurane gas (0.5%-4.5%) in oxygen. To reduce pain, rats received Metacam (2 mg/kg) subcutaneously (s.c.), immediately prior to placement in the Kopf stereotaxic unit, and Tramadol (20 mg/kg) at the end of surgery. Marcaine (2 mg/kg) was directly administered into the scalp immediately after the rats’ heads were shaved. The scalp was sterilized with Hibitane, followed by alternating swabs of alcohol (70% solution) and Betadine (iodine solution) to the incision site. Two 23-gauge, 13 mm cannula-guides were implanted bilaterally, targeting the ventral hippocampus (5.3 mm posterior, ± 5.2 mm lateral to bregma, 4.5 mm ventral to dura, with the cannula-guides angled 6° laterally), according to brain
atlas coordinates (Paxinos & Watson, 1998). The guides were secured to the skull using jeweler’s screws and dental cement. Pins were placed in each cannula-guide to prevent blockages. Immediately following surgery, Polysporin was applied around the incision site to prevent infection, and lactated ringer solution (5-10 ml, s.c.) was administered. Subjects were then housed individually in a new cage with fresh bedding and placed partly under a heat lamp to aid in temperature regulation. Once recovered from anesthesia (i.e., re-establishment of body righting reflex and spontaneous locomotor activity), subjects were transferred to a recovery room where they remained for a minimum of three days. The recovery room temperature was set to approximately 26º C. During recovery, rats were injected with analgesics Tramadol (20 mg/kg, s.c.) and Metacam (1 mg/kg, s.c.), once per day for three days. Following recovery, rats were relocated to their regular colony room for at least one week prior to testing.

2.1.4 Infusions

Following post-surgical recovery, a random number generator was used to assign rats to one of two infusion groups: 1) 0.9% physiological saline (SAL) or 2) NPY (1.5 µg/side). To control for potential drug carry over effects, half of the rats received the same treatment (SAL/SAL and NPY/NPY) before the EPM and SPBT, respectively, whereas the other half received the opposite treatments (SAL/NPY and NPY/SAL) before each test. At least 1 week elapsed between the rats’ first and second infusions. All dosages were determined using previous literature (Trent & Menard, 2011, 2013).

On each of the three consecutive days prior to testing, rats were habituated to the infusion procedures by brief removal and replacement of their cannula pins in the infusion room. On testing day, the rats were gently hand-held while cannula pins were removed. Two 30-gauge stainless-steel internal injectors were lowered 2.0 mm beyond the tip of the guide cannulae.
Polyethylene tubing (PE50) was used to connect each injector to two 10 µL constant rate Hamilton micro-syringes mounted onto an infusion pump (KD Scientific, MA). The infusion rate was set at 1 µL/minute for a final volume of 1 µL per side. At the end of the infusion, the injectors were left in place for an additional 1 minute to allow for diffusion away from the tips. The displacement of an air bubble inside the polyethylene tubing was monitored to confirm drug flow. Behavioural testing started 15 minutes after the end of the infusions.

2.1.5 Behavioural Testing

Rats were allowed to recover from the cannula implantation for at least one week prior to commencement of behavioral testing. All infusions and testing occurred between 0900 and 1700. Behavioral tests were video recorded for later analysis using Observer 7 software (Noldus Information Technology, MA).

2.1.5.1 Elevated Plus-Maze

The EPM test provides an ethological conflict between safety provided by the closed arms and goal directed exploration (Pellow et al., 1985). The maze consists of two pairs of opposing arms, raised 50 cm from the floor by supports under each arm. One arm pair is open (50 cm x 10 cm), while the other is walled on three sides (50 cm x 10 cm x 50 cm) with open ceilings. The 5-minute test began when the rat was placed in the center of the maze, facing one of the closed arms. Rats were allowed to freely explore the maze while a video-monitor simultaneously recorded their behaviour. The following behaviors were coded: 1) number of open arm entries, 2) time spent in the open arms, 3) number of closed arm entries, and 4) total number of closed and open arm entries. In this test, increases in open-arm exploration (open arm entries and time spent in the open arms) that are not accompanied by changes in general locomotor activity (closed arm entries and total entries) indicate selective anxiolytic-like effects.
(Pellow et al., 1985; Rodgers & Johnson, 1995). An arm entry is defined as a rat having all four paws in one of the arm sections of the maze. The maze was thoroughly cleaned with dilute ethanol solution (3%) and allowed to dry after each rat was tested. All tests were conducted between 0900 and 1300 hours.

2.1.5.2 Shock-Probe Burying Test

The SPBT assesses the rat’s innate defensive response following contact with a noxious stimulus (Treit et al., 1981). The apparatus consists of an electrified copper-wired stationary Plexiglas probe (6 X 0.5 X 0.5 cm) inserted through a hole into a transparent Plexiglass chamber (40 X 30 X 40 cm) that contains 5 cm of bedding material (wood chips) spread evenly on its floor. An electrified probe can be inserted through a small hole located 5 cm above the bedding material. An electrical current is distributed through two copper wires wrapped around the probe using a 2000 V shock source, with the shock intensity set at 2.5 mA. Just prior to testing, the rats were individually habituated to the test chamber without the shock-probe present for 15 minutes for four consecutive days prior to testing. On the test day, the probe was inserted and secured 6 cm into the chamber. The rats were individually placed in the chamber facing away from the shock-probe, and the 15 minute test commenced immediately after the rat received its first contact-induced shock. At the end of testing, fecal boli were removed and the bedding was replaced and smoothed to equal thickness.

After being shocked, rats’ innate defensive response consists of rapidly pushing bedding with their forepaws and shoveling bedding with their head towards and over the probe (Treit et al., 1981). Behaviours of interest were captured by a digital video-camera and later coded by an experimenter who was unaware of the group status. Variables measured were: a) the duration of burying (i.e., the time spent pushing bedding material toward the shock probe using the head and
rapid pushing using the front paws), b) the duration of immobility (i.e., resting and/or sleeping with no movement except that need for respiration), and c) number of probe-contact induced shocks received (Treit et al., 1981). In addition, the rats’ physical reactivity to the shocks was measured on a four-point scale (Treit et al., 1981): 1) small head or forepaw flinch, 2) whole body flinch without movement away from probe, 3) whole body flinch with slow movement away from probe, and 4) whole body flinch with rapid movement away from the probe. A mean shock reactivity score was calculated for each rat by dividing the total shock reactivity scores by the total number of shocks received. The duration of burying was used as the primary index of anxiety in this test. Reductions in burying that are not associated with changes in associative learning and memory (indexed by probe avoidance, i.e., the number of shocks received), pain sensitivity (indexed by mean shock-reactivity) and locomotor activity (indexed by duration of immobility) are indicative of anxiety reduction in this test (Treit et al., 1981).

2.1.6 Histology

Following surgery and behavioural testing, rats were sacrificed using a CO₂ chamber. Their brains were extracted and individually stored in 10% formalin solution for at least 48 hours. The brains were frozen using a cryostat to make 40 μm coronal slices that were mounted onto glycerin subbed glass slides. The slides were assessed for the location of cannulae and were transcribed correspondingly to atlas sheets (Paxinos & Watson, 1998). All histological analysis was performed while blind to the corresponding behavioural data. Only the behavioural results from accurate bilateral placements were used for statistical analysis.

2.1.7 Statistical Analysis

Data are presented as means +/- standard error of means (SEM). The data from the EPM and the SPBT were analyzed using a one-way analysis of variance (ANOVA). Significant
ANOVA were followed up for pair-wise comparisons, using the Least Significant Difference (LSD) test.

2.2 Experiment 2: Investigating the effects of the NPY Y₁ receptor in the ventral hippocampus

All experimental procedures were the same as in Experiment 1, with the following exceptions. Forty-six rats were implanted with bilateral cannula guides towards the ventral hippocampus. These rats were randomly assigned to one of four infusion groups: 1) SAL + SAL, 2) SAL + NPY (1.5 µg/side), 3) BIBO 3304 (0.15 µg/side) + SAL, or 4) BIBO 3304 + NPY (1.5 µg/side). The NPY Y₁ antagonist, BIBO 3304 trifluoroacetate was obtained from Tocris Bioscience in Bristol, UK. BIBO 3304 was dissolved in sterile water rather than saline due to poor solubility. Rats were given their second infusions 15 minutes after the end of the first infusion, and behavioural testing began 15 minutes following the second infusion.

2.3 Experiment 3: Investigating the effects of the NPY Y₂ receptor in the ventral hippocampus

All experimental procedures were the same as Experiment 2, with the following exceptions. Forty-two rats were surgically implanted with bilateral cannula guides, and were randomly assigned to one of four infusion groups: 1) SAL + SAL, 2) SAL + NPY (1.5 µg/side), 3) BIIE 0246 (0.5 µg/side) + SAL, or 4) BIIE 0246 + NPY. The NPY Y₂ antagonist, BIIE 0246 was obtained from Tocris Bioscience in Bristol, UK. BIIE 0246 (1 mg) was dissolved in 300 µL of 100% ethanol. This solution was then topped up with 2mL of distilled water and divided into aliquots, which were frozen until use. The infusion volume for BIIE 0246 was set at 1.15 mL/side, yielding a final concentration of 0.5µg/1.15µL per side.
Chapter 3: Results

3.1 Experiment 1: Infusions of NPY into the ventral hippocampus

The accurate cannulae placements into the ventral hippocampus are shown in Fig. 1. Of the 16 rats, 4 rats had infusion tips placed outside the ventral hippocampus resulting in a final \( N = 12 \).

3.1.1 Elevated plus-maze

After the exclusion of missed cannula placements, the following \( n \)’s were obtained: \( n_{SAL} = 6 \), \( n_{NPY} = 5 \). As seen in Fig. 2, infusions of NPY did not significantly affect the percentage of open-arm entries, \( F(1,9) = .653, p = .44 \), \( \eta_{partial}^2 = .07 \) or the percentage of time spent in the open arm, \( F(1,9) = .046, p = .83 \), \( \eta_{partial}^2 = .08 \). NPY did not induce any changes in global locomotor activity as evidenced by the lack of significant differences either on the number of closed arm entries in NPY-infused rats, \( F(1,9) = 1.532, p = .25 \), \( \eta_{partial}^2 = .145 \), or total entries made, \( F(1,9) = .303, p = .60 \), \( \eta_{partial}^2 = .03 \) (Table 1).

I also tried to analyze the data from rats with at least one cannula tip outside of the ventral hippocampus. However, due to the low sample size, this analysis was not possible (\( n_{SAL} = 2 \), \( n_{NPY} = 2 \)). While these data are not presented as part of the current thesis, the procedures used in Experiment 1 are identical to a pilot study I conducted in my undergraduate thesis (Yoon, 2013). I collapsed the data from my undergraduate study with data from Experiment 1 to further investigate whether NPY infusions outside of the ventral hippocampus affected open-arm or locomotor activities (\( N_{collapsed} = 30 \)). The pattern of results for rats with cannula guides aimed to the ventral hippocampus did not differ from the current analysis (i.e., did not significantly differ across open-arm or locomotor activities). Furthermore, the data from rats with cannula tips
outside of the ventral hippocampus did not significantly differ whether the rats were infused with NPY or SAL (see Appendix, Fig 10).
Figure 1. Placements of the infusion tip in the ventral hippocampus in Experiment 1. The circles indicate the approximate infusion tip placement. Infusions of SAL are indicated by unfilled circles, while filled circles represent infusions of NPY. Atlas plates were adapted from Paxinos & Watson, 1998.
Figure 2. Mean ± SEM of percentages of open-arm entries and time spent in the open arm using bilateral infusions of SAL (n = 6) and NPY (1.5µg/side, n = 5) into the ventral hippocampus.
**Table 1.** Mean (± SEM) plus-maze locomotive behaviours in rats infused with either SAL or NPY into the ventral hippocampus.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>SAL (n = 6)</th>
<th>NPY (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed Arm Entries</td>
<td>12.50 ± 0.07</td>
<td>11.83 ± 1.33</td>
</tr>
<tr>
<td>Total Arm Entries</td>
<td>14.33 ± 1.56</td>
<td>13.83 ± 1.19</td>
</tr>
</tbody>
</table>

Note: Rats received bilateral infusions of either saline or NPY (1.5 µg/side) into or outside of the ventral hippocampus. Closed and total arm entries represent the mean number of times rats entered a closed arm and the total number of entries made into either the closed or open arms, respectively.

### 3.1.2 Shock-Probe Burying Test

One of the rats was euthanized following testing in the EPM due to health complications. The following n’s were obtained for rats with correct cannula tip placements: n_{SAL} = 4 and n_{NPY} = 7. As can be seen in Fig. 3, infusions of NPY into the ventral hippocampus significantly reduced burying duration, $F(1,9) = 12.00, p = .007, \eta_{partial}^2 = .57$. Importantly, this reduction in burying was not accompanied by significant differences in the duration of time spent immobile, $F(1,9) = .112, p = .746, \eta_{partial}^2 = .01$, the number of shocks received by the rat, $F(1,9) = 0.46, p = .515, \eta_{partial}^2 = .05$, and the mean shock reactivity, $F(1,9) = .12, p = .735, \eta_{partial}^2 = .01$ (Table 2). These results indicate that the reduction in burying occurred independently of drug-induced changes in locomotor activity, associative memory, or pain sensitivity, respectively.
Two analyses were performed after collapsing the current burying data with data from my undergraduate thesis (see Appendix). First, I analyzed the data from rats with cannula guides aimed outside of the ventral hippocampus to test for neuroanatomical specificity ($N_{collapsed} = 22$). No significant differences in mean burying duration were found between SAL or NPY infused rats (see Appendix, Fig. 11). Secondly, to determine whether the prior infusion in the EPM affected behaviours in the SPBT, I re-grouped the two data sets (current and archival) according to the prior infusion test history of the rat (i.e., SAL/SAL, SAL/NPY, NPY/SAL, or NPY/NPY prior to the EPM/SPBT, respectively). A significant treatment effect on the duration of burying was found, such that both SAL/NPY and NPY/NPY group buried significantly less than the SAL/SAL and NPY/SAL groups (see Appendix, Fig. 12). There were no other significant between group differences. The pattern of results indicates that there were no carry over effects from the first infusion (prior to the EPM) to the 2nd infusion (prior to the SPBT).
Figure 3. Mean (± SEM) durations of burying for rats in Experiment 1. Rats that received infusions of NPY (1.5 µg/side; n = 7) into the ventral hippocampus had significantly suppressed mean burying durations compared to rats that received SAL (n = 4). * p < .05 compared to rats infused with SAL.
Table 2. Mean (± SEM) of potentially confounding variables in the SPBT in rats infused with either SAL or NPY in the ventral hippocampus.

| Behaviour               | SAL  
|-------------------------|------|
|                         | (n = 10) | NPY  
|                         | (n = 12) |
| Immobility              | 10.45 ± 6.54 | 13.20 ± 4.95 |
| Total Shocks            | 3.00 ± 0.84  | 2.29 ± 0.64  |
| Mean Shock Reactivity   | 2.08 ± 0.17  | 2.16 ± 0.13  |

Note: Rats received bilateral infusions of either saline or NPY (1.5 µg/side) into or outside of the ventral hippocampus. Immobility, total shocks, and mean shock reactivity are measured as time in seconds, count, and score between 1 and 4, respectively.
3.2 Experiment 2: Co-infusions of the Y₁ receptor antagonist into the ventral hippocampus

The cannulae placements for the 48 rats used in Experiment 2 can be found in Figure 5. Nine rats’ data were excluded from analysis due to cannula placements outside of the ventral hippocampus, resulting in a final $N = 39$.

3.2.1 Elevated Plus-Maze

An additional four rats’ data were excluded due to problems during the infusions (e.g., no bubble movement during the infusion and/or fluid efflux from the proximal tip of a cannula guide). The following group $n$’s were obtained: $n_{SAL/SAL} = 9$, $n_{SAL/NPY} = 10$, $n_{BIBO3304/SAL} = 8$, and $n_{BIBO3304/NPY} = 8$. A one-way ANOVA did not find any significant between-groups differences on any of the behaviours measured in the test: percentage of open-arm entries, $F(3,31) = .500$, $p = .69$, $\eta_{partial}^2 = .05$ (See Fig. 6); percentage of open-arm time, $F(3,31) = .01$, $p = .99$, $\eta_{partial}^2 < .01$; closed arm entries, $F(3,31) = .064$, $p = .979$, $\eta_{partial}^2 = .01$; and total number of entries made, $F(3,31) = .20$, $p = .90$, $\eta_{partial}^2 = .02$ (Table 4).

3.2.2 Shock Probe Burying Test

One rat failed to contact the shock-probe, yielding the following group $n$’s: $n_{SAL/SAL} = 9$, $n_{SAL/NPY} = 9$, $n_{BIBO3304/SAL} = 11$, and $n_{BIBO3304/NPY} = 9$. A one-way ANOVA was conducted using infusion group as the independent variable and burying duration as the dependent variable, and found a significant effect for infusion groups, $F(3,34) = 3.00$, $p = .044$, $\eta_{partial}^2 = .21$ (See Fig. 7). Post-hoc analyses revealed significantly reduced burying in rats in the SAL/NPY group compared to the SAL/SAL ($p = .024$), BIBO 3304/SAL ($p = .013$), and BIBO 3304/ NPY ($p = .022$) groups, respectively (Figure 7). The latter three groups did not differ from each other. No other significant comparisons were found for the total number of shocks received, $F(3,34) =$
.691, $p = .564$, $\eta_{partial}^2 = .06$; duration of immobility, $F(3,34) = .958$, $p = .424$, $\eta_{partial}^2 = .08$; and mean shock reactivity, $F(3,34) = .846$, $p = .479$, $\eta_{partial}^2 = .07$ (Table 5).
Figure 4. Placements of the infusion tip in the ventral hippocampus in Experiment 2. Infusions of SAL/SAL are indicated by unfilled circles, SAL/NPY by filled circles, BIBO 3304/SAL by unfilled triangles, and BIBO 3304/NPY with filled triangles. Atlas plates were adapted from Paxinos & Watson, 1998.
Figure 5. Mean ± SEM of percentages of open-arm entries and time spent in the open arm using bilateral infusions of SAL/SAL (n = 9), SAL/NPY (1.5 µg/side, n = 10), BIBO 3304/SAL (0.15 µg/side, n = 8) and BIBO 3304/NPY (n = 8) into the ventral hippocampus.
Figure 6. Mean (± SEM) durations of burying in rats bilaterally infused with SAL/SAL (n = 9), SAL/NPY (1.5 µg; n = 9), BIBO 3304/SAL (0.15 µg; n = 11), or BIBO 3304/NPY (n = 9). The duration of burying was significantly suppressed compared to all other groups in rats infused with SAL/NPY bilaterally into the ventral hippocampus. *p < .05 relative to treatment groups other than SAL/NPY.
Table 3. Mean (± SEM) of measures of locomotor activity in the EPM in Experiment 2.

<table>
<thead>
<tr>
<th>Infusions into the Ventral Hippocampus</th>
<th>SAL/SAL (n = 9)</th>
<th>SAL/NPY (n = 10)</th>
<th>BIBO 3304/SAL (n = 8)</th>
<th>BIBO 3304/NPY (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed Arm Entries</td>
<td>12.22 ± 0.78</td>
<td>12.10 ± 0.96</td>
<td>12.13 ± 1.08</td>
<td>12.63 ± 0.92</td>
</tr>
<tr>
<td>Total Arm Entries</td>
<td>13.44 ± 1.11</td>
<td>13.70 ± 0.83</td>
<td>14.75 ± 1.79</td>
<td>13.88 ± 1.25</td>
</tr>
</tbody>
</table>

Note: Rats received bilateral infusions of SAL/SAL, SAL/NPY (1.5 µg/side), BIBO 3304/SAL (0.15 µg/side) or BIBO 3304/NPY into the ventral hippocampus prior to testing in the EPM. Closed and total arm entries represent the mean number of times rats entered a closed arm and the total number of entries made into either the closed or open arms, respectively.

Table 4. Mean (± SEM) of potentially confounding variables in the SPBT in Experiment 2.

<table>
<thead>
<tr>
<th>Infusions into the Ventral Hippocampus in the SPBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behaviour</td>
</tr>
<tr>
<td>Immobility</td>
</tr>
<tr>
<td>Total Shocks</td>
</tr>
<tr>
<td>Mean Shock Reactivity</td>
</tr>
</tbody>
</table>

Note: Rats either received bilateral infusions of SAL/SAL, SAL/NPY (1.5 µg/side), BIBO 3304/SAL (0.15 µg/side) or BIBO 3304/NPY into the ventral hippocampus prior to testing in the SPBT. Immobility, total shocks, and mean shock reactivity are measured as time in seconds, count, and score between 1 and 4, respectively.
3.3 Experiment 3: Co-infusions of the Y$_2$ receptor antagonist into the ventral hippocampus

The cannulae placements for the 42 rats used in Experiment 2 can be found in Figure 8. Eleven rats’ data were excluded from analysis due to cannula placements outside of the ventral hippocampus, resulting in a final N = 31.

3.3.1 Elevated-Plus Maze

The following group n’s were obtained following the exclusion of rats with misplaced cannula placements: $n_{\text{SAL/SAL}} = 8$, $n_{\text{SAL/NPY}} = 8$, $n_{\text{BIBO3304/SAL}} = 7$, and $n_{\text{BIBO3304/NPY}} = 8$. A one-way ANOVA did not find any significant between-groups differences on any of the behaviours measured in the test: percentage of open-arm entries, $F(3, 27) = 1.927$, $p = .15$, $\eta_{\text{partial}}^2 = .18$ (See Fig. 9); percentage of open-arm time, $F(3,27) = .77$, $p = .52$, $\eta_{\text{partial}}^2 < .08$; closed arm entries, $F(3, 27) = .25$, $p = .86$, $\eta_{\text{partial}}^2 = .03$; and total number of entries made, $F(3, 27) = .76$, $p = .53$, $\eta_{\text{partial}}^2 = .08$ (Table 7).

3.3.2 Shock Probe Burying Test

The group n’s remained the same as the EPM: $n_{\text{SAL/SAL}} = 8$, $n_{\text{SAL/NPY}} = 8$, $n_{\text{BIBO3304/SAL}} = 7$, and $n_{\text{BIBO3304/NPY}} = 8$. A one-way ANOVA was conducted using infusion group as the independent variable and burying duration as the dependent variable, and found a significant effect for infusion groups, $F(3,27) = 6.38$, $p = .002$, $\eta_{\text{partial}}^2 = .42$ (See Fig. 10). Post-hoc analyses revealed significantly reduced burying in rats in the SAL/NPY group compared to the SAL/SAL ($p = .002$) and BIIE 0246/SAL ($p = .003$), but did not differ from the BIIE 0246/NPY group ($p = .556$; see Figure 10). In addition, the BIIE 0246/NPY group exhibited significantly suppressed burying compared to the SAL/SAL ($p = .008$) and BIIE 0246/SAL ($p = .013$) groups. No other significant comparisons were found for the total number of shocks received, $F(3, 27) = .73$, $p =
.542, $\eta_{\text{partial}}^2 = .08$; duration of immobility, $F(3, 27) = 2.13, p = .119, \eta_{\text{partial}}^2 = .19$; and mean shock reactivity, $F(3, 27) = .126, p = .944, \eta_{\text{partial}}^2 = .01$ (Table 8).
Figure 7. Placements of the infusion tip in the ventral hippocampus in Experiment 3. Infusions of SAL/SAL are indicated by unfilled circles, SAL/NPY by filled circles, BIIE 0246/SAL by triangles, and BIIE 0246/NPY with squares. Atlas plates were adapted from Paxinos & Watson, 1998.
Figure 8. Mean ± SEM of percentages of open-arm entries and time spent in the open arm using bilateral infusions of SAL/SAL (n = 8), SAL/NPY (1.5 µg/side, n = 8), BIIE 0246/SAL (0.5 µg/side, n = 7) and BIIE 0246/NPY (n = 8) into the ventral hippocampus.
Figure 9. Mean (±SEM) durations of burying in rats bilaterally infused with SAL/SAL (n = 8), SAL/NPY (1.5 µg; n = 8), BIBO 3304/SAL (0.15 µg; n = 7), or BIIE 3304/NPY (n = 8). The duration of burying was significantly suppressed compared to all other groups in rats infused with SAL/NPY and BIIE 0246/SAL bilaterally into the ventral hippocampus compared to rats that received SAL/SAL or BIIE 0246/SAL. *p < .05 relative to treatment groups other than SAL/NPY.
Table 5. Mean (± SEM) of measures of locomotor activity in the EPM in Experiment 3.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Infusions into the Ventral Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAL/SAL ( (n = 8) )</td>
</tr>
<tr>
<td>Closed Arm Entries</td>
<td>11.00 ± 0.85</td>
</tr>
<tr>
<td>Total Arm Entries</td>
<td>12.50 ± 0.83</td>
</tr>
</tbody>
</table>

Note: Rats received bilateral infusions of SAL/SAL, SAL/NPY (1.5 µg/side), BIBO 3304/SAL (0.15 µg/side) or BIBO 3304/NPY into the ventral hippocampus prior to testing in the EPM. Closed and total arm entries represent the mean number of times rats entered a closed arm and the total number of entries made into either the closed or open arms, respectively.

Table 6. Mean (± SEM) of potentially confounding variables in the SPBT in Experiment 3.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Infusions into the Ventral Hippocampus in the SPBT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infusions into the Ventral Hippocampus</td>
</tr>
<tr>
<td></td>
<td>SAL/SAL ( (n = 8) )</td>
</tr>
<tr>
<td>Immobility</td>
<td>3.78 ± 1.39</td>
</tr>
<tr>
<td>Total Shocks</td>
<td>2.88 ± 0.52</td>
</tr>
<tr>
<td>Mean Shock Reactivity</td>
<td>1.59 ± 0.31</td>
</tr>
</tbody>
</table>

Note: Rats either received bilateral infusions of SAL/SAL, SAL/NPY (1.5 µg/side), BIBO 3304/SAL (0.15 µg/side) or BIBO 3304/NPY into the ventral hippocampus prior to testing in the SPBT. Immobility, total shocks, and mean shock reactivity are measured as time in seconds, count, and score between 1 and 4, respectively.
Chapter 4: Discussion

4.1 Summary of Main Findings

In Experiment 1, I tested the hypothesis that infusions of NPY into the ventral hippocampus would have anxiolytic-like effects in the EPM and the SPBT. Although an increase in open arm entries or time spent in the open arms was not observed in NPY-treated rats, treatments of NPY did significantly reduce the duration of time rats spent burying the electrified probe in the SPBT. This pattern of results was replicated in Experiment 2. I again observed null results in the EPM and robust NPY-induced decreases in shock-probe burying in the NPY-treated rats relative to saline-treated rats. The anxiolytic-like effects of NPY on defensive burying were completely reversed by pre-infusions of the Y₁ receptor antagonist, BIBO 3304, which did not affect burying and other measured behaviours when administered alone. Similar results were observed in Experiment 3, in that no effects were observed in the EPM and NPY alone replicated the suppression of defensive burying. However, the pre-infusion of the Y₂ receptor antagonist, BIIE 0246, did not alter the effects of NPY on the duration of defensive burying. BIIE 0246 on its own also did not affect any of the measured behaviours in the EPM or SPBT. These are the first results to demonstrate the anxiolytic-like effect of NPY in the ventral hippocampus on burying behaviour, and further that this effect is associated with Y₁, but not the Y₂, receptors at that site.

4.1.1 Selective suppression of defensive burying

Importantly, intra-ventral hippocampal NPY-induced reductions in burying were behaviourally specific, in that there were no apparent between-group differences in potential confounding variables across all three experiments. If intra-hippocampal NPY produced changes in pain sensitivity, this could alter levels of burying behavior. This possibility seems unlikely
because there were no between-group differences in either mean shock-reactivity scores or the total number of contact-induced probe shocks received. The absence of between-group differences in the number of probe shocks further suggests that local application of NPY into the ventral hippocampus did not alter the rats’ ability to associate the shock with the electrified probe (i.e., NPY-treated rats displayed normal levels of shock-probe avoidance). Finally, there was no evidence that the drug had sedative actions and/or locomotor effects, as indexed by the absence of group differences in the duration of immobility in the SPBT, the total number of entries and the number of closed arm entries made in the EPM.

4.1.2 Neuroanatomical specificity

The neuroanatomical specificity of my findings were addressed by analyzing the data of rats with cannula guides placed outside of the ventral hippocampus. This analysis was performed to ensure that extra-hippocampal NPY did not affect anxiety-like behaviours in either of the tests used. I did not find an anxiolytic-like effect of extra-hippocampal NPY in either the EPM or the SPBT in Experiment 1, suggesting that the anxiolytic-like effect of NPY may limited to its actions in the ventral hippocampus. However, it is important to note that of one of the two groups was very small ($n = 3$). It was not possible to conduct a meaningful statistical analysis of this type for data from rats with misplaced cannulae in Experiments 2 and 3. This is because the group $n$’s from those experiments were very small (i.e., $n = 1-4$). Nonetheless, all animals that received NPY infusions outside of the hippocampus displayed levels of open-arm exploration and burying durations within the range of saline-treated controls (i.e., open arm activity percentages between 0% and 15%, duration of burying between 80-340 seconds).
4.2 Test-specific effects of NPY infusions into the ventral Hippocampus

My findings in the SPBT (but not the EPM) are in line with a vast literature implicating the ventral hippocampus in anxiety regulation. This dissociation was unexpected, as prior studies showed that either permanent and temporary lesions of the ventral hippocampal reduce rats’ anxiety-like behaviours across a wide range of tests, including the EPM and SPBT (Degroot & Treit, 2002). Infusing the GABA₄ agonist, muscimol into the ventral hippocampus increased rats’ open-arm exploration in the EPM (Trent & Menard, 2010) and suppressed burying behaviour in the shock-probe test (McEown & Treit, 2010), as did local stimulation of the α₂ GABA₄ receptor subunits in the ventral hippocampus (McEown & Treit, 2013). Thus, it seems clear that the ventral hippocampus contributes to the expression of anxiety-related behaviours in both tests. Furthermore, open-arm avoidance is sensitive to the anxiolytic-like actions of NPY given i.c.v. (Bacchi et al., 2006; Britton, Akwa, Spina, & Koob, 2000; Karlsson, Holmes, Heilig, & Crawley, 2005; Nakajima et al., 1998; Sørensen et al., 2004) or locally administered into other regions of the brain, including the dorsal hippocampus (Smiałowska et al., 2007), locus coeruleus (Kask, Rägo, & Harro, 1998), periaqueductal gray (Kask et al., 1999) and the lateral septum (Trent & Menard, 2013).

It remains unclear why I observed effects in the SPBT but not in the EPM. Interestingly, most of the cannulae tips in the current study were situated in area CA1 of the ventral hippocampus (as reflected in Figures 1, 5, and 8). The ventral hippocampal CA1 sub-field is ultimately connected with the anterior hypothalamus, whereas CA3 ultimately sends information to the lateral hypothalamus (Risold & Swanson, 1997). These different projection paths might serve different roles in anxiety regulation. In support, infusions of muscimol into the anterior hypothalamus suppressed burying without altering open-arm avoidance, while infusions of
muscimol into the lateral hypothalamus altered open-arm avoidance without affecting burying (Hakvoort, Schwerdtfeger & Menard, 2008). Thus, it might be that the test specific effects I observed reflect perturbations of ventral hippocampal CA1 (but not CA3) and subsequent downstream changes in the anterior (but not lateral) hypothalamus. Further work is needed to confirm or refute this possibility.

4.3 Y1 and Y2 receptors’ contributions to anxiety regulation in the ventral hippocampus

The current findings are in line with previous investigations of NPY and the Y1 receptor’s contributions to anxiety regulation. The Y1 receptor has been extensively implicated anxiolytic-like actions i.c.v. in addition to several structures in the brain. For example, i.c.v. infusions of a receptor-specific agonist for the Y1 receptor, [D-His26]NPY, resulted in an anxiolytic-like response in both the elevated plus-maze and open field tests (Sørensen, Lindberg, Wörtwein, Bolwig, & Woldbye, 2004). Furthermore, infusions of the Y1 receptor agonist (i.e., p[Leu31,Pro34]NPY) resulted in an anxiolytic-like effect comparable to native NPY (Heilig et al., 1993b). In another study, infusions of NPY into the basolateral nucleus of the amygdala resulted in an increased duration of social interaction in rats, which was suppressed by the Y1 receptor antagonist, BIBO 3304 (Sajdyk, Vandergriff, & Gehlert, 1999). Similarly, BIBO 3304 attenuated the anxiolytic-like effect of NPY when infused into the lateral septum (Trent & Menard, 2011). Thus, the anxiolytic-like effect that I observed in the SPBT following the infusion of NPY, and the attenuation of this effect with a pre-infusion of BIBO 3304, adds to a wide base of evidence regarding the contributions of NPY and the Y1 receptor in the regulation of anxiety-like behaviours.

On the other hand, the current study suggests that the Y2 receptor does not contribute to the regulation of anxiety-like behaviours in the ventral hippocampus. This was unexpected, as
the Y2 receptors are found in greater densities in area CA1 of the ventral hippocampus, where most of my cannulae tips landed, compared to the Y1 receptor (Parker & Herzog, 1999). Furthermore, the Y2 receptor has been strongly implicated in anxiety regulation (e.g., Bacchi et al., 2006; Nakajima et al., 1998; Tasan et al., 2010). A possible limitation leading to the null findings regarding the Y2 receptor may have been the dosage of BIIE 0246 used. Due to the paucity of investigations of the ventral hippocampal Y2 receptors, it was difficult to determine a precise, appropriate dose of the Y2 receptor antagonist to infuse for the ventral hippocampus. The dosage used in Experiment 3 was based on a prior investigation from the Menard lab that used infusions of 0.5 µg/side of BIIE 0246 to the lateral septum (Trent & Menard, 2013). This dosage of the Y2 receptor antagonist was sufficient to block the anxiolytic-like effects of NPY in at least one of the indices of anxiety used. It is possible that a higher dose of the Y2 receptor antagonist may have been required to sufficiently block Y2 receptor action to observe any effects in the behavioural tests.

4.4 Limitations and Future Directions

Future research should investigate the neuroanatomical subdivisions of the ventral hippocampus to address the issue of test-specificity of infusions of NPY that I observed. Earlier, I noted that projections from the CA1 and CA3 subfields of the ventral hippocampus have different ultimate destinations (i.e., the anterior hypothalamus and lateral hypothalamus, respectively). Perturbations in each of these hypothalamic areas, using muscimol, resulted in a dissociation between shock-probe burying behaviour and open-arm avoidance. Because most of my cannula guides were aimed at the CA1 subfield, it is not possible to determine whether the test-specific effect I observed (i.e., selective decrease in burying in the SPBT but null effects in the EPM) is a ventral hippocampus-wide effect or one limited to CA1. An investigation using
precise microinfusions to the CA1 and CA3 subfields might shed light on whether the different neuroanatomical connections between these two areas in the ventral hippocampus indeed contribute to the test-specific effect I observed. Using specific Y₁ and Y₂ agonists and antagonists may provide novel findings regarding receptor specificity of these areas, in addition to neuroanatomical specificity. While both the CA1 and CA3 contain Y₁ and Y₂ receptors, it is possible that these receptors are associated with different effects in each subfields as was found for the Y₁ and Y₂ receptors in CA1 and the dentate gyrus of the dorsal hippocampus (Smiałowska et al., 2007).

Another issue to address is the appropriate dose of receptor antagonists. It is possible that the dose of the Y₂ receptor antagonist used was not large enough for a relatively large structure like the ventral hippocampus. Due to the high density of Y₂ receptors in the ventral hippocampus and its strong ties to anxiety-related behaviours, it may be worthwhile investigating multiple dosages of the Y₂ receptor antagonist. Furthermore, the use of an Y₂ receptor-specific agonist such as NPY₁₃-₃₆ may clarify further whether the Y₂ receptor has a role in anxiety regulation in the ventral hippocampus. It is also important to consider the variety of ethological models of anxiety that are used to test the effects of NPY in these ventral hippocampal subfields. I only used two ethological rat models of anxiety in the current investigation, which only represents a fraction of extant assessments for anxiety-like behaviours. Tasks such as the novelty-induced suppression of feeding are useful in assessing both appetitive and anxiety-related behaviours by assessing latency to approach novel and familiar foods (Merali, Levac, & Anisman, 2003).

4.5 Conclusion

In summary, I found that infusions of NPY into the ventral hippocampus reduced anxiety-like behavior in a test-specific fashion. Specifically, infusions of NPY resulted in a
selective anxiolytic-like effect in the SPBT. I did not observe a similar anxiolytic-like action in the EPM. The test-specific anxiety reduction may be associated with specific ventral hippocampal sub-regions. The anxiolytic-like effect of NPY in the burying test was blocked by pre-infusions of the Y₁ receptor antagonist, BIBO 3304, but not by the Y₂ receptor antagonist, BIIE 0246, suggesting that the SPBT-related anxiolytic-like actions are associated with the Y₁ receptor. These findings should be further explored using other animal models of anxiety and sub-field specific infusions of NPY.
Chapter 5: References


Appendix: Collapsed data between undergraduate and Master’s Theses

Experiment 1: Analysis of EPM behaviours

Of the 41 rats’ data from Experiment 1 and my undergraduate thesis data, 11 rats had infusion tips placed outside the ventral hippocampus resulting in a final $N = 30$. Following the exclusion of missed cannula placements, the following $n$’s were obtained: $n_{SAL} = 17$, $n_{NPY} = 13$. As seen in Fig. 10A, infusions of NPY did not significantly affect the percentage of open-arm entries, $F(1,28) = .282, p = .60, \eta_{partial}^2 = .01$ or the percentage of time spent in the open arm, $F(1,28) = .559, p = .46, \eta_{partial}^2 = .01$. NPY did not induce any changes in global locomotor activity as evidenced by the lack of significant differences either on the number of closed arm entries in NPY-infused rats, $F(1,28) = .483, p = .49, \eta_{partial}^2 = .01$, or total entries made, $F(1,28) = 1.39, p = .25, \eta_{partial}^2 = .05$ (Table 7).

I also analyzed the data from rats with at least one cannula tip outside of the ventral hippocampus: $n_{SAL} = 3$, $n_{NPY} = 8$. As seen in Fig. 10B, infusions of NPY outside of the ventral hippocampus did not affect open arm activity, indicated by the percentage of open-arm entries, $F(1,9) = 1.679, p = .23, \eta_{partial}^2 = .16$, or the percentage of time spent in the open arm, $F(1,9) = 1.333, p = .28, \eta_{partial}^2 = .13$. No changes in global locomotor activity was observed evidenced by closed-arm entries, $F(1,9) < .001, p = .99, \eta_{partial}^2 < .01$ and the total entries made, $F(1,9) = .43, p = .53, \eta_{partial}^2 = .05$, between the two groups (Table 7).
Figure 10. Mean ± SEM of percentages of open-arm entries and time spent in the open arm using bilateral infusions of SAL ($n = 17$) and NPY (1.5µg/side, $n = 13$) into the ventral hippocampus (A), and either unilateral or bilateral infusions of SAL ($n = 3$) and NPY ($n = 8$) outside of the ventral hippocampus (B).
Table 7. Mean (± SEM) plus-maze locomotive behaviours in rats infused with either SAL or NPY into and outside of the ventral hippocampus.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Infusions into the Ventral Hippocampus</th>
<th>Infusions outside the Ventral Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAL (n = 17)</td>
<td>NPY (n = 13)</td>
</tr>
<tr>
<td>Closed Arm Entries</td>
<td>11.76 ± 0.61</td>
<td>11.08 ± 0.80</td>
</tr>
<tr>
<td>Total Arm Entries</td>
<td>15.29 ± 0.92</td>
<td>13.69 ± 0.98</td>
</tr>
</tbody>
</table>

Note: Rats received bilateral infusions of either saline or NPY (1.5 µg/side) into or outside of the ventral hippocampus.

Experiment 1: Analysis of SPBT behaviours

Due to video software failure during data collection in my undergraduate thesis, data from 8 rats’ were lost and could not be included in the analysis. When combining the burying data from my undergraduate thesis with the current data, the following n’s were obtained for rats with correct cannula tip placements: n_{SAL} = 10 and n_{NPY} = 12. As can be seen in Fig. 11A, infusions of NPY into the ventral hippocampus significantly reduced burying duration, $F(1,20) = 26.6, p < .001, \eta^2_{partial} = .57$. Importantly, this reduction in burying was not accompanied by significant differences in the duration of time spent immobile, $F(1,20) = .103, p = .75, \eta^2_{partial} = .05$, the number of shocks received by the rat, $F(1,20) = 2.25, p = .15, \eta^2_{partial} = .10$, and the mean shock reactivity, $F(1,20) = .03, p = .86, \eta^2_{partial} < .01$ (Table 8). These results indicate that the
reduction in burying occurred independently of drug-induced changes in locomotor activity, associative memory, or pain sensitivity, respectively.

Data from 8 rats with at least one cannula tip located outside of the ventral hippocampus were analyzed as neuroanatomical controls ($n_{SAL} = 5$ and $n_{NPY} = 3$). Burying duration was not affected by infusions of NPY outside the ventral hippocampus, $F(1,6) = .28, p = .62, \eta_{partial}^2 = .04$ (Fig. 11B). In addition, no effects were observed for infusions of NPY of outside the ventral hippocampus in immobility, $F(1,6) = .953, p = .37, \eta_{partial}^2 = .14$, total shocks received, $F(1,6) = .029, p = .871, \eta_{partial}^2 = .01$, and mean shock reactivity, $F(1,6) = .211, p = .66, \eta_{partial}^2 = .03$ (Table 8).

To determine whether the effects of NPY on burying were influenced by the animals’ prior treatment history, I re-grouped the rats according to the treatments they received prior to the EPM and SPBT, respectively. This yielded the following group compositions: SAL/SAL ($n = 5$), NPY/SAL ($n = 5$), SAL/NPY ($n = 6$) and NPY/NPY ($n = 5$). There was a significant treatment effect on the duration of burying, $F(3,21) = 10.75, p < .001, \eta_{partial}^2 = .64$. Pairwise comparisons (LSD test) revealed that the SAL/NPY and NPY/NPY groups did not differ from each other, and both of those groups spent significantly less time burying compared to either the SAL/SAL or the NPY/SAL groups (Fig. 12). The latter 2 groups did not differ. No significant differences were found in any other recorded behaviours (i.e., immobility, total shocks, and mean shock reactivity; Table 9).
Figure 11. Mean (± SEM) durations of burying for rats in Experiment 1. Rats that received infusions of NPY (1.5 µg/side; $n = 12$) into the ventral hippocampus (A) had significantly suppressed mean burying durations compared to rats that received SAL ($n = 10$). The duration of burying did not differ between rats that received SAL ($n = 5$) or NPY ($n = 3$) infusions outside of the ventral hippocampus (B). * $p < .001$ compared to rats infused with SAL.
Table 8. Mean (± SEM) of potentially confounding variables in the SPBT in rats infused with either SAL or NPY in or outside the ventral hippocampus.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Infusions into the Ventral Hippocampus</th>
<th>Infusions outside the Ventral Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAL (n = 10)</td>
<td>NPY (n = 12)</td>
</tr>
<tr>
<td></td>
<td>SAL (n = 5)</td>
<td>NPY (n = 3)</td>
</tr>
<tr>
<td>Immobility</td>
<td>36.60 ± 20.54</td>
<td>45.41 ± 18.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.41 ± 9.83</td>
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<td></td>
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<td>11.51 ± 7.60</td>
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<tr>
<td>Total Shocks</td>
<td>3.30 ± 0.42</td>
<td>2.50 ± 0.34</td>
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<tr>
<td></td>
<td>3.60 ± 1.17</td>
<td>3.33 ± 0.33</td>
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<tr>
<td>Mean Shock Reactivity</td>
<td>1.89 ± 0.19</td>
<td>1.94 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>2.23 ± 0.35</td>
<td>2.50 ± 0.50</td>
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</tbody>
</table>

Note: Rats received bilateral infusions of either saline or NPY (1.5 µg/side) into or outside of the ventral hippocampus. Immobility, total shocks, and mean shock reactivity are measured as time in seconds, count, and score between 1 and 4, respectively.

Table 9. Mean (± SEM) of potentially confounding variables in the SPBT in rats that received SAL in both the EPM and SPBT, SAL in the EPM and NPY in the SPBT, NPY in the EPM and SAL in the SPBT, or NPY in both tests.

<table>
<thead>
<tr>
<th>Infusions into the Ventral Hippocampus in the EPM / SPBT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Behaviour</td>
</tr>
<tr>
<td>Immobility</td>
</tr>
<tr>
<td>Total Shocks</td>
</tr>
<tr>
<td>Mean Shock Reactivity</td>
</tr>
</tbody>
</table>
Note: Rats received either bilateral SAL or NPY (1.5 µg/side) prior to testing in the EPM, and either SAL or NPY prior to the SPBT.

**Figure 12.** Mean (± SEM) durations of burying for rats that received SAL just prior to testing in the EPM and SAL, in the following week, just prior to testing in the SPBT ($n = 5$), SAL then NPY (1.5 µg/side; $n = 6$), NPY then SAL ($n = 5$), and NPY prior to both the EPM and the SPBT ($n = 6$). Rats that received NPY prior to the SPBT buried significantly less than rats that received SAL, regardless of infusion history in the EPM. *$p < .005$. 