The Role of Oxytocin in the Stress and Anxiety Response

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Centrally released oxytocin (OT) is believed to attenuate stress-induced activation of the hypothalamic pituitary adrenal (HPA) axis as well as being anxiolytic. Therefore, it is expected that OT deficient (OT-/-) mice that do not synthesize or release OT centrally or peripherally will display enhanced HPA axis activation, as well as increased anxiety-related behavior compared to wildtype (OT+/+) mice. To test this hypothesis, OT-/- mice were exposed to shaker stress, (psychogenic stressor), cholecystokinin- (CCK) administration (physical stressor), or the elevated plus maze (EPM), a behavioral test of anxiety.

Female OT-/- mice released more corticosterone than OT+/+ mice in response to shaker stress. Shaker stress exposure activated Fos in OT neurons of the paraventricular nucleus of the hypothalamus (PVN) of male and female OT+/+ mice and corticotropin-releasing hormone (CRH) within the PVN of male and female mice of both genotypes. In addition, shaker stress exposure revealed that Fos expression in the medial nucleus of the amygdala (MeA) was lower in female OT-/- than OT+/+ mice. Genotypic differences in corticosterone release and Fos activation of the MeA in response to shaker stress exposure were not observed in male mice. Furthermore, similar genotypic (and/or sex) differences were not revealed in response to CCK-administration.

OT is also anxiolytic in female mice. Female OT-/- mice tested in the EPM displayed increased anxiety-related behavior compared to OT+/+ mice. In response to EPM exposure Fos expression in the MeA was greater in female OT-/- mice than OT+/+ mice. Surprisingly, male OT-/- mice tested in the EPM displayed decreased anxiety-related behavior compared to OT+/+ mice, but did not display genotypic differences in the Fos expression within the MeA.

The results of this thesis suggest that OT is anxiolytic and attenuates HPA activation in female, but not male mice. Furthermore, it appears that OT plays a modulatory role in the processing of psychogenic stressors, but may not be involved in the processing of physical or systemic stressors. More specifically, it is possible that OT plays a role in behavioral and physiological responses that depend upon neuronal processing within the MeA.

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I. Overview

A. Introduction

A healthy physiological and behavioral response to acute stress is crucial for our ability to deal with everyday challenges, either systemic or psychological. The initial response to an acute stress is protective, enhancing immune function, promoting memory of dangerous events, increasing blood pressure and heart rate to meet the physical and emotional demands to react to the stressor. Failure to terminate the acute response to stress may contribute to greater vulnerability to illness. Overactivity of the stress system is believed to play an important role in the pathogenesis of certain chronic diseases, such as affective or panic disorders, anorexia, depression, coronary heart disease, and functional gastrointestinal disturbances [31].

An organism's adaptive response to stressful stimuli is mediated, in part, by the hypothalamic pituitary adrenal (HPA) axis. There are many neurotransmitters and neuropeptides that regulate HPA activation to stress. Oxytocin (OT) is a neuropeptide, which is synthesized within the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus and is released from the hypothalamo-neurohypophyseal system in response to a number of stressors. The significance of this increased peripheral release of OT is not known. However, OT is also widely distributed throughout the central nervous system [19] and many studies implicate an inhibitory role of central OT on HPA activation in response to stress and anxiety behavior.

Initial studies in humans have focused on the role of peripheral OT in female reproduction and lactation. However, OT is present in the brains and peripheral circulation of both males and females. The fact that OT is present in males and females may indicate a functional role of OT beyond that of female reproduction. Studies performed in human subjects suggest that elevated OT may attenuate the HPA axis stress response. For instance, adrenocorticotropin hormone (ACTH) and cortisol release are attenuated in lactating women versus non-lactating women following exercise

stress [1]. Furthermore, in response to psychosocial stress, overall blood pressure [118], ACTH and cortisol levels [71] were blunted in women following breast-feeding (when plasma OT levels are elevated) compared to women that did not breast-feed. However, these studies do not correlate OT expression with stress hormone levels and there are a variety of confounding factors during the peri-partum period, such as the release of other hormones (i.e. prolactin), making it difficult to ascribe the blunted stress hormone effect to OT alone.

Similar findings to those of humans have been reported in animal models of stress and anxiety. OT is released within the brain and into the circulation of male, as well as virgin female rats in response to stressors that contain a psychogenic component. These stressors include forced swimming in male [216,217] and female rats [209], various forms of social stress in male [48,50] and female rats [138], and shaker stress in male rats [142]. Infusion of synthetic OT into the lateral ventricles of estrogen-treated ovariectomized rats decreased the corticosterone response to psychogenic restraint stress [210] and noise stress [211]. Infusion of an OT receptor antagonist into the lateral ventricles augmented the basal and stress-induced release of andrenocorticotropin hormone (ACTH) and corticosterone in female rats forced to swim [140] and in male and female rats exposed to an elevated plus-maze (EPM) [140] or to repeated airpuffs [138]. In addition, OT is believed to be anxiolytic in female laboratory rats [11,140,211] and mice [129]. Central administration of OT to estrogen-primed ovariectomized rats [211] or mice [129] decreased anxiety-related behavior in the elevated plus-maze (EPM). OT infused into the amygdala of ovariectomized estrogen treated rats significantly increased open field activity (decreased anxiety) and increased the time spent in open arms of the EPM (anxiolytic effect) [11]. However, the anxiolytic effect of OT has not been reported in male rats or mice. These sex differences may be due to gonadal steroids. Estrogen has been reported to facilitate OT mRNA expression [150,185], OT receptor binding [221], and stress- and anxiety-related effects of OT [128,129]. Collectively the data suggest that OT

inhibits the response of the HPA axis to stress in male and female rodents, as well as produces anxiolysis in female rats and mice.

Although many studies conducted prior to the development of the OT deficient mouse suggest that exogenous administration of OT attenuates the stress and anxiety response, or that administration of an OT antagonist enhances the stress and anxiety response, it has not been possible to determine whether the absence of OT affects the stress and anxiety responses. The central hypothesis of this thesis is that the absence of OT will result in an enhanced corticosterone response to stress and/or anxiety. Therefore, OT deficient mice will display an increased response of the HPA axis to stress and greater anxiety-related behavior than wildtype mice. In addition, OT deficient mice will display differences in the degree of activation of stress- and anxiety-related brain pathways compared to wildtype mice.

B. Oxytocin

1. Gene and Peptide Structure

OT was first discovered in 1909 using posterior pituitary gland extracts [40]. Dale described the powerful role of OT in producing uterine contractility. In 1953 du Vigneaud described the structure of the OT peptide [44]. OT contains nine amino acids consisting of a six amino acid ring structure, created by a disulfide bridge between Cys residues in the 1 and 6 position, and a three amino acid side chain (Figure 1).

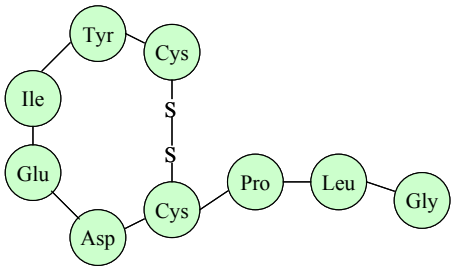

Figure 1. Oxytocin Peptide

The mouse OT gene, encoding the pre-propeptide, consists of two introns and three exons [67], similar to that observed in the human [176] and the rat [89]. The first exon encodes a translocator signal, the oxytocin peptide, the tripeptide processing signal (GKR), and the first nine residues of neurophysin; the second exon encodes the central part of neurophysin I; and the third exon encodes the C-terminal region of neurophysin I [67] (Figure 2).

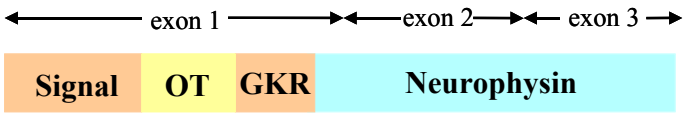

Figure 2. Structure of the Mouse Oxytocin Gene

The OT pre-prohormone is synthesized in the ribosomes of OT neurons, cleaved in the endoplasmic reticulum, and packaged into secretory granules in the golgi apparatus. The mature OT and neurophysin peptides are stored in the axon terminals until the neuron is activated to release the peptide. Neurophysin is responsible for the proper targeting, packaging and storage of OT into secretory granules prior to release. The dissociation of the OT-neurophysin complex is facilitated as the complex is released from the secretory granules and enters the plasma.

2. Location and Projection of Magnocellular and Parvocellular Oxytocin Neurons

OT is located and synthesized in the magnocellular and parvocellular neurons of the paraventricular nucleus (PVN) as well as the magnocellular neurons of the supraoptic nucleus (SON) of the hypothalamus. The majority of magnocellular OT neurons in the PVN and SON project to the posterior lobe of the pituitary and release OT into the peripheral circulation upon stimulation [159]. In the rat, OT has a half-life of 1-3 minutes [59,80,93] and basal concentrations are ~1-10nM in plasma [68]. OT released into the peripheral system targets multiple tissues such as the mammary gland [79], uterus [79], and kidneys [7] amongst other organs. OT is also locally synthesized in

many peripheral tissues, such as the heart [91], the ovary [88] and uterus [116] in the female reproductive system, and the testis and prostate of the male reproductive system [87].

Parvocellular OT efferents of the PVN project to extra-hypothalamic brain areas and release OT into the brain. OT is present in cerebrospinal fluid at concentrations between 10-50pM [68] and has a half-life of approximately 28 min [93]. OT projections target multiple areas of the brain, including the olfactory nucleus [19], the limbic system [19,188,189], and the brain stem [19,179,188,189] (Refer to Table 1). At this time, the OT projections of the mouse have not been well defined.

Brain Regions Containing OT Efferents

Olfactory system	
Anterior olfactory nucleus	[19]
Olfactory tubercle	
Piriform cortex	
Entorhinal/perirhinal area	
Basal ganglia	
Caudoputamen	
Ventral pallidum	
Limbic system	
Lateral septal nucleus	
Bed nucleus of the stria terminalis	[19,82]
Hippocampus	[19,188]
Central amygdala	[19,188,189]
Medial amygdala	[188,189]
Dorsal subiculum	
Ventral subiculum	
Thalamus and Hypothalamus	
Periventricular thalamic nucleus	
Ventromedial hypothalamic nucleus	
Medial preoptic area	
Supraoptic nucleus	
Paraventricular nucleus	
Supramammillary nucleus	[188,189]
Brain Stem	
Substantia nigra	[188,189]
Tegmental area	
Dorsal raphe nucleus	[19, 188,189]
Nucleus of the solitary tract	[179, 188,189
Dorsal motor nucleus of the vagus	[19, 179, 188,
Locus coeruleus	[19, 188,189]

Table 1. Oxytocin Efferents of the Paraventricular Nucleus of the Hypothalamus

The references cited in this table are the results of tracing studies performed in male rats. Similar studies in mice and female rats have not been published.

3. Oxytocin Receptor

The OT receptor gene sequence has been identified in numerous mammalian species, including the human [101], pig [62], rat [169], sheep [88], and mouse [108]. Similar to the human [83] and the rat [169], the mouse OT receptor gene contains 3 introns and 4 exons [108] (Figure 3). Exons 1 and 2 correspond to the 5'-prime noncoding region. Exon 3 encodes a portion of the 5'-prime noncoding region, the start codon (ATG), and the first 6 of the 7 transmembrane regions. Exon 4 contains the sequence encoding the seventh transmembrane domain, the stop codon (TGA), the COOH terminus, and the entire 3'-noncoding region [108].

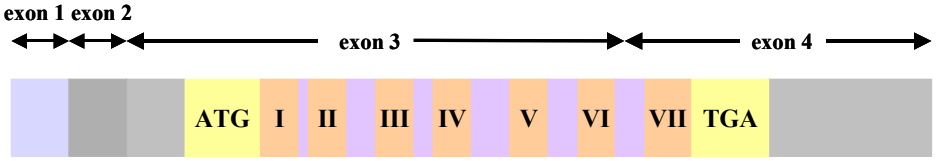

Figure 3. Structure of the Mouse Oxytocin Receptor Gene

OT receptor mRNA is expressed in various tissues of the rat including the uterus [111], mammary gland [136,190], heart [91], kidney [7], and the brain [146,195,219]. In addition, the OT receptor is also expressed in the mammary gland [63] and brain of the mouse [63,85,86]. Within the brain of the rat and mouse, expression of the OT receptor gene has been identified in many different areas. These areas include the olfactory system, cortex, basal ganglia, limbic system, thalamus, hypothalamus, and brain stem (refer to Table 2).

	Rat		Mouse	
Brain Regions	**Male**	**Female**	**Male**	**Female**
Olfactory system				
Olfactory bulb				[63+]
Anterior olfactory nucleus	[146*,195#]	[195#,219*]	[85#,86#]	[63+,85#]
Olfactory tubercle	[146*]	[219*]		
Piriform cortex		[219*]	[85#]	[63+,85#]
Entorhinal/perirhinal area			[86#]	[63+]
Basal ganglia				
Caudoputamen	[195#]	[195#,219*]	[85#]	[85#]
Ventral pallidum	[195#]	[195#,219*]		
Limbic system				
Lateral septal nucleus	[146*,195#]	[195#,219*]	[85#,86#]	[63+,85#]
Bed nucleus of the stria terminalis	[146*,195#]	[195#,219*]	[85#]	[85#]
Hippocampus	[146*,195#]	[195#,219*]	[85#,86#]	[85#]
Central amygdala	[195#]	[195#,219*]	[85#]	[85#]
Medial amygdala	[195#]	[195#,219*]	[86#]	
Dorsal subiculum	[195#]	[195#,219*]		
Ventral subiculum	[195#]	[195#,219*]		
Thalamus and Hypothalamus				
Periventricular thalamic nucleus	[195#]	[195#,219*]	[86#]	
Ventromedial hypothalamic nucleus	[146*,195#]	[195#,219*]	[86#]	[63+]
Medial preoptic area				[63+]
Supraoptic nucleus	[146*]	[219*]		
Paraventricular nucleus	[146*]	[219*]		
Supramammillary nucleus	[146*]	[195#,219*]		[63+]
Brain Stem				
Substantia nigra	[146*]	[219*]		
Tegmental area				[63+]
Dorsal raphe nucleus		[219*]		[63+]
Nucleus of the solitary tract				[63+]
Dorsal motor nucleus of the vagus	[146*,195#]	[195#,219*]		[63+]
Locus coeruleus	[146*]			

Table 2. Oxytocin Receptor Localization in the Rat and Mouse.

Numbers in the table refer to studies cited in the reference list. Empty spaces refer to brain areas that were not evaluated and/or did not display oxytocin receptors. References include studies that measured mRNA expression*, oxytocin receptor binding#, or oxytocin receptor gene expression using a Lac-Z reporter mouse+.

C. Stress

1. Definition and Classification of Stress

Stress is a concept that is difficult to define fully because its interpretation tends to vary according to individual disciplines. In 1936 Hans Selye, a pioneer in the physiological and pathophysiological principles in the exploration of stress, defined stress as "the nonspecific response of the body to any demand upon it, including bacterial infection, toxins, and various physical stimuli (i.e. surgery, exercise)" [183]. However, Selye's definition of stress as a "nonspecific" response of the body has been challenged. Although he did not define stress, Walter Cannon was the first to introduce the term "homeostasis" in reference to the stress response [22]. According to Cannon, homeostasis is the product of multiple physiological systems that maintain steady-state in an organism [22]. According to Cannon, a nonspecific stress response would not have provided an advantage in natural selection and would not have evolved [22]. The definition has been refined over time to describe stress as selective pressure from the physical and social environment that threaten or challenge an organism's homeostasis and elicit physiologically and behaviorally adaptive responses that are specific to the stressor [30,31,206].

Stressors can be defined as conditions that endanger, or are perceived to endanger, the well-being of an individual. The current literature broadly categorizes stressors as psychogenic (based on either a conditioned or an unconditioned response) or physical/systemic stimuli [73,77]. Psychogenic stressors affect emotional processes and may result in behavioral changes such as anxiety or fear. Systemic stressors include cold, heat, hypoglycemia, hemorrhage, pain, and chemical or noxious stimuli. The stressors described above are commonly used in animal research. However, many stressors are both physical and psychogenic. Exposure to stressors result in a series of coordinated responses composed of alterations in behavior, autonomic function and the secretion

of multiple hormones including adrenocorticotropin hormone (ACTH) and cortisol/corticosterone (discussed below).

2. Hypothalamic Pituitary Adrenal Axis

A key regulator of the stress response is the hypothalamic-pituitary-adrenal (HPA) axis (Figure 4). In response to stress, neural inputs from the central nervous system converge on the paraventricular nucleus (PVN) of the hypothalamus and signal for increased synthesis and release of corticotropin-releasing hormone (CRH) [207]. In turn, CRH increases synthesis and release of adrenocorticotropin (ACTH) from the anterior pituitary. Vasopressin (AVP), which is co-expressed in CRH neurons of the PVN, is also regulated in response to stress and acts synergistically with CRH to stimulate ACTH release [207]. Peripherally released ACTH stimulates synthesis of glucocorticoids. Glucocorticoids in turn negatively feedback to the pituitary and hypothalamus to reduce the synthesis and release of ACTH and CRH respectively, and also feed back at higher brain centers to modulate the neural inputs to the hypothalamus [207].

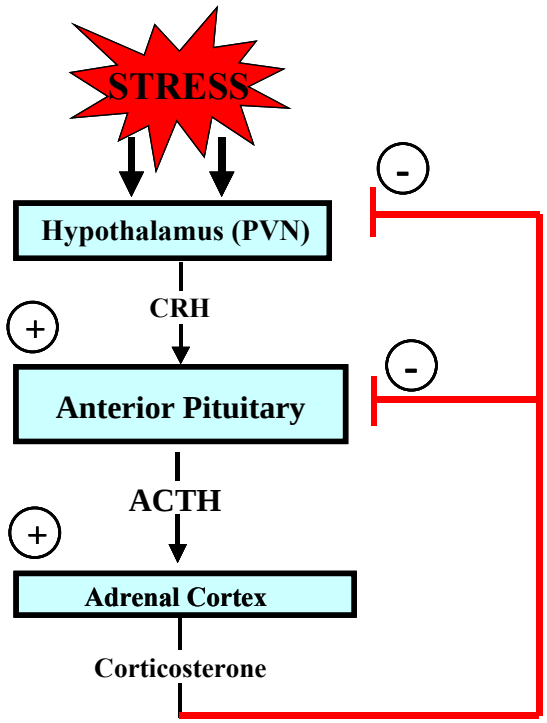

Figure 4. Schematic Representation of the Hypothalamic Pituitary Adrenal Axis

3. Neurocircuitry

Multiple brain structures are involved in the organization of responses to stressful stimuli. Among them are the hypothalamus, limbic brain areas (i.e. the lateral septum, the hippocampus, the amygdala), hindbrain regions (i.e. the nucleus of the tractus solitarius and the locus coeruleus), the parabrachial nucleus, and raphe nucleus. One of the most prevalent hypotheses regarding neurocircuit regulation of the HPA axis proposes the categorization of psychogenic (processive, exteroceptive, neurogenic) and physical (interoceptive, systemic) responses to stress. It has been theorized that the psychogenic class of stressors require forebrain or limbic processing and integration prior to HPA axis activation, while the physical class of stressors is dependent upon reflexive, direct hind-brain pathways to the PVN to activate the HPA axis. Stimuli that fall within the category of psychogenic stress include novelty and restraint, while physical stressors include hemorrhage, cold exposure, immune challenge and pain. Each stressor is believed to activate a

"signature" pathway that is unique to that particular stressor. While stressors that are classified as psychogenic tend to activate a combination of distinctly different neuronal pathways from stressors that are classified as physical, there is some overlap in the brain areas activated by each stressor.

a) Stress-Related Hindbrain Projections to the Paraventricular Nucleus of the Hypothalamus

Generically speaking, stressors that consist of a physical or systemic stimulus directly activate the PVN through hindbrain projections. Stimuli that demand immediate physiological responses have a direct unimpeded pathway to the PVN region, by way of the brainstem, eliciting an ACTH and corticosterone response. These hindbrain PVN-projecting neurons are positioned to evoke rapid, reflexive activation of the HPA axis, which is faster than psychogenic stress-induced activation.

Nucleus of the Solitary Tract

The nucleus of the solitary tract (NTS), which is located in the medulla, receives and integrates sensory information from most major organs of the body (Figure 5). Fos activation (discussed in the introduction of Chapter V) within the NTS increases following most stressors classified as physical, including administration of the nauseogenics cholecystokinin (CCK) [145] and lithium chloride [110], immune challenge by interleukin-1 [51,178] or lipopolysaccharide administration [109], hypoxia [193], hypovolemia [107], hypotension [107], and footshock [178]. The PVN receives its major catecholaminergic (i.e. noradrenergic and adrenergic) input from the NTS [38]. Fibers from this area innervate the medial parvocellular zone of the PVN [38]. The catecholaminergic input represents a major HPA excitatory pathway, promoting CRH [158] and ACTH [158,192] release and CRH gene transcription [157]. However, the NTS is also activated during psychogenic stressors such as restraint [36], swim [36], and fear conditioning induced by footshock [155]. The NTS also receives afferents from limbic forebrain circuits, including the

prefrontal cortex, central amygdala, and several hypothalamic nuclei [182,196]. These responses support the role of the NTS as a relay for sensory and reflexive information to the PVN and other forebrain structures.

The rat NTS contains OT axon terminals [179,188,189] and OT acts within the NTS to alter parasympathetic output in the rat. In addition to stress-induced Fos activation within the NTS, CCK [14], interleukin-1 [20,51], and lithium chloride [145] also activate Fos within OT neurons of the PVN. Furthermore, lesioning ascending NTS neurons to the PVN reduced stress-induced Fos activation of OT positive neurons [20,51]. Therefore, it appears that the NST drives activation of OT neurons within the PVN. In turn, OT efferents have been identified within the NTS and are capable of acting at the level of the NTS during the stress response [179,188,189].

Locus Coeruleus

Another source of noradrenergic input to the hypothalamus is the locus coeruleus (LC), a dorsal pontine structure that receives viscerosensory and somatosensory input via the spinal cord in response to systemic stimuli (Figure 5). The LC serves as an important integrator of the behavioral and physiological response to stress. While the LC has limited direct input to the PVN [38], it provides noradrenergic input to the prefrontal cortex, hippocampus, and amygdala, all of which influence HPA axis activation [2]. OT efferents [19,188,189] and receptors [195] are located in the LC and chronic OT treatment results in the suppression of the LC response to stress [154]. Exposure to restraint and swim stress increased Fos expression [27,36] and tyrosine hydroxylase (a catecholamine precursor) mRNA expression in the locus coeruleus [187]. Therefore, HPA activation may occur through multisynaptic pathways involving the locus coeruleus, and PVN projecting forebrain pathways.

Parabrachial Nucleus

The PVN also receives projections from the parabrachial nucleus of the pons (Figure 5). The parabrachial nucleus serves as a site for the relay of viscerosensory information from the NTS to the

PVN [106,175], bed nucleus of the stria terminalis [106,175], and amygdala [106,175]. The parabrachial nucleus relays information on cardiovascular tone and pain perception to the PVN, however the precise role of this structure in HPA integration is not known.

b) Stress-Related Forebrain Projections to the Paraventricular Nucleus of the Hypothalamus

Psychogenic stressors, which require active processing by the brain to consciously determine whether a stimulus is a threat, arrive at the PVN via a multisynaptic pathway. These forebrain structures, including regions such as the hippocampus, bed nucleus of the stria terminalis, amygdala, and septum, are critical for emotional responses and conditioned stress. Possible interaction of OT with the following brain areas are discussed in reference to the experiments performed in Chapter V.

Bed Nucleus of the Stria Terminalis

The bed nucleus of the stria terminalis (BNST) plays an integrative role in the regulation of the HPA axis response to stress by linking many forebrain regions such as the amygdala and hippocampus with the hypothalamic and brainstem regions (Figure 5). The BNST provides GABAergic input to the parvocellular PVN, suggesting an inhibitory action on PVN neurons [37]. However, the action of the BNST (inhibitory versus excitatory) is dependent upon the division of the BNST (anterior versus posterior) that is stimulated during the stress response. The BNST is divided into anterior and posterior components based on cyto- and chemoarchitecture [95,96]. The posterior BNST receives glutamatergic projections from the subiculum [37]. Lesions of the posterior BNST enhance expression of CRH mRNA [75], whereas stimulation of the subnuclei of the posterior division of the BNST reduces corticosterone release [46]. These data support an inhibitory action of the BNST on the HPA axis via excitation of inhibitory BNST projections to the parvocellular PVN. The anterior division of the BNST receives GABAergic projections from the amygdala and projects to the PVN as well as the brainstem [43]. Lesions of the anterior division of the BNST decrease

expression of CRH mRNA in the PVN [75] and stimulation of the anterior division of the BNST result in increased corticosterone secretion [46]. These data support an excitatory action of the posterior BNST on the HPA axis through disinhibition of inhibitory BNST projections to the parvocellular PVN. The BNST is a prime structure to integrate limbic information from inhibitory and/or excitatory sources into signals for HPA axis inhibition or activation.

Amygdala

The amygdala, which contains oxytocinergic terminals, is a limbic system structure that plays a part in the mediation of the neuroendocrine and autonomic responses to stress (Figure 5). The amygdala has been implicated in assigning emotional significance to sensory information. Specifically, the amygdala appears to be an essential component of circuitry underlying stress and fear-related responses. Hypothalamic and brainstem inputs to the amygdala arise from regions involved in behavior and autonomic systems [171]. Cortical and thalamic inputs, which are glutamatergic (excitatory), supply sensory information to the amygdala [147].

In the rat, amygdala nuclei are divided into three groups; the basolateral nuclei, the cortical nuclei, and the centromedial nuclei [130]. Although many subnuclei of the amygdala are implicated in HPA axis modulation, this review will only discuss the role of the centromedial nuclei, which include the BNST and the central and medial nuclei of the amygdala, in the modulation of HPA activation during stress (discussed in Chapter V in greater detail).

The medial nucleus of the amygdala (MeA) plays a role in HPA axis integration in response to numerous psychological stressors. Selective stimulation of the MeA increases corticosterone release in anesthetized rats [47] and may increase adrenal sensitivity to ACTH [172]. Furthermore, MeA Fos induction can be observed following stimuli that activate psychogenic pathways, including restraint [36,42], novelty [49] and fear conditioning through administration of footshock [156], but is less far pronounced in conjunction with physical responses to stimuli such as cytokine stimulation [177] or ether inhalation [49]. While the MeA has very few direct projections to the PVN, it has an

extensive network of GABAergic projections to PVN-projecting regions, including the anterior division of the BNST and the medial preoptic area, as well as other hypothalamic nuclei that project to the PVN [24]. The BNST, medial preoptic area, and PVN surrounding hypothalamic nuclei are predominantly GABAergic [37], suggesting that MeA-PVN relays are composed of sequential GABA projections. The stimulatory effect of the MeA on corticosterone release can be blocked by lesions of the BNST [52,53], supporting the importance of these relays in HPA integration. Therefore the MeA likely activates the PVN through disinhibition.

Unlike the MeA, the central amygdala (CeA) is implicated in the integration of the HPA axis to mostly physical stressors. This hypothesis is supported by Fos mapping data showing preferential induction of the CeA by stressors such as hemorrhage [194], cytokine infusions [177] and lithium chloride injection [110,218], while stimuli such as novelty, restraint [36], footshock [177] or air-puff startle [194] show minimal CeA Fos response. However, the CeA has little direct interaction with the PVN [65,125]. The CeA has connections with brainstem structures innervating the PVN, including the nucleus of the solitary tract, parabrachial nucleus, and the dorsal motor nucleus of the vagus [182,196]. In addition, there is evidence for forebrain relay projections to the posterior division of the BNST [43,161]. The BNST contains large populations of GABAergic neurons; in combination with the predominantly GABAergic phenotype of CeA projection neurons, the CeA-BNST-PVN circuit may utilize two GABA synapses, and thus activate the PVN by disinhibition.

Hippocampus

The hippocampus is involved in terminating the HPA axis responses to stress (Figure 5). Hippocampal lesions prolong corticosterone and/or ACTH release following exposure to restraint [74,76], footshock [99], and open field exposure [76]. However, hippocampal lesions are without effect on HPA axis responses to ether [76] or hypoxia [16], indicating that the involvement of the hippocampus in HPA axis integration is dependent upon the type of stressor. Therefore it appears

that the hippocampus plays a role in inhibiting HPA axis activation in response to psychogenic, but not physical, stressors.

The hippocampus can inhibit HPA activation via glucocorticoid negative feedback. Two corticosteroid receptors have been identified, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) [132,197]. Low concentrations of corticosterone are believed to activate MR, while higher corticosterone concentrations are believed to activate GR during stress. Both the MR and the GR are expressed in the hippocampus [104,162]. The MRs of the hippocampus mediate tonic inhibitory influence of corticosterone on HPA activity [8] and show a stress-induced increase in density [60]. GRs of the hippocampus mediate negative feedback due to stress-induced corticosterone release [162] and show a stress-induced decrease in GR mRNA [72,78]. Glucocorticoid receptor activity within the hippocampus modulates HPA axis activation in response to stress.

Hippocampal inhibition of the HPA axis is specifically mediated by a restricted set of neurons in the ventral subiculum. Lesions of the ventral subiculum enhance responsiveness to restraint and open field exposure, but not to ether vapors [74,76]. Projections from the ventral subiculum of the hippocampus are predominantly glutamatergic [204]. Ventral subicular efferents contact PVN-projecting regions, such as the posterior BNST, medial preoptic area, and the dorsomedial hypothalamic nucleus [37]. The BNST [37], medial preoptic area [37], and dorsomedial hypothalamic nucleus [144] contain populations of GABAergic neurons that may serve to relay inhibitory information to PVN neurons [15].

Lateral Septum

The lateral septum is responsible for the modulation of neuroendocrine, behavioral and autonomic responses to anxiety and stress (Figure 5). Lesions of the lateral septum are known to produce extreme anxiety and aggression, also known as “septal rage” [164]. Fos mapping shows neurons of the lateral septum are robustly induced by a variety of psychogenic stressors, such as

novelty [49], fear-conditioned behavior [21], and social stress [126], but show little induction following activation of physical stress pathways [49,178,194]. However, lateral septal neurons do not project directly to the PVN, but innervate the medial preoptic area, anterior and lateral hypothalamus [164]. The lateral septum sends GABAergic projections to PVN-projecting regions that are predominantly GABAergic. Therefore, like the amygdala, the lateral septum produces HPA activation through the disinhibition of inhibitory projections to the PVN.

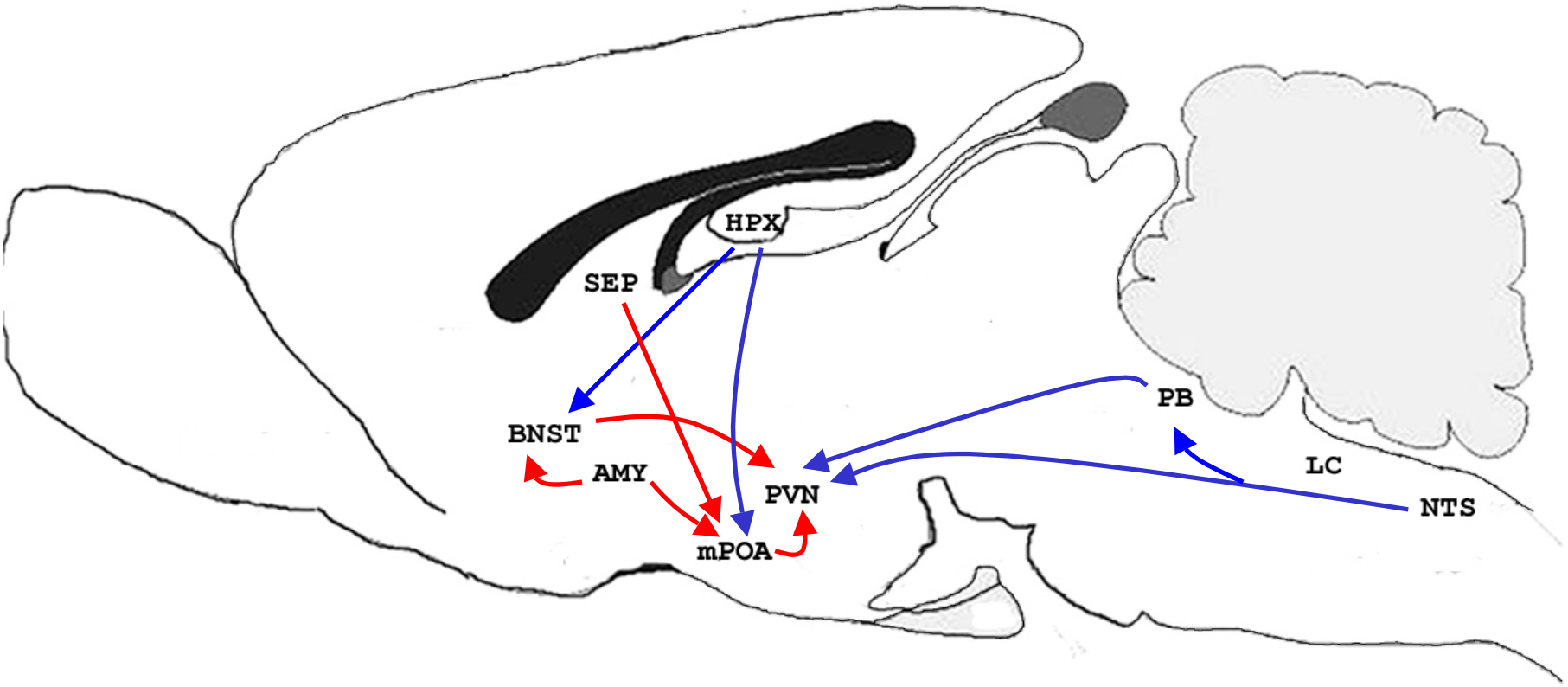

Figure 5. Projections to the Paraventricular Nucleus of the Hypothalamus from Brain Areas Involved in the Stress Response

Blue projections are predominantly excitatory and red projections are predominantly inhibitory. Amygdala (AMY), bed nucleus of the stria terminalis (BNST), hippocampus (HPX), locus coeruleus (LC), medial preoptic area (mPOA), nucleus of the solitary tract (NTS), parabrachial nucleus (PB), paraventricular nucleus of the hypothalamus (PVN), lateral septum (SEP).

D. Anxiety

1. Definition and Classification of Anxiety

Anxiety is a common human emotional reaction that occurs in response to environmental and/or physiological stressors. At mild levels anxiety is considered "normal", enhancing the senses and mobilizing the body to respond to the stressor. However, anxiety is detrimental when it interferes with a person's ability to function in normal daily activities, or when anxiety is inappropriately triggered by little or no external stressful stimuli. The anxiety response in humans has been defined by psychological symptoms such as worry, restlessness, or fear and physiological symptoms such as sweating, elevated heart rate, or trembling [9]. The American Psychiatric Association describes several forms of anxiety disorders, which currently include: generalized anxiety disorder, obsessive-compulsive disorder, phobias, panic disorder, and post-traumatic stress disorder [9]. Each type of anxiety disorder exhibits a unique combination of symptoms that in some cases overlap. For example, generalized anxiety disorder is characterized by excessive worry and negative emotional affect. Post-traumatic stress disorder is also characterized by negative emotional affect as well as increased stress reactivity. Overwhelming fear during a panic attack is a feature of panic disorder and phobias result in extreme fear and avoidance of a specific object or situation. Currently, there is little understanding of the underlying cause of anxiety at a neurobiological level.

Based on the behavioral symptoms of many of the anxiety disorders it seems likely that neural pathways involved in the fear response are also involved in at least some anxiety disorders. However, the current literature provides conflicting opinions on the distinction between anxiety and fear. Anxiety is defined as an emotional anticipation of an aversive situation that is difficult to predict or control, and is likely to occur. It is usually considered a more general state of distress prompted by generalized cues, lasting for long periods of time once activated, and physiological arousal that lacks adaptive significance [28]. On the other hand, fear is usually elicited by an

identifiable, threatening stimulus with escape or avoidance as a goal [28]. To date much of the literature attempting to define the neurobiology of anxiety has relied on studies of fear-related behavior.

To gain a better understanding of the neurobiology of anxiety, behavioral animal models have been developed to reproduce some of the symptoms observed in human anxiety disorders. Behavioral animal models of human anxiety disorders rely on of a variety of ethological behaviors of rodents that have been interpreted to be "anxiety-like" [56,184].

Animal tests of anxiety can be divided into two categories. These include tests based on conditioned fear or unconditioned fear, in which anxiety is generated by exposure to a novel environment or situation [120,160]. Conditioned anxiety animal models test the ability of an animal to suppress a behavior in response to the delivery of an unavoidable form of punishment. Therefore the behavior of the animal tends to be repressed [32]. Conditioning models allow for experimental control over behavioral baselines, but require extensive behavioral training of the animals and multiple experimental controls for non-specific treatment effects on learning/memory, appetite, and/or perception of the punishment. Conditioned anxiety tests can be further categorized as conflict or non-conflict based tasks. In a non-conflict based task the rodent is re-exposed to an environment or situation that resulted in fear or anxiety-related behavior. Conditioned tasks based on conflict usually involve punishment (i.e. shock) in response to innate behaviors (i.e. eating or drinking).

The second classification of anxiety testing relies on unconditioned responses. Procedures based on unconditioned behavior can be distinguished by the expression or inhibition of responses. Tests falling into the former category include those that result in the expression of unconditioned defensive reactions, such as freezing, startle, or ultrasonic vocalization to an anxiogenic stimulus. However, the majority of unconditioned anxiety tests result in behavioral inhibition in response to an anxiogenic stimulus. For instance, the elevated plus-maze (discussed in Materials and Methods), an ethologically based test, measures a mouse's natural tendency to explore an unfamiliar environment

opposed with its innate fear of novel or aversive environments [13,167]. Unlike conditioned behavior animal models, unconditioned response paradigms do not require training and are less susceptible to variability in motivational processes.

2. Neurocircuitry of the Anxiety Response

Although there is a close correspondence between fear and anxiety, the study of anxiety has relied heavily on the use of fear conditioned animal models. Much of the literature reporting neuroanatomical activation of anxiety-related pathways makes use of stimulus-specific fear models as opposed to less stimulus specific models, which may be more relevant to human generalized anxiety disorders. However, the use of fear-conditioning in animals has been extremely valuable in understanding the brain systems that are involved in anxiety. Possible interaction of OT with many of the following brain areas is discussed in reference to the experiments performed in Chapter V.

a) Afferents and Stimulus Processing of Anxiety

In response to anxiety or fear stimuli, sensory information (i.e. auditory, visual, somatosensory) is relayed from peripheral receptor cells to the dorsal thalamus, which is the neuronal interface between sensory stimuli and forebrain structures [113,115] (Figure 6). Afferent sensory inputs from the thalamus are then relayed directly to the amygdala and cortical brain regions, such as the primary visual (occipital), auditory (temporal), or tactile (post-central gyrus) cortex, which then relay sensory information to the amygdala [90]. However, olfactory sensory input has input to the amygdala either directly or through the entorhinal cortex (a portion of the hippocampal formation). From the entorhinal cortex there are direct projections to the amygdala and the hippocampus which projects to the amygdala [64]. The amygdala, in turn sends reciprocal projections to the hippocampus [171]. The connections between the entorhinal cortex, hippocampus and the amygdala provide a neuroanatomical substrate for the interaction between storage and recall of the memory of a fear- or anxiety-inducing stimulus and the emotion related to that stimulus [64]. The amygdala also

has strong reciprocal projections to the thalamus [171]. The thalamocortico-amygdala connections, could account for an unconscious fear and anxiety response [114].

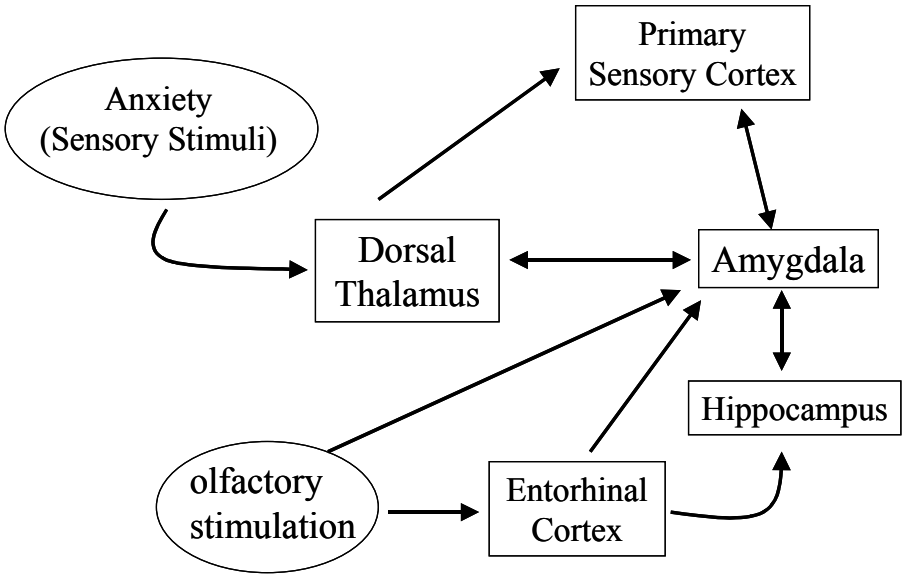

Figure 6. Afferents and Stimulus Processing of Anxiety.

b) Efferents and the Anxiety Response

The amygdala receives highly processed sensory information from all modalities through its lateral and basolateral nuclei. The basolateral nucleus of the amygdala sends extensive projections to the striatum and the BNST [171]. These projections may be responsible for motor responses critical in the "fight or flight" responses to threatening stimuli. The remainder of the afferents of the lateral and basolateral nuclei project to the central nucleus of the amygdala, which then project to a variety of brain areas that mediate the emotional and physiological reactions to fear and anxiety [41,171] (Figure 7).

Sympathetic activation and hormonal release associated with anxiety and fear is mediated by stimulation of the hypothalamus via projections from the central nucleus of the amygdala (CeA), bed nucleus of the stria terminalis (BNST) and locus coeruleus (LC). The hypothalamus integrates information it receives from a variety of brain structures into a coordinated pattern of sympathetic

and neuroendocrine responses. Stimulation of the lateral hypothalamus directly by the CeA or through the (LC) activates the sympathetic system resulting in increased in blood pressure and heart rate, sweating, piloerection, and pupil dilation [171]. Activation of the PVN from CeA projections relayed through the BNST [37] results in HPA activation. Lastly, CeA projections to the dorsal motor nucleus of the vagus result in parasympathetic inhibition associated with anxiety, including gastrointestinal and genitourinary disturbances [28].

It should be noted that through the use of immunohistochemical labeling for Fos it has been determined that the medial nucleus of the amygdala is also activated during exposure to the elevated plus maze [45,186]. The medial amygdala (MeA) receives projections from the entorhinal cortex, frontal cortex, and hypothalamus, and projects to the BNST, hypothalamic nuclei, and thalamus [171]. Therefore, it is possible that exposure to certain forms of anxiety-inducing stimuli activate the MeA. However, activation of afferent and efferent pathways of the MeA have not been investigated extensively.

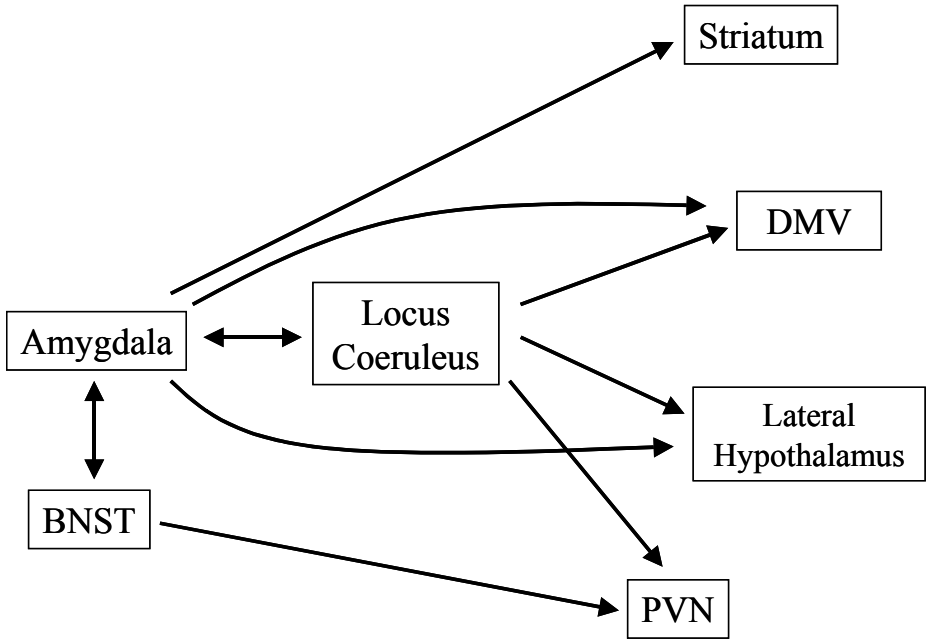

Figure 7. Efferents of the Amygdala to Anxiety-Related Brain Areas

Abbreviations, bed nucleus of the stria terminalis (BNST), dorsal motor nucleus of the vagus (DMV), paraventricular nucleus of the hypothalamus (PVN).

E. Involvement of Oxytocin in Stress and Anxiety

Peripheral and central release of OT accompanies secretion of corticosterone and ACTH in response to certain forms of stress. Central and peripheral OT is released in male and female rats in response to stressors that contain a psychogenic component. These stressors include forced swimming in male [216,217] and female rats [209], various forms of social stress in male [48,50] and female rats [138], and shaker stress in male rats [142]. Chronic infusion of synthetic OT into the lateral ventricles of ovariectomized female rats decreased the corticosterone response to a psychogenic (noise) stress and reduced anxiety-related behavior [211]. Infusion of an OT receptor antagonist into the lateral ventricles disinhibited the basal and stress induced release of ACTH and corticosterone in male and female rats exposed to an elevated plus-maze [140], repeated airpuffs [138], and female rats forced to swim [140]. Thus activation of central OT signaling mechanisms is believed to exert inhibitory control over the stress response.

Parvocellular OT neurons are activated in response to emotional stress, and the presence of OT receptors throughout anxiety related brain areas, including the hypothalamus and amygdala, suggest a potential role for OT in the modulation of anxious behavior. Following central administration of OT, an anxiolytic-like effect has been described in rats. Central administration of OT into the lateral ventricles of rats [211] and estrogen-treated ovariectomized mice [129] resulted in decreased anxiety-related behavior in the elevated plus maze. Oxytocin infused into the central nucleus of the amygdala, but not the ventromedial nucleus of the hypothalamus, resulted in decreased anxiety-related behavior of rats in the elevated plus maze and open field, indicating brain region-specific effects [11]. However, aside from the findings of Bale *et al.* there is little functional information regarding the role OT in anxiety-related behavior.

F. Oxytocin Deficient Mouse

1. Production of the Oxytocin Deficient Mouse

Similar to the human OT gene, the mouse gene for OT-neurophysin I consists of three exons: the first exon encodes a signal peptide, the nine peptide OT hormone, the tripeptide processing signal (GKR), and the first nine amino acids of neurophysin; the second exon encodes the central part of the neurophysin (residues 10-76); and the third exon encodes the COOH-terminal region of neurophysin (residues 77-93/95) [67]. Currently there are three different versions of the oxytocin deficient mouse (OT-/-). Gross *et al.* replaced all three exons of the oxytocin (OT) gene, eliminating the preproOT/neurophysin coding sequence [66,213]. Nishimori *et al*. deleted the first exon of the OT gene [141]. This deletion resulted in the elimination of the initiation ATG codon, the processing signal, the OT peptide, and the first few amino acids of neurophysin. Young *et al.* replaced the second and third exons of the OT gene with a neomyocin resistance cassette, resulting in the deletion of the carrier polypeptide [223]. Therefore, although OT is transcribed, it is not packaged or transported out of the cell. The OT-/- mouse created by Young et al. was used for all of the experiments discussed in this thesis (refer to Chapter II, section A).

2. Central Peptide Expression

Through different methods of evaluation it has been confirmed that all three mutations of the OT gene sequence resulted in the elimination of OT. Using *in situ* hybridization histochemistry it was determined that oxytocin transcripts were absent from the PVN and SON of the hypothalamus of OT deficient mice in which all three exons were deleted compared to wildtype mice of the same 129/Sv-Black Swiss background [143]. CRH transcript levels were not different in the PVN of OT-/- and OT+/+ mice [143]. However, *in situ* hybridization histochemistry also revealed decreased AVP mRNA in the PVN and SON of OT-/- mice compared to OT+/+ mice [143]. The AVP gene is closely linked to the OT gene [67]. While it is not known why AVP expression is reduced in the OT-/- mouse, it is possible that genomic response elements within the OT gene that regulate the expression of AVP are altered in the OT-/- mouse.

In a different version of the OT-/- mouse Nishimori *et al*. also performed *in situ* hybridization histochemistry to confirm that OT mRNA was not synthesized [141]. OT/neurophysin mRNA was not present in the PVN and SON of OT-/- mice. In addition, AVP mRNA content in the PVN and SON of OT-/- mice is not altered in OT-/- mice. Although this transgenic mouse does not synthesize OT, the synthesis of AVP mRNA is intact.

Young *et al*. also performed *in situ* hybridization histochemistry in addition to immunocytochemistry to confirm that the production of OT was reduced [223]. Using a probe for exon 1 of the OT gene, OT transcripts in the PVN of OT heterozygous and OT deficient mice were 53% and 1% of the wildtype OT mRNA, respectively [223]. Immunohistochemistry using antibodies to neurophysin confirmed the findings obtained using *in situ* hybridization histochemistry. Abundant staining of OT neurophysin was evaluated in the PVN, SON, and posterior pituitary of wildtype mice [223]. However, there was no detectable OT protein in the PVN and SON of OT-/- mice [223]. Using *in situ* hybridization histochemistry Young *et al*. also measured the expression of other genes normally expressed in the PVN and SON. Corticotropin-releasing hormone mRNA expression was not altered in the PVN of OT-/- mice compared to OT+/+ mice [223]. Compared to OT+/+ mice AVP mRNA is reduced by 26% in the PVN and 30% in the SON of OT-/- mice [223]. However, AVP related behavioral differences have not been observed in OT-/- mice.

G. Specific Objectives of the Research

The overall objective of this research was to evaluate the role of OT in the stress and anxiety response. Anxiety-related behavior, the corticosterone response and neuronal activation in response to stress- and anxiety-related stimuli were studied in OT deficient mice in order to elucidate the role of OT in stress and anxiety responses. Prior to the development of the oxytocin deficient mouse,

researchers have relied on exogenous administration of OT, OT antagonists, or OT antisense oligonucleotides to study the effects of OT on the stress and anxiety response. Although pharmacological studies have provided a tremendous amount of information regarding the role of OT in the stress and anxiety responses, there are limitations and a lot of variability in these studies. Therefore, OT deficient mice that do not synthesize or release central or peripheral OT are a unique animal model to test the role of OT in the stress and anxiety responses. The specific objectives of this study are:

1. **To evaluate HPA axis activation in response to psychogenic and systemic stressors in the OT deficient mouse.**
    A. To determine the effect of OT on the corticosterone response to a psychogenic stressor. This was achieved by exposing male and female OT+/+ and OT-/- mice to shaker stress, which has been defined as a psychogenic stressor, and evaluating the corticosterone response.
    B. To determine the effects of OT on the corticosterone response to a physical stressor. This was achieved by administering systemic cholecystokinin (CCK), a physical stressor, to male OT+/+ and OT-/- mice and evaluating the corticosterone response.
2. **To evaluate anxiety-related behavior of the OT deficient mouse.**
    A. To determine the effect of OT on anxiety-related behavior. This was achieved by placing male and female OT+/+ and OT-/- mice in the elevated plus maze.
    B. To determine if the effect of OT is dependent upon binding at the OT receptor. This was achieved by infusing OT into the lateral ventricles of OT-/- mice, or an OT antagonist into the lateral ventricles of OT+/+ mice, prior to testing in the elevated-plus maze. In addition, an OT antagonist was infused into the lateral ventricles of OT-/- mice followed by infusion of OT, prior to testing in the elevated plus maze.

3. To evaluate activation of stress- and anxiety-related pathways following exposure to anxiety- or stress-related stimuli in OT deficient mice.

A. To determine if OT signaling pathways contribute in the modulation of the HPA axis response to a psychogenic stressor. This was achieved by exposing OT+/+ and OT-/- mice to shaker stress, a psychogenic stressor. Brain tissue was harvested and then processed for immunocytochemistry for Fos and CRH, AVP, or OT, and quantifying Fos activation in the PVN and stress-related limbic brain areas.

B. To determine if OT signaling pathways contribute in the modulation of the HPA axis response to a systemic stressor. This was achieved by administering cholecystokinin, a nauseogenic, to OT+/+ and OT-/- mice. Brain tissue was harvested and then processed for immunocytochemistry for Fos and CRH, AVP, or OT, and quantifying Fos activation in the PVN and stress-related limbic brain areas.

C. To determine if OT signaling pathways contribute in the modulation of anxiety-related behavior. This was achieved by exposing OT+/+ and OT-/- mice to the elevated-plus maze. Brain tissue was harvested and then processed for immunocytochemistry for Fos and CRH, AVP, or OT, and quantifying Fos activation in the PVN and anxiety-related limbic brain areas.

II. Materials and Methods

The protocols were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

A. Breeding and Maintenance of Wildtype and Oxytocin Deficient Mice

Male and female wildtype (OT+/+) and OT deficient (OT-/-) mice of C57BL/6 strain were used for these studies. The OT-/- mice were developed by Scott Young, (National Institute of Mental Health, Bethesda, MD) [222] and breeding pairs for this study were purchased from Jackson Laboratories (Bar Harbor, ME). OT+/+ mice were created by breeding OT+/+ male and female mice. OT-/- mice were created by breeding female OT+/- mice with male OT-/- mice. This breeding paradigm was used to eliminate the necessity for cross-fostering pups. In addition, all pups are exposed to peripheral circulating OT *in utero* during development, limiting the variability in the prenatal development of OT+/+ and OT-/- pups.

Animals were bred and housed in the viral free quarters of the University of Pittsburgh Animal Facility under a 12-h light/dark cycle (lights on at 0700 h). Mice were housed in standard suspended cages in groups of up to four animals per cage with free access to water and food (Prolab RMH 3000 5P00, LabDiet/Purina). During testing animals were removed from group housing and acclimated to single housing for a week prior to the test day unless otherwise stated.

B. Genotype Determination of Mice Using Polymerase Chain Reaction (PCR)

To identify the genotype of the mice approximately 0.5 cm of mouse-tail was digested and DNA was extracted and prepared for polymerase chain reaction (PCR). The DNA sample was dissolved in 100µl of 10mM Tris-HCl and 1mM EDTA. 50µl PCR reactions containing 2µl (100ng) of the DNA sample, 5.0µl 10X PCR buffer minus Mg (Gibco BRL, Gaithersburg, MD), 2.5µl 10mM dNTPs (Invitrogen, Carlsbad, CA), 2.5µl 50mM $MgCl_2$ (Gibco BRL, Gaithersburg, MD), 0.5µl Taq

DNA polymerase (Gibco BRL, Gaithersburg, MD) and 1-2µl primers were heated for 5 min at 95°C and then cycled 30 (35 for OT) times at 94°C for 40 sec (45 sec for OT) and 63°C (55°C for OT) for 1 min.

Primer pairs, synthesized at the University of Pittsburgh Sequence facility, were designed for PCR that detect either the wild-type allele (OT, 332 bp) or the mutant allele (neomycin resistance cassette, 430 bp). Primer pairs for the wild-type allele are (forward) TCG CTC TGC CAC AGT CCG GAT TC and (reverse) TCA GTG TTC TGA GCT GCA AAC C, and for the mutant allele are (forward) AGA GGC TAT TCG GCT ATG ACT G and (reverse) TTC GTC CAG ATC ATC CTG ATC.

C. Plasma Corticosterone Analysis

Trunk blood was collected into heparinized tubes on ice, centrifuged at 4°C, and plasma was stored at –20°C until assay. Plasma corticosterone levels were measured by radioimmunoassay using a commercially available kit purchased from Diagnostic Products Corporation (Los Angeles, CA). The range of this is assay is 0ng/ml - 2000ng/ml and the minimum detection limit for corticosterone is 5.7ng/ml. The intra-assay precision (coefficient of variation) for the assay is 3.7% $\pm$ 0.2 and the ED50 is 122.4ng/ml $\pm$ 2.8.

D. Immunocytochemistry

Mice were anesthetized by intraperitoneal (ip) injection of ketamine/xylazine, and perfused with 0.15M saline followed by 4% paraformaldehyde fixative (0.1M sodium phosphate buffer containing 4% paraformaldehyde, 1.4% L-lysine, and 0.2% sodium metaperiodate) 60-75 minutes after experimental testing. Fixed brains were removed from the skull, post-fixed in 4% paraformaldehyde at 4°C for 12-18 hours, and transferred to 25% sucrose solution (4°C) for 24-72 hours before sectioning. Coronal tissue sections were cut (35µm thick) using a freezing stage

microtome. Tissue was stored in cryoprotectant [205] at –20°C until immunocytochemical processing.

Sections were removed from cryoprotectant and rinsed in several changes of 0.1M sodium phosphate buffer, treated for 30 minutes in 1% sodium borohydride (Sigma, St. Louis, MO), and rinsed again in sodium phosphate buffer. Antisera were diluted in sodium phosphate buffer containing 0.3% Triton X-100 and 1% normal donkey serum. Tissue sections were incubated for 48h at 4°C in rabbit anti-c-fos (1:50,000; provided by Drs. Philip Larson and Jens Mikkelsen, Planum Institute, Denmark), rinsed, and then incubated in biotinylated donkey anti-rabbit IgG (1:600; Jackson ImmunoResearch Laboratories, West Grove, PA) for 1h at room temperature. Sections were rinsed and processed using the Vectastain Elite avidin-biotin immunoperoxidase method (Vector Laboratories, Burlington, CA). A sodium acetate buffer solution of diaminobenzadine (DAB), nickel sulfate, and H_2O_2 was used to generate blue-black nuclear cFos immunolabeling.

The tissue was then processed for OT (rabbit anti-OT, 1:30,000; Chemicon, Temecula, CA), AVP (rabbit anti-AVP, 1:20,000; Chemicon, Temecula, CA), or CRH (rabbit anti-CRH, 1:10,000; Peninsula Laboratories, Belmont CA). The tissue was incubated in antisera diluted in 0.1M sodium phosphate buffer containing 1% normal donkey serum and 0.3% Triton X-100 for 48-72 hours at 4°C. Tissue was rinsed in several changes of 0.1M sodium phosphate buffer and then incubated in biotinylated donkey anti-rabbit IgG (1:600) for 1 h at room temperature. Sections were then rinsed and processed using the Vectastain Elite avidin-biotin immunoperoxidase method (Vector Laboratories, Burlington, CA). OT, AVP, and CRH immunolabeling was generated using a non-enhanced DAB reaction to create brown immunoprecipitate. Immunolabeled tissue sections were mounted onto Superfrost Plus glass slides (Fisher Scientific, Pittsburgh, PA), cleared in graded alcohols and xylene, and coverslipped using Histomount (VWR, Bridgeport, NJ).

A quantitative analysis of cFos expression in OT, AVP, and CRH magnocellular and/or parvocellular neurons of the PVN and OT and AVP magnocellular neurons of the supraoptic nucleus

of the hypothalamus (SON) was conducted in anatomically matched tissue sections. For this purpose, anatomically matched brain sections containing OT-positive neurons (in OT +/+ mice only, since OT immunolabeling is absent in OT -/- mice; 4-5 brain sections from bregma –0.58 to –1.22), AVP-positive neurons (in all mice; 4-5 brain sections from bregma –0.58 to –1.22), and CRH-positive neurons (in all mice; 2-3 brain sections from bregma –0.58 to -0.94mm) were selected for analysis [57]. Unlike in rats, in which parvocellular and magnocellular divisions of the PVN are easily distinguished, there is as yet no conventional method to separate these two divisions in mice. OT, AVP, and CRH cells were considered Fos-positive when their nuclei contained blue-black immunoreactivity. The average numbers of activated, phenotypically identified neurons within the PVN and SON per section (both sides) were calculated for each mouse.

A quantitative analysis was also conducted in the bed nucleus of the stria terminalis (BNST; combined counts of the anterodorsal, anterolateral and anteroventral nuclei of the medial and lateral BNST), medial (MeA) and central nuclei (CeA) of the amygdala, and the medial preoptic area (mPOA) of OT+/+ and OT-/- mice. For each mouse, brain sections containing the anatomically matched levels of the BNST (3-4 brain sections in bregma 0.62 to 0.26mm), the MeA (4-6 sections in bregma –0.82 to –1.70mm), the CeA (6-8 sections in bregma –0.82 to 1.70mm), and the mPOA (3-5 brain sections in bregma 0.5 to –0.10mm) were selected for analysis [57]. Cells were considered cFos positive when their nuclei contained blue-black immunoreactivity. The average number of cells per section (both sides) in each brain region was calculated for each mouse.

E. Shaker Stress

Shaker stress, originally described as an environmental stress, was chosen because it is a predominantly psychogenic stressor [137] that releases corticosterone and OT, but not AVP, in rats [69,142]. Mice were individually placed in an opaque plastic beaker (27cm diameter and 36cm height) that was fixed to a shaking platform (Dubnoff Shaker Model 3575). Mice were subjected to

shaker stress (180cycles/min) for 10 minutes, and then returned to their home cage and sacrificed via rapid decapitation for corticosterone assessment or perfused for immunocytochemical processing at the times specified in each of the experiments. The duration of shaker stress was chosen based on studies performed in rats showing increased peripheral and central OT and corticosterone release after 5+ min of shaker stress [69]. Control mice that did not receive shaker stress were sacrificed at the same time. Studies were conducted between 0800-1200h, which is the nadir of corticosterone secretion in rodents [29,224]. To keep environmental stress to a minimum during the experiments, mice were housed, exposed to shaker stress, and sacrificed in different rooms. On the day of the experiment mice were tested as paired cohorts according to genotype and treatment group.

F. Cholecystokinin Administration

Cholecystokinin (CCK; Bachem, King of Prussia, PA) was chosen as a systemic stressor because it is an anxiogenic/nauseogenic agent that results in the activation of the HPA axis [97], and OT activation [200] and release [198]. Mice of both genotypes were either normal fed or overnight food deprived for 18h. The following morning mice were injected i.p. with saline or CCK (10μg/kg dissolved in 0.9% saline). The dose used in this study was based on prior studies determining that 10μg/kg CCK produced anorexia in fasted OT+/+ and OT-/- mice and activated PVN OT neurons of OT+/+ mice [122]. Mice were sham injected daily for seven days prior to the day of experimentation to control for the possible increase in corticosterone due to the pain of injection. To keep environmental stress to a minimum, sacrifice took place in a different room from drug administration.

G. Lateral Ventricle Cannulation Surgery and Infusions

OT+/+ and OT-/- mice were anesthetized with ketamine (1mg/10g bodyweight, ip) and xylazine (0.025mg/10g bodyweight, ip) for stereotaxic placement of cannulae into the lateral ventricles. The top of the animal's head was shaved and a 1-mm midline incision was made across

the top of the skull. After cleaning the periosteum, a 1mm hole was drilled 1.00-mm lateral and 0.5mm posterior to bregma and the tip of a 26-gauge stainless steel infusion cannula was placed 2.00 mm below the skull surface in the lateral ventricle. The cannula was secured to the skull with dental cement and a stylus was inserted to maintain patency. Mice were allowed to recover for one week prior to behavioral testing. Patency of the guided cannula was confirmed by the movement of an air bubble placed in the PE-10 tubing connecting a 25µl Hamilton syringe to the guide cannula. Prior to euthanizing, we infused 2µl of India ink dissolved in aCSF to confirm cannula placement. Only mice that showed correct placement of the cannula were included in the analyses.

H. Elevated Plus Maze Testing

One of the best-documented and pharmacologically validated animal models of anxiety is the elevated plus maze (EPM) task. The elevated plus maze is a conflict test based on the tendency of rats [152] and mice [119] to explore an unfamiliar environment versus the tendency of rodents to avoid the aversive properties of a brightly-lit, open, elevated space. The apparatus has four arms extending from a central platform that form the shape of a plus. The mouse EPM is positioned on a platform 40 cm above the floor, consists of two opposite facing open arms (30x5cm) and two closed arms (30x5 cm with 15cm high walls) with a central area (5x5cm). The animal is placed in the center, at the junction of the four arms, and allowed to explore the maze during a 5 minute test session. The critical independent variables are: (1) percentage of entries into the open arms, (2) percentage of time spent in the open arms, (3) number of entries in the closed arms, (4) number of total arm entries, and on occasion (5) time spent in the center (risk assessment). This index represents the tendency of the animal to explore the aversive open, brightly lit, elevated environment, rather than remaining in the preferred enclosed, dark environment. An animal exhibiting a decrease in open arm entries or time would be considered to have an increased level of anxiety. The EPM has been validated in mice and rats through the administration of anxiolytic drugs, which increase the

percentage of open arm entries, and anxiogenic drugs, which decrease the percentage of open arm entries [119,152].

Approximately one week prior to testing, mice were transferred from group to individual housing. During a 4-day acclimation and on the test day, mice were brought to the holding area outside of the behavioral testing room for 1h. Mice were tested in the elevated plus maze (EPM) between 1300-1700h and mice were paired by genotype and/or treatment during each test session. Mice were placed on the central platform, facing an open arm and the number and duration of entries into open or closed arms were videotaped for each mouse for 5 min and later scored by a single observer blinded to treatment and genotype of each mouse. An arm entry was defined as all four paws entering an arm of the EPM.

I. Determination of the Estrous Cycle

Vaginal smears were obtained by flushing 50μl of distilled, autoclaved water into the vaginal cavity and retrieving it with a plastic pipette tip. The fluid was then immediately placed onto a glass slide (Fisher Scientific, Pittsburgh, PA) and viewed under a microscope at low magnification. The vaginal cytology at each stage of the estrous cycle is as follows: proestrus, signaled by the appearance of polynucleated epithelial cells; estrus, represented by masses of cornified cells; metestrus, represented by scattered leukocytes and dispersed non-nucleated cells; diestrus, represented by many leukocytes with some epithelial cells. This was repeated everyday for 2-3 estrous cycles (12-14 days) at 10am each morning to accurately determine the vaginal cycle of each mouse [34].

J. Statistical Analysis

Results are presented as group mean $\pm$ SE. Statistical analysis was performed using StatView software (Abacus Concepts, Inc., Berkeley, CA). Data was assessed for normal distribution prior to statistical comparison. All data presented in this thesis were normally

distributed. Therefore, pairwise differences were analyzed by a two-tailed *t* test and differences between genotypes and treatments were determined using a two-way analysis of variance (ANOVA). A repeated measures ANOVA was conducted to determine differences between genotypes and/or treatments across time. If the ANOVA revealed a statistically significant difference in the overall F ratio, *post-hoc* pairwise comparisons were done using the Bonferroni/Dunn test. A statistically significant effect was accepted when $p < 0.05$.

III. Stress and the Oxytocin Deficient Mouse

Results of this chapter have been published in part [3,124].

Centrally released OT is believed to attenuate the response to stress in laboratory rodents [61,210,211]. OT is released within the brain and/or into the circulation of male and female rats in response to various psychogenic and physical stressors. These stressors include restraint stress in male rats [94], forced swimming in male [94,216,217] and female rats [209], 24h dehydration [94], hemorrhage [94], various forms of social stress in male [48,50] and female rats [138], and shaker stress in male rats [142]. Infusion of synthetic OT into the lateral ventricles of estrogen-treated ovariectomized rats decreased the corticosterone response to psychogenic restraint stress [210] and noise stress [211], and reduced anxiety-related behavior [211]. Infusion of an OT receptor antagonist into the lateral ventricles augmented the basal and stress-induced release of andrenocorticotropin hormone (ACTH) and corticosterone in male and female rats exposed to an elevated plus-maze (EPM) [140], repeated airpuffs [138], and female rats forced to swim [140].

To assess the role of the central oxytocinergic system in the stress response, plasma corticosterone concentrations were measured after the termination of multiple stressors, both psychogenic and physical, in male and female OT knockout and wildtype mice. Therefore, if central OT attenuates the HPA axis response to stress, it was hypothesized that OT knockout mice will have higher concentrations of corticosterone in plasma than wildtype mice in response to stress.

A. Experimental Design

1. Psychogenic Stress

Experiment 1A. Corticosterone Response to Shaker Stress in Group Housed Female Mice

The purpose of this experiment was to determine if shaker stress would release corticosterone in mice and to determine the time course of corticosterone release following termination of stress. The second purpose of the experiment was to determine if OT plays a role in modulating the

corticosterone response to shaker stress. Group-housed female OT+/+ and OT-/- mice were subjected to shaker stress and sacrificed 10 (OT+/+ n=4, OT-/- n=6) or 30 (OT+/+ n=4, OT-/- n=6) minutes post-stress. Control mice (OT+/+ n=3, OT-/- n=6), not exposed to shaker stress but maintained the same as mice receiving stress, were sacrificed at the same times. Trunk blood was obtained for measures of corticosterone. Compared to control mice, corticosterone concentrations increased following shaker stress 10 and 30 minutes after stress. Because genotype differences were similar 10 and 30 minutes following shaker stress, subsequent studies only examined corticosterone levels in mice 10 min after termination of shaker stress.

Experiment 1B. Corticosterone Response to Shaker Stress in Individually Housed Mice

Housing conditions (group housed vs. individually housed) have been shown to influence corticosterone levels in rats. Therefore, *Experiment IA* was repeated in OT+/+ (n=7) and OT-/- (n=7) mice that were individually housed for the week prior to and during the experiment in contrast to the first study in which animals were group housed. Mice were individually housed in an effort to reduce variability in corticosterone levels measured within experimental groups. Mice were subjected to shaker stress and sacrificed 10 minutes following termination of the stressor. Control OT+/+ (n=8) and OT-/- (n=8) mice, which were not exposed to shaker stress but were maintained and handled as mice receiving stress, were sacrificed at the same time. Trunk blood was obtained for measures of plasma corticosterone.

Experiment 2. Diurnal Plasma Corticosterone Concentrations in Female Mice

The purpose of this experiment was to determine whether genotypic differences in the corticosterone response to shaker stress are due to alterations in the diurnal rhythm of corticosterone in OT-/- mice. To determine minimum and maximum plasma corticosterone release, female OT+/+ (N=13) and OT-/- (N=14) mice were sacrificed at the expected circadian nadir (AM, 2 hours after lights on) or at circadian peak (PM, 1 hour before lights off) of a 12/12h light/dark cycle [135]. Trunk blood was collected at sacrifice to determine plasma corticosterone levels.

Experiment 3. Corticosterone Response to Central Administration of Corticotrophin Releasing Hormone in Female Mice

To determine if the genotypic difference in corticosterone response to shaker stress occurs at the HPA axis, ovine corticotrophin releasing hormone (oCRH) was administered into the lateral ventricles of female mice of both genotypes. Female OT+/+ (N=10) and OT-/- (N=12) mice received artificial cerebrospinal fluid (aCSF) or 30ng oCRH (Bachem, Torrence, CA) into the lateral ventricles in a volume of 2μl over 2 minutes. The dose and route of administration was chosen because it was reported to activate the HPA axis and release ACTH [18]. Thirty minutes following infusion mice were sacrificed and blood was collected for determination of corticosterone levels.

Experiment 4. Corticosterone Response to Repeated Shaker Stress in Female Mice

Repeated exposure to shaker stress has been found to dampen the corticosterone response and attenuate release of plasma OT in rats [69]. Therefore, the purpose of this experiment was to determine if repeated exposure to shaker stress would eliminate the genotypic differences in the corticosterone response. Plasma corticosterone concentrations were compared after acute shaker stress in mice that were exposed to daily shaker stress for 9 days. OT+/+ and OT-/- mice were divided into four groups. Conditioned stressed mice (OT+/+ n=10, OT-/- n=15) received shaker stress daily, for 9 days. On the 10th day of the experiment, animals were sacrificed following shaker stress. Naïve stressed mice (OT+/+ n-11, OT-/- n=12) were handled for 9 days and received acute stress on the 10th day of the experiment. Conditioned control mice (OT+/+ n=5, OT-/- n=7) received 10 minutes of shaker stress daily, for 9 days. On the 10th day of the experiment, animals were sacrificed without being exposed to shaker stress. Naïve control mice (OT+/+ n=9, OT-/- n=8) were handled daily for 9 days, but not exposed to shaker stress, and were sacrificed on the 10th day of the experiment. Trunk blood was collected at sacrifice to determine plasma corticosterone levels.

Experiment 5. Corticosterone Response to Shaker Stress Across the Estrous Cycle

To determine if the corticosterone response to shaker stress is influenced by the stage of the estrous cycle, OT+/+ and OT-/- mice were sacrificed after shaker stress at each of the following stages of the estrous cycle: diestrus (OT+/+ n=6, OT-/- n=6), proestrus (OT+/+ n=5, OT-/- n=8), and estrus (OT+/+ n= 6, OT-/- n=6). Trunk blood was collected at sacrifice to determine corticosterone. Vaginal cytology was used to predict the stage of the cycle at which mice were to be studied with shaker stress. Only mice with regular cycles were used in these experiments (94% of the total number of OT+/+ mice and 91% of the total number of OT-/- mice tested).

Experiment 6. Corticosterone Response to Shaker Stress in Male Mice

The purpose of this experiment was to determine if exposure to shaker stress would result in genotypic differences of the corticosterone response in male mice. Male mice were exposed to shaker stress and sacrificed 10 (OT+/+ n=8, OT-/- n=8) or 30 (OT+/+ n=8, OT-/- n=7) minutes following termination of shaker stress. Control mice (OT+/+ n=8, OT-/- n=10), which did not receive shaker stress, were sacrificed at the same time. Trunk blood was collected for measurement of plasma corticosterone.

2. Physical/Systemic Stress

Experiment 7. Corticosterone Response to Cholecystokinin Administration in Fasted and Non-fasted Mice

The purpose of this experiment was to determine genotypic differences in the corticosterone response to cholecystokinin (CCK) in fasted and non-fasted mice. Male fasted or non-fasted (normal fed) OT+/+ and OT-/- mice were injected with CCK (n=6 of each genotype fasted and n=6 of each genotype non-fasted) or saline (n=6 of each genotype fasted and n=6 of each genotype non-fasted), sacrificed 30 min later, and corticosterone levels were determined.

B. Results

1. Psychogenic Stress

Experiment 1A.. Corticosterone Response to Shaker Stress in Group Housed Female Mice

In group-housed mice, plasma corticosterone concentrations significantly increased in both genotypes at 10 and 30 minutes post-termination of a 10-minute shaker stress (ANOVA, $F_{(2,25)}=26.60$, $p < 0.0001$) compared to non-stressed mice (Figure 8). The increase in corticosterone was similar 10 and 30 minutes following shaker stress. Furthermore, OT-/- mice released more corticosterone than OT+/+ mice 10 min ($p < 0.0002$) and 30 min ($p < 0.04$) post-termination of shaker stress (Figure 8).

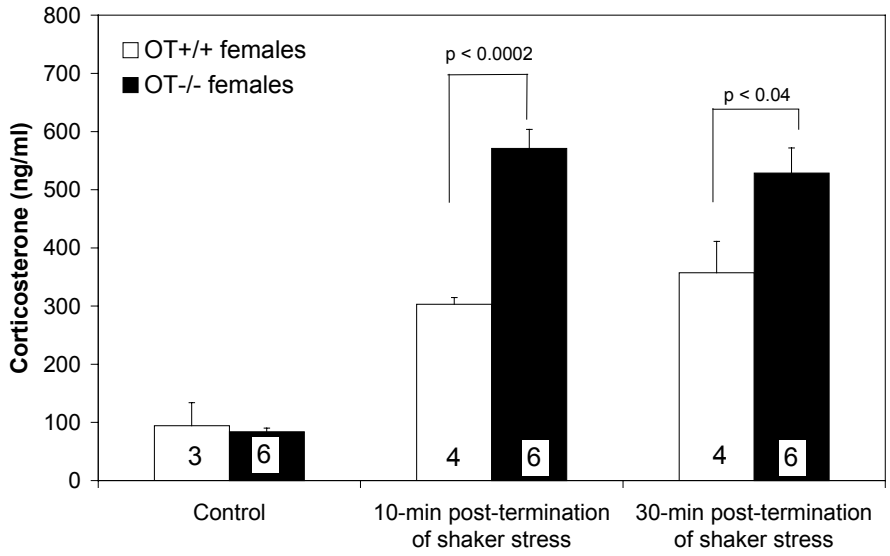

Figure 8. Corticosterone Response to Shaker Stress in Group-Housed Female Mice.

Plasma corticosterone concentrations of group housed female wildtype (OT+/+) and OT deficient (OT-/-) mice were measured 10 or 30 min following termination of a 10 min shaker stress or in control mice that did not receive shaker stress. Shaker stress resulted in a rise in plasma corticosterone concentrations in both genotypes, but the corticosterone increase was greater in OT-/- versus OT+/+ mice at 10 min and 30 min (ANOVA, $F_{(2,25)}=26.60$, $p<0.0001$). The number of mice per group is located in the data bars. Figure was reprinted with permission from reference [124].

Experiment 1B. Corticosterone Response to Shaker Stress in Individually Housed Mice

As was the case with group-housed mice, plasma corticosterone concentrations increased in both genotypes 10 minutes after termination of shaker stress compared to mice not receiving this stress (Figure 9). Moreover, corticosterone concentrations increased to a greater degree in OT-/- mice versus OT+/+ mice (ANOVA, $F_{(1,12)}=51.15$, $p < 0.02$).

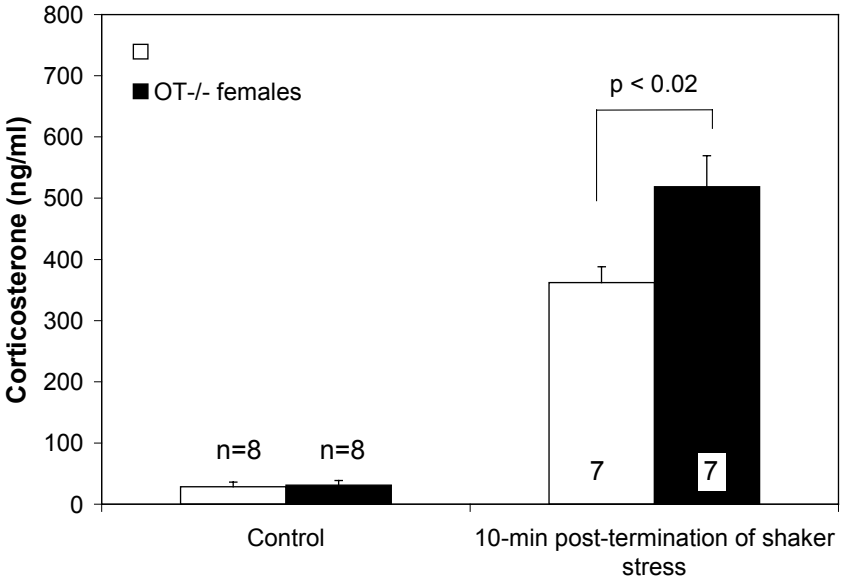

Figure 9. Corticosterone Response to Shaker Stress in Individually Housed Female Mice.

Plasma corticosterone concentrations were measured in individually housed female (OT+/+) and OT deficient (OT-/-) mice 10 min following termination of a 10 min shaker stress or in control mice that did not receive shaker stress. Plasma corticosterone concentrations increased in both genotypes after shaker stress, but to a greater degree in OT-/- versus OT+/+ mice at 10 min (ANOVA, $F_{(1,12)}$=51.15, p<0.02). The number of mice per group is located in or above the data bars. Figure was reprinted with permission from reference [124].

Experiment 2. Diurnal Plasma Corticosterone Concentrations in Female Mice

Analysis of plasma corticosterone levels at the expected circadian nadir and peak revealed an increase in basal corticosterone in both OT+/+ (ANOVA, $F_{(1,11)} = 29.61$, $p < 0.0002$) and OT-/- mice (ANOVA, $F_{(1,12)} = 40.65$, $p < 0.0001$) at the circadian peak (Figure 10). Minimum and maximum plasma corticosterone levels did not differ between female OT+/+ and OT-/- mice.

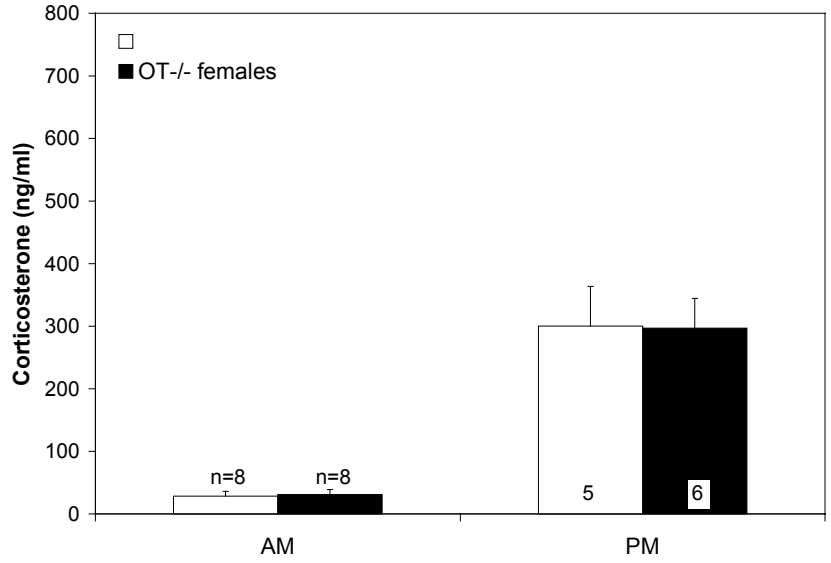

Figure 10. Diurnal Plasma Corticosterone Concentrations in Female Mice.

Diurnal plasma corticosterone concentrations were measured in individually housed female wildtype (OT+/+) and OT deficient (OT-/-) mice. Female OT+/+ and OT-/- mice were sacrificed at circadian nadir (AM, 2 hours after lights on) or at circadian peak (PM, 1 hour before lights off) on a 12/12 light/dark cycle. Analysis of plasma corticosterone revealed an increase in baseline corticosterone of female OT+/+ (ANOVA, $F_{(1,11)} = 29.61$, $p = 0.0002$) and OT-/- mice (ANOVA, $F_{(1,12)} = 40.65$, $p < 0.0001$) at the circadian peak. Plasma corticosterone values did not differ between female OT+/+ (ANOVA, $F_{(1,14)} = 0.51$, $p = 0.82$) and OT-/- mice (ANOVA, $F_{(1,9)} = 0.002$, $p = 0.96$). The number of mice per group is located in or above the data bars.

Experiment 3. Corticosterone Response to Central Administration of Corticotrophin Releasing Hormone in Female Mice

Plasma corticosterone was released in female OT+/+ ($p < 0.03$) and OT-/- ($p < 0.001$) mice thirty minutes following administration of oCRH compared to aCSF treated mice (Figure 11). However, plasma corticosterone concentrations increased to the same degree in female mice of both genotypes (ANOVA, $F_{(1,18)} = 0.059$, $p = 0.45$).

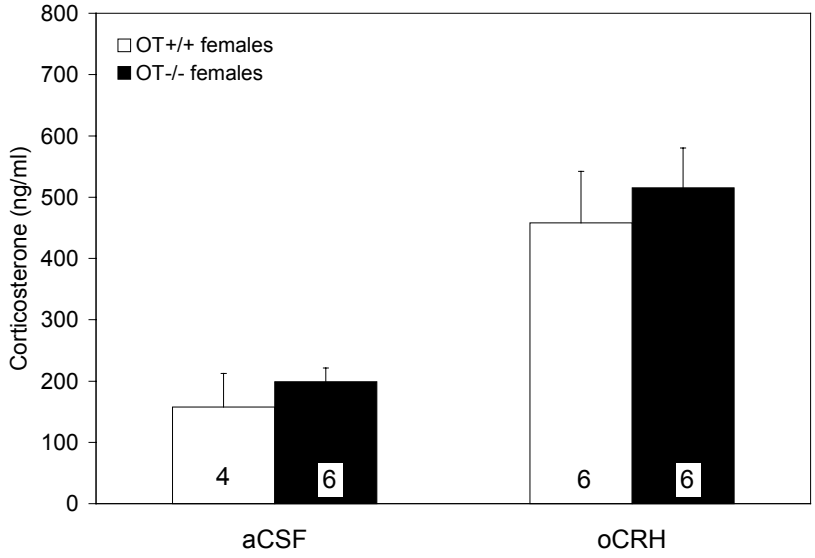

Figure 11. Corticosterone Response to Central Administration of Corticotrophin Releasing Hormone in Female Mice.

Plasma corticosterone concentrations were measured in female wildtype (OT+/+) and OT deficient (OT-/-) mice 30 minutes following administration of ovine corticotropin releasing hormone (oCRH) or artificial cerebrospinal fluid (aCSF). Administration of oCRH into the lateral ventricles of OT+/+ and OT-/- mice resulted in an increase in plasma corticosterone compared to aCSF treated mice of both genotypes. Plasma corticosterone increased to the same degree in female OT+/+ and OT-/- mice (ANOVA, $F_{(1,18)} = 0.059$, $p = 0.45$). The number of mice per group is located in the data bars.

Experiment 4. Corticosterone Response to Repeated Shaker Stress

The corticosterone response of mice repeatedly exposed to shaker stress was compared to mice receiving a single episode of acute shaker stress. Basal corticosterone levels of conditioned control mice were the same as naïve control mice of both genotypes (Figure 12). The corticosterone response of conditioned stressed OT+/+ ($p < 0.04$) and OT-/- ($p < 0.01$) mice was attenuated compared to naïve stressed (Figure 12). However, repeated exposure to shaker stress did not abolish the difference in corticosterone release between genotype. Conditioned stressed OT-/- mice had higher plasma corticosterone concentrations compared to OT+/+ mice ($p < 0.01$, Figure 12), despite repeated exposure to shaker stress.

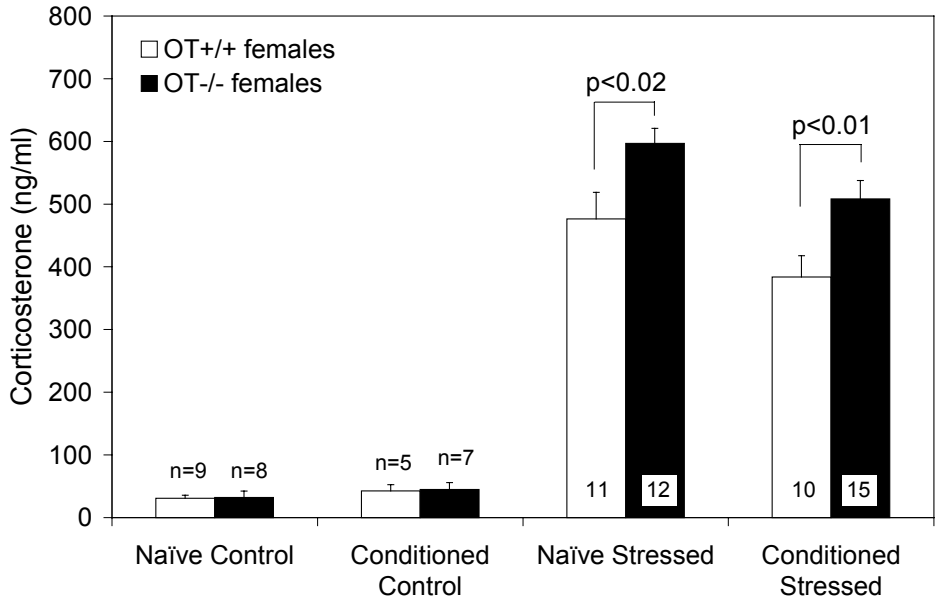

Figure 12. Corticosterone Response to Repeated Shaker Stress in Female Mice

Plasma corticosterone response in female wildtype (OT+/+) and OT deficient (OT-/-) mice following repeated shaker stress. Conditioned control OT+/+ and OT-/- mice released similar amounts of corticosterone as naïve control mice. Naïve stressed OT-/- mice released more corticosterone than OT+/+ mice following shaker stress. Conditioned stressed OT-/- mice released more corticosterone than OT+/+ mice following shaker stress. In addition, conditioned control OT+/+ ($p < 0.04$) and OT-/- ($p < 0.01$) mice displayed an attenuated corticosterone response to shaker stress compared to mice of the same genotype. The number of mice per group is located in or above the data bars. Figure was reprinted with permission from reference [124].

Experiment 5. Corticosterone Response to Shaker Stress Across the Estrous Cycle

OT-/- mice released more corticosterone than OT+/+ mice when exposed to shaker stress (ANOVA, $F_{(1,30)}=32.08$, $p < 0.0001$; Figure 13). Significant genotypic differences in the corticosterone response to shaker stress were observed at each stage of the estrous cycle. Corticosterone increase in response to shaker stress was higher in OT-/- compared to OT+/+ mice in the diestrus ($p < 0.03$), proestrus ($p < 0.004$), and estrus ($p < 0.003$) stages of the estrous cycle (Figure 13). Plasma corticosterone levels following shaker stress across the estrous cycle (ANOVA, $F_{(2,30)} = 9.03$, $p < 0.008$). Corticosterone levels in response to shaker stress were lower in mice in estrus that in diestrus ($p < 0.003$) or proestrus ($p < 0.003$). However, there was not an interaction between genotype and stage of the estrous cycle in reference to the corticosterone response to shaker stress (ANOVA, $F_{(2,30)} = 0.881$, $p>0.05$). Vaginal cytology verified 4-day estrous cycles in both genotypes.

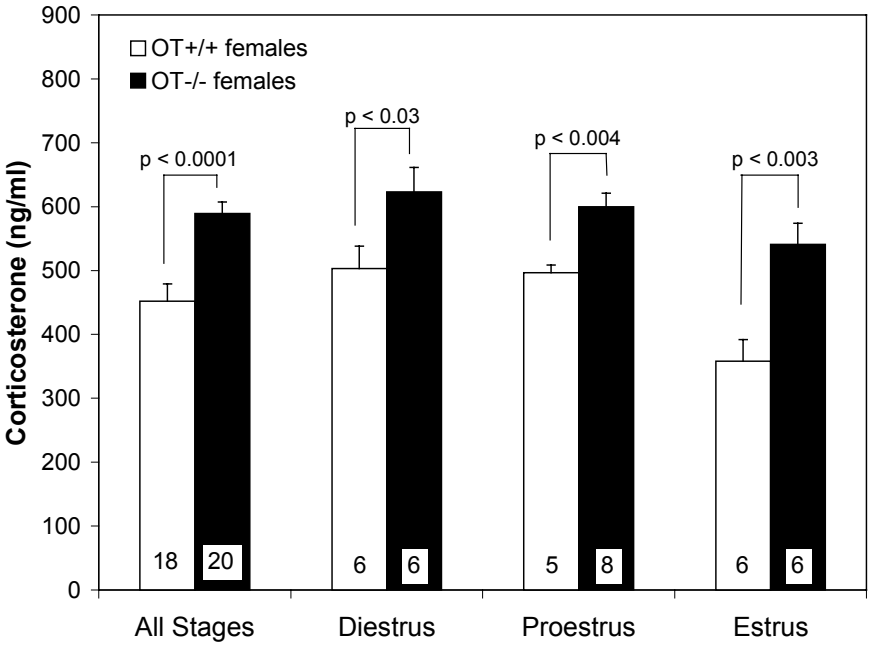

Figure 13. Corticosterone Response to Shaker Stress Across the Estrous Cycle

Plasma corticosterone concentrations were measured across the estrous cycle of female wildtype (OT+/+) and OT deficient (OT-/-) mice following shaker stress. OT-/- mice released more corticosterone than OT+/+ mice when exposed to shaker stress. Genotypic differences in the corticosterone response to shaker stress were not influenced by the stage of the estrous cycle. Corticosterone increase in response to shaker stress was higher in OT-/- compared to OT+/+ mice during the diestrus, proestrus, and estrus stages of the estrous cycle. The number of mice per group is located in or above the data bars. Figure was reprinted with permission from reference [124].

Experiment 6. Corticosterone Response to Shaker Stress in Male Mice

Plasma corticosterone concentrations increased following termination of a 10-minute shaker stress compared to control mice that did not receive shaker stress (ANOVA, $F_{(2,43)}= 218.03$, $p < 0.0001$; Figure 14). Unlike female mice, plasma corticosterone concentrations increased to a similar degree in male OT+/+ and OT-/- mice (ANOVA, $F_{(2,43)} = 0.09$, $p = 0.77$).

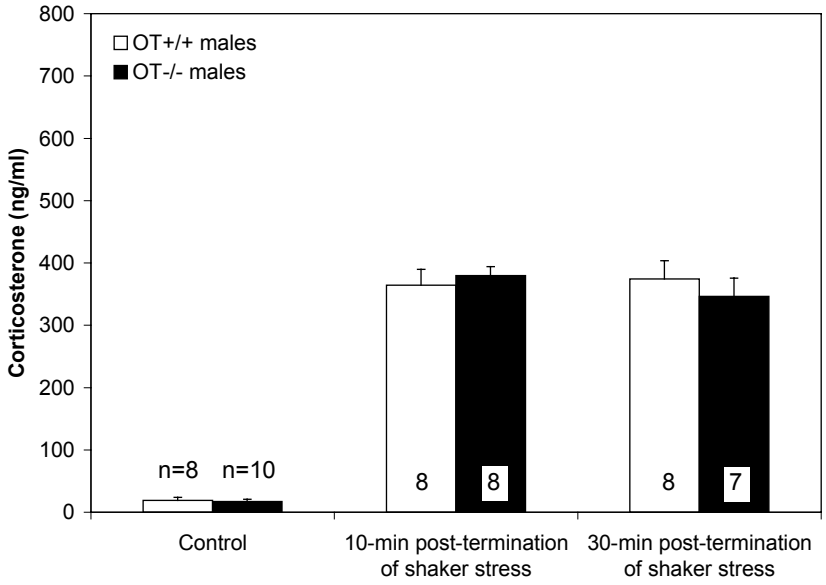

Figure 14. Corticosterone Response to Shaker Stress in Male Mice

Plasma corticosterone concentrations were measured in male wildtype (OT+/+) and OT deficient (OT-/-) mice at 10 min or 30 min following termination of a 10 min shaker stress or in control mice that did not receive shaker stress. Shaker stress resulted in a rise in plasma corticosterone concentrations to a similar degree in female mice of both genotypes (ANOVA, $F_{(2,43)} = 0.09$, $p = 0.77$). The number of mice per group is located in or above the data bars.

2. Physical/Systemic Stress

Experiment 7. Corticosterone Response to Cholecystokinin Administration in Fasted and Non-fasted Male Mice

Intraperitoneal administration of CCK in fasted (ANOVA, $F_{(1,20)}=43.46$, $p<0.0001$; Figure 15) or normal fed (ANOVA,$F_{(1,19)}=145.31$, $p<0.0001$; Figure 16) male mice resulted in an increase in corticosterone release compared to saline treated mice. However, plasma corticosterone concentrations increased to the same degree in OT+/+ and OT-/- fasted (ANOVA, $F_{(1,20)}=0.05$, $p=0.82$) and normal fed (ANOVA, $F_{(1,19)}=0.4$, $p=0.53$) mice.

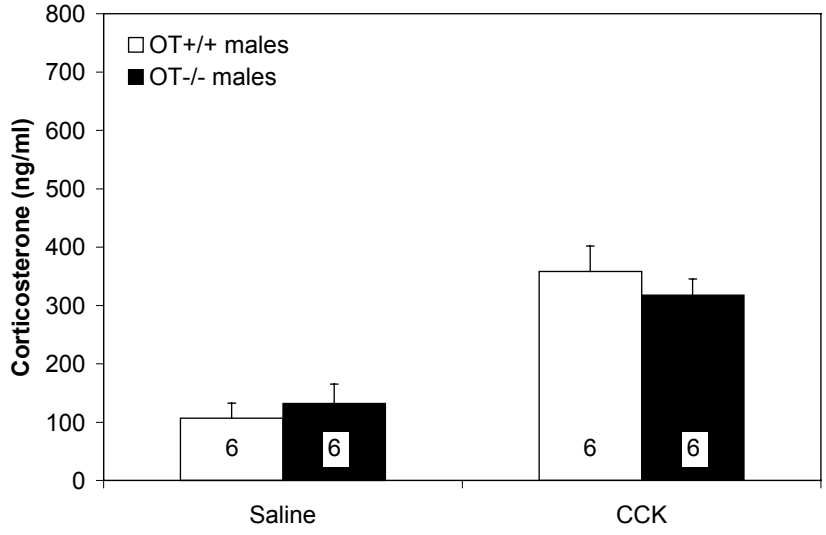

Figure 15. Corticosterone Response to Cholecystokinin Administration in Fasted Male Mice.

Plasma corticosterone concentrations were measured in fasted male wildtype (OT+/+) and OT deficient (OT-/-) mice injected with cholecystokinin (CCK). Intraperitoneal administration of CCK resulted in increased corticosterone release in fasted (ANOVA, $F_{(1,20)}=43.46$, $p<0.0001$) male mice compared to mice administered saline. Plasma corticosterone increased to the same degree in OT+/+ and OT-/- fasted mice (ANOVA, $F_{(1,20)}=0.05$, $p>0.05$). The number of mice per group is located in or above the data bars.

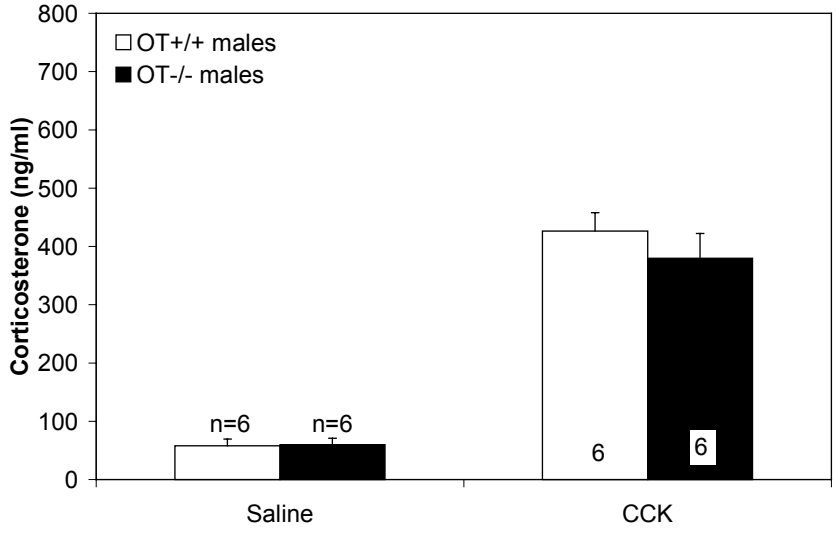

Figure 16. Corticosterone Response to Cholecystokinin Administration in Non-fasted Male Mice.

Plasma corticosterone concentrations were measured in non-fasted male wildtype (OT+/+) and OT deficient (OT-/-) mice injected with cholecystokinin (CCK). Intraperitoneal administration of CCK resulted in increased corticosterone release in non-fasted (ANOVA, $F_{(1,19)}=145.31$, $p<0.0001$) male mice compared to mice administered saline. Plasma corticosterone increased to the same degree in OT+/+ and OT-/- non-fasted mice (ANOVA, $F_{(1,19)}=0.40$, $p>0.05$). The number of mice per group is located in or above the data bars.

C. Discussion

The glucocorticoid (corticosterone) response was measured in male and female OT-/- and OT+/+ mice that were exposed to shaker stress or administered cholecystokinin. Because OT is believed to attenuate the response of the HPA axis to stress, it was hypothesized that OT-/- mice that lack OT would release more corticosterone following stress exposure than OT +/+ mice. This study demonstrated that the absence of OT is associated with higher plasma corticosterone concentrations in female, but not male, mice exposed to shaker stress, a psychogenic stress. However, administration of cholecystokinin, a physical stressor, did not result in enhanced corticosterone release in male OT-/- mice compared to OT+/+ mice. Genotypic differences in corticosterone levels were revealed in response to a psychogenic stressor (shaker), but not a physical stressor (CCK administration). Based on the existing literature examining differences in psychogenic- versus physical-stress induced neuronal activation, it is likely that shaker stress and CCK administration activate distinctly different brain areas. Therefore, the inhibitory role of OT upon HPA activation may be dependent upon the neuronal pathways activated upstream of the HPA axis.

Shaker stress was selected for investigation because it is an a psychogenic stress that has been reported to release corticosterone and OT, but not AVP, in rats [69,142]. Mice, like rats, released corticosterone after shaker stress. If OT is modulatory, mice that do not produce or release OT should display different levels of corticosterone compared to wildtype mice. This was the case, in that female OT-/- mice released more corticosterone than OT+/+ mice in response to shaker stress. Therefore, OT-/- mice, which are not capable of producing OT, displayed an inability to attenuate the HPA axis response to shaker stress.

Exposure to shaker stress resulted in increased corticosterone response in both group and individually housed female OT-/- mice compared to OT+/+ mice. Housing conditions (i.e. group vs. individual) have been shown to influence the stress and anxiety response in rodents. Group housed rats show lower levels of corticosterone [17] and decreased anxiety-related behavior compared to

individually housed rats [148]. Furthermore, OT is a neuropeptide associated with social behavior [220]. Chronic administration of OT into the brains of male rats increased the amount of social interaction with other male rats [214]. To ensure that the genotypic differences in plasma corticosterone were due to shaker stress exposure and not due to social environment, female OT+/+ and OT-/- mice were both group and individually housed prior to testing. Therefore, the enhanced corticosterone release evaluated in female OT-/- mice compared to OT+/+ mice was not due to housing conditions or social environment.

Although the mice in these studies were sacrificed at the same time of day (circadian nadir) to eliminate the problem of diurnal variation, baseline corticosterone levels were measured. This would confirm that the observed genotypic differences in response to stress were due to variations in neuronal processing upstream of the HPA axis and not due to alterations in the physiology of the HPA axis. The peak and nadir circadian concentrations of corticosterone occur during dark and light hours, respectively, in both genotypes and the magnitude of the circadian peak does not differ between genotypes. Because greater variability in basal corticosterone concentrations can be expected at the circadian peak of corticosterone secretion, studies were conducted in the early morning, at the circadian nadir of corticosterone secretion when fluctuations in plasma corticosterone secretion are at a minimum. Higher plasma concentrations of corticosterone in stressed OT-/- versus OT+/+ mice are not likely to be explained by spontaneous plasma corticosterone fluctuation. In support of this concept is the finding that plasma corticosterone concentrations in non-stressed mice did not differ between genotypes. Therefore, OT is not necessary to establish normal diurnal corticosterone production and genotypic differences in the corticosterone response to shaker stress are not due to abnormalities in diurnal variation.

Central administration of CRH into female OT+/+ and OT-/- mice, also confirmed that the physiology of the HPA axis is normal in OT-/- mice. CRH, which is synthesized within the medial parvocellular neurons of the PVN, can stimulate the release of ACTH from the anterior lobe of the

pituitary gland [4,207]. In turn, ACTH signals the adrenal gland to release corticosterone. Therefore, central administration of CRH is a reliable pharmacological stimulus of the HPA axis. Infusion of CRH into the lateral ventricles resulted in increased corticosterone secretion in female mice. Female OT+/+ and OT-/- mice did not display genotypic differences in their corticosterone release in response to CRH administration. This implies that function and activation of the HPA axis is similar in mice of both genotypes. However, it should be noted that central administration of CRH into the lateral ventricles results in increased anxiety-related behavior in mice [133] and rats [149] tested in the elevated plus maze. Therefore, it is difficult to determine if the corticosterone response of the mice tested in this study is due to central CRH administration, CRH-induced anxiety, or a combination of the two. Thus, it may be useful to repeat this study using peripheral administration of CRH to activate ACTH release (and in turn corticosterone) from the anterior pituitary.

Several reports about the adaptation of the HPA axis to repeated stress in rats have been published [70,84,98,121]. Repeated exposure of rats to the same stressor results in the habituation of the corticosterone response to that stressor. The corticosterone response to shaker stress was attenuated if rats were repeatedly exposed to this stress [69]. Similarly, in the present study, mice of both genotypes that were habituated to daily shaker stress for nine days had a lower plasma corticosterone response to shaker stress on the 10^{th} day compared to responses of naïve mice subject to the same stress only once. Despite the attenuated corticosterone response observed in mice of both genotypes, OT-/- mice still had a significantly higher plasma corticosterone response to shaker stress compared to OT+/+ mice. These findings support the view that endogenous OT attenuates the corticosterone response to repeated or acute shaker stress. However, OT by itself cannot account for the attenuation of corticosterone to repeated stress exposure, since habituation was also observed in OT-/- mice.

Importantly the genotypic difference in the corticosterone response following shaker stress was independent of stage of the estrous cycle. OT-/- mice exposed to shaker stress released more

corticosterone than OT+/+ mice during each stage of the estrous cycle. Estrous cyclicity (as determined by vaginal smears) was similar in both genotypes. Therefore, the stress-induced hyper-responsiveness of OT-/- mice compared to OT+/+ mice was present regardless of stage of the cycle. Gonadal steroids are known to affect HPA axis function under basal conditions and following stressful stimuli. Both basal [10,25] and stress-induced release of plasma corticosterone [202] have been reported to vary across the estrous cycle of rats and to be highest at proestrous, the time of maximal estrogen secretion [58]. It was not the primary intent of this study to determine if the magnitude of the stress-induced corticosterone release differed across the estrous cycle of mice. However, this study determined that stress-induced release of corticosterone differed across the estrous cycle of mice. Corticosterone concentrations were lower during estrus than diestrous or proestrous in OT+/+ and OT-/- mice following shaker stress. However, the number of mice per experimental group was not sufficient to determine if stress-induced corticosterone release differed across the estrous cycle of mice of each genotype.

Male mice administered shaker stress had increased plasma corticosterone compared to control mice, but the magnitude of the response was similar in male OT+/+ and OT-/- mice. Male OT-/- mice, unlike female OT-/- mice, do not release more corticosterone than OT+/+ mice to shaker stress. However, the reasons for the sex differences in the corticosterone response to shaker stress are not understood. The HPA axis is sensitive to gonadal steroids. Many studies have shown that female rats display HPA hyperactivity compared to male rats [25,35,112,165] and that HPA hyperreactivity is associated with variations in gonadal hormone levels [102,103,165]. Pretreatment of estrogen to ovariectomized female rats enhances corticosterone secretion following stress [25,55,202]. In contrast, testosterone administration inhibits HPA activation in response to stress [201,203]. OT mRNA expression [150,185] and OT receptor binding [221] are facilitated by estradiol, and areas of the brain involved in the stress response, such as the amygdala, BNST, and the mPOA are hormone responsive. Specifically, estrogen pre-treatment of ovariectomized mice results

in increased OT receptor binding in the amygdala [221] and lateral septum [129,221], both areas that are traditionally activated by psychogenic stressors. Furthermore, regions of high specific binding in the male rat brain, such as the BNST and the central amygdala, display low OT binding in the mouse brain [86]. It is possible that differences in OT receptor expression, and in turn neuronal activation during psychogenic stressors result in these sex differences. Therefore, it is possible that estradiol and/or progesterone modulate OT actions in the stress response of mice.

Because male OT-/- mice that were exposed to a psychogenic stressor did not release more corticosterone than OT+/+ mice, a physical stressor known to activate the OT system in the rat was studied. Peripheral administration of CCK is a potent stimulus of the HPA axis [97], as well as an effective anorexigenic agent [198]. The 10μg/kg dose of CCK has been found to increase ACTH levels [97] and activate OT, but not AVP, neurons in the PVN of rats [142]. Similarly, in male mice CCK was found to increase corticosterone levels. Moreover, this large dose of CCK is capable of producing visceral illness in OT+/+ and OT-/- mice. Although mice do not have a complete emetic center, and do not vomit, they do exhibit behavioral changes. OT+/+ and OT-/- mice exhibited decreased mobility and increased grooming, which is also observed in rats after administration of a nauseogenic [199]. CCK administration resulted in increased corticosterone levels in OT+/+ and OT-/- mice compared to saline treated controls. However, a genotypic difference in the corticosterone response to CCK was not observed. This coincides with the behavioral findings. 1, 3, and 10μg/kg CCK resulted in decreased food intake of fasted OT+/+ and OT-/- mice [122]. However, OT-/- mice consumed equivalent amounts of food as OT+/+ mice following CCK injection. Therefore, OT does not appear to influence the behavioral response or HPA activity in response to choleystokinin administration in male mice.

In summary, the absence of normally functioning OT systems in female OT-/- mice results in increased corticosterone release in response to psychogenic stressors. Therefore, the differences in

stress-induced corticosterone release in female OT+/+ and OT-/- mice are not due to differences in HPA axis function. Morning and evening baseline corticosterone levels were similar in both genotypes. Moreover, CRH administration into the lateral ventricles of female OT+/+ and OT-/- mice resulted in an equivalent increase in plasma corticosterone in both genotypes. Therefore, the differences in stress-induced corticosterone release in female OT+/+ and OT-/- mice are not due to differences in HPA axis function. Moreover, it has been determined that estradiol facilitates OT binding and enhances oxytocinergic actions, but the genotypic differences in corticosterone release following a psychogenic stressor were not dependent upon the stage of the estrous cycle. However it is possible that the inhibitory role of OT in HPA axis activation is dependent upon the presence of estradiol and/or progesterone since similar genotypic corticosterone differences were not found in male mice exposed to psychogenic stressors. Lastly, the inhibitory role of OT on HPA activation is also dependent the type of stressor. While genotypic differences in the corticosterone response were elucidated during exposure to psychogenic stress, similar genotypic differences in the corticosterone response to cholecystokinin-induced stress were not observed. These findings suggest that genetic deletion of OT alters the HPA axis response to specific types of psychogenic stressors in mice. It appears that the effects of OT on the stress response are dependent upon neuronal activation up-stream of the HPA axis. Therefore, further study of the neuronal activation is necessary to determine the role of oxytocinergic pathways on the HPA axis response in male and female mice exposed to psychological and physical stressors. This thesis will attempt to examine the role of oxytocinergic pathways in the stress response (discussed in Chapter V).

IV. Anxiety and the Oxytocin Deficient Mouse

Results of this chapter have been published in part [3,123].

OT is believed to be anxiolytic in female laboratory rats [11,139,211] and mice [129]. Central administration of OT to estrogen-primed ovariectomized rats [211] and mice [129] decreased anxiety-related behavior in the elevated plus-maze (EPM). OT infused into the amygdala of ovariectomized estrogen treated rats resulted in increased open field activity (decreased anxiety) and an increase in the time spent in the open arms of the EPM (anxiolytic effect) [11]. Collectively the data support a possible anxiolytic role for OT in female rats or mice.

To assess the role of central oxytocinergic systems in anxiety-related behavior, OT-/- and OT+/+ mice were studied. If central OT reduces anxiety, then OT-/- mice that lack OT pathways will display greater anxiety-related behavior than OT+/+ mice. Experiments were conducted using the EPM, which has been validated as a test of anxiety in rats [152] and mice [119]. Synthetic OT was administered into the lateral cerebral ventricles of OT -/- mice and their behavior was compared in the EPM with OT -/- mice that received injections of artificial cerebrospinal fluid (aCSF). In addition, OT+/+ mice were tested in the EPM in the presence and absence of a centrally administered competitive OT receptor antagonist, d[Dtyr(Et)^2,Thr^4]ornithine vasotocin (Atosiban), to determine whether endogenous OT was anxiolytic and if the anxiolytic function of OT was at the OT receptor.

A subobjective of the present study was to determine if the anxiety-related behavior of male mice was similar to that of female mice. Male OT-/- mice have been reported to demonstrate less anxiety-like behavior than OT+/+ mice during EPM testing [212]. This observation in male mice contrasts with studies suggesting an anxiolytic effect of OT in female rats [11,211] and mice [129]. Therefore, male mice of each genotype were also tested for anxiety related behavior in the EPM.

A. Experimental Design

Experiment 1. Behavior of Female OT+/+ and OT-/- Mice in the Elevated Plus Maze

The purpose of this experiment was to determine genotype differences in anxiety-related behavior in female mice. OT+/+ (n=7) and OT-/- mice (n=8) were tested in the EPM and the number and duration of entries into open or closed arms were recorded for 5 min.

Experiment 2. Behavior of Female OT+/+ and OT-/- Mice in the Elevated Plus Maze Following Administration of an Oxytocin Antagonist

The purpose of this experiment was to determine if blocking endogenous OT would alter anxiety-related behavior. Atosiban (20 ng, n=7 or 100ng, n= 6) or an equivalent volume of aCSF (n=9) was infused into the lateral ventricles of female OT+/+ mice. Five min post-infusion, mice were placed in the EPM and the number and duration of entries into open or closed arms were recorded for 5 min.

Experiment 3. Behavior of Female OT+/+ and OT-/- Mice in the Elevated Plus Maze Following Administration of Synthetic Oxytocin or Vasopressin

The purpose of this experiment was to determine if administering OT to OT-/- mice would influence anxiety-related behavior and whether the effect is specific for OT. Female OT-/- mice received infusions of OT (2ng, n=9), aCSF (n=8), or AVP (2ng, n=7) into the lateral ventricles. AVP, a peptide closely related to OT, was used as a second control. Five min post-infusion, mice were placed in the EPM and the number and duration of entries into open or closed arms were recorded for 5 min.

Experiment 4. Behavior of Female OT+/+ and OT-/- Mice in the Elevated Plus Maze Following Administration of an Oxytocin Antagonist Prior to Oxytocin

The purpose of this experiment was to determine whether the anxiolytic function of OT was at the OT receptor. On the day of testing, female OT-/- mice (n=8) received central infusions of Atosiban (100ng) followed by a second infusion of OT (2 ng) five minutes later. These doses were chosen based on the findings of *Experiments 2 and 3*. Control OT-/- female mice (n=7) received an infusion of aCSF followed by a second infusion of aCSF five minutes later. Five min post-infusion,

mice were placed in the EPM and the number and duration of entries into open or closed arms were recorded for 5 min.

Experiment 5. Behavior of Male OT+/+ and OT-/- Mice in the Elevated Plus Maze

The purpose of this experiment was to determine genotype differences in anxiety-related behavior in female mice. Naïve OT+/+ (n=8) and OT-/- male mice (n=8) were placed in the EPM and the number and duration of entries into open or closed arms were recorded for 5 min.

B. Results

Experiment 1. Behavior of Female OT+/+ and OT-/- Mice in the Elevated Plus Maze

Female OT-/- mice displayed more anxiety-like behavior than OT+/+ mice in the EPM. The percentage of entries ($p < 0.0002$) and time spent ($p < 0.003$) in the open arms of the EPM was less in OT-/- mice than OT+/+ mice (Figure 17). The increase in anxiety-like behavior was not due to altered locomotor activity, as overall activity in the closed and total arm entries was not different between genotype (Figure 17).

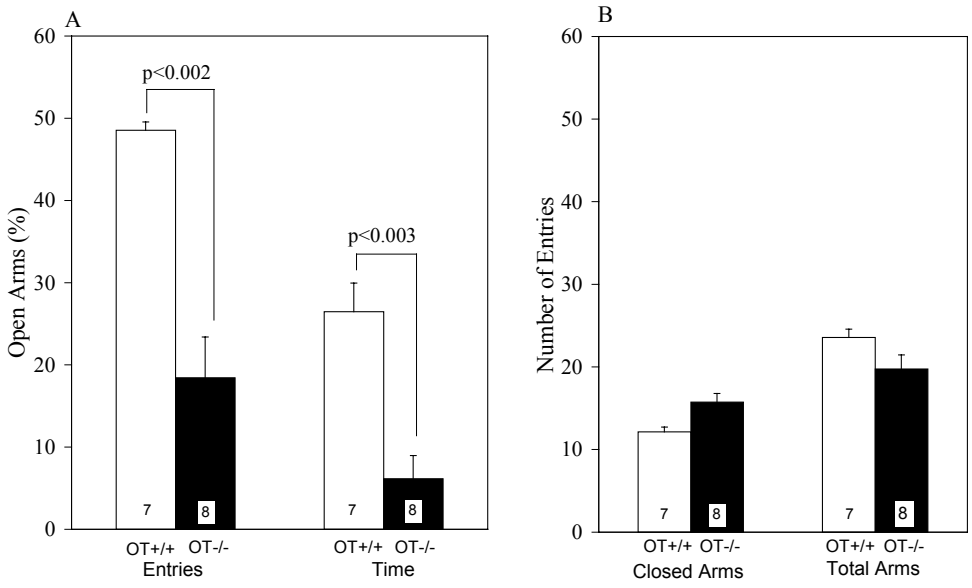

Figure 17. Behavior of Female Wildtype and Oxytocin Deficient Mice in the Elevated Plus Maze.

Behavior was observed and scored in female oxytocin deficient (OT-/-) and wildtype (OT+/+) mice in the elevated plus-maze. (A) The percentage of entries ($p < 0.0002$, 2 tailed *t*-test) and time spent ($p < 0.003$, 2 tailed *t*-test) in the open arms of the maze was less in OT-/- mice than OT+/+ mice. (B) The number of closed arm or total arm entries was not significantly different between genotypes. The number of mice per group is located in or the data bars. Figure was reprinted with permission from reference [123], Copyright 2004, The Endocrine Society.

Experiment 2. Behavior of Female OT+/+ and OT-/- Mice in the Elevated Plus Maze Following Administration of an Oxytocin Antagonist

Administration of an OT receptor antagonist, 20 or 100ng, into the lateral ventricles of female OT+/+ mice increased anxiety-like behavior in OT+/+ mice. The decrease in entries (ANOVA, $F_{(2,19)} = 5.35$, $p < 0.004$) and time spent (ANOVA, $F_{(2,19)} = 3.82$, $p < 0.012$) in the open arms of the plus maze was significant for the 100ng dose (Figure 18). The decrease in anxiety-related behavior was not due to altered locomotor activity because overall activity in the closed and total arm entries were not different between aCSF and Atosiban treated OT+/+ mice (Figure 18).

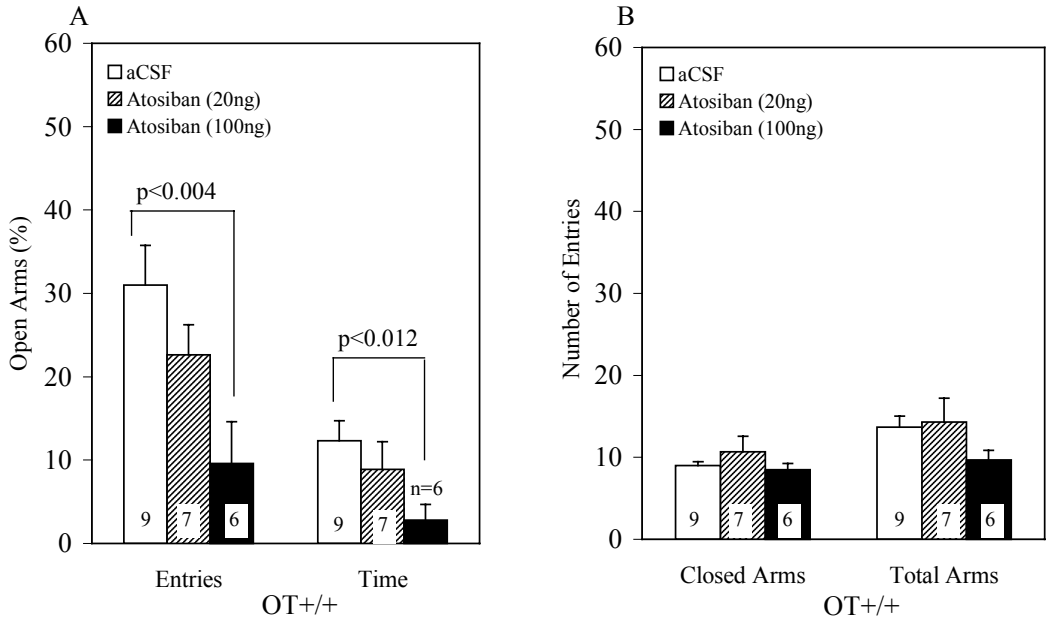

Figure 18. Behavior of Female Wildtype Mice in the Elevated Plus Maze Following Administration of an Oxytocin Antagonist.

(A) Administration of an oxytocin antagonist (Atosiban, 20 and 100ng) into the lateral ventricles of wildtype (OT+/+) mice decreased the percentage of entries (ANOVA, $F_{(2,19)} = 5.35$, $p < 0.004$) and time spent (ANOVA, $F_{(2,19)} = 3.82$, $p < 0.012$) in the open arms of the plus maze in a dose dependent manner. (B) The number of closed arm or total arm entries did not differ between genotype. The number of mice per group is located in the data bars. Figure was reprinted with permission from reference [123], Copyright 2004, The Endocrine Society.

Experiment 3. Behavior of Female OT-/- Mice in the Elevated Plus Maze Following Administration of Synthetic Oxytocin or Vasopressin

Administration of synthetic OT 2ng into the lateral ventricle of female OT-/- mice enhanced the percentage of entries ($p < 0.003$) and time spent ($p < 0.004$) in the open arms of the plus maze compared to OT-/- mice that received aCSF icv (Figure 19). However, administration of AVP 2ng into the lateral ventricle of female OT-/- mice did not alter the percentage of entries or time spent in the open arms of the plus-maze compared to aCSF treated OT-/- mice (Figure 20). Neither OT (Figure 19) nor AVP (Figure 20) impaired locomotor function.

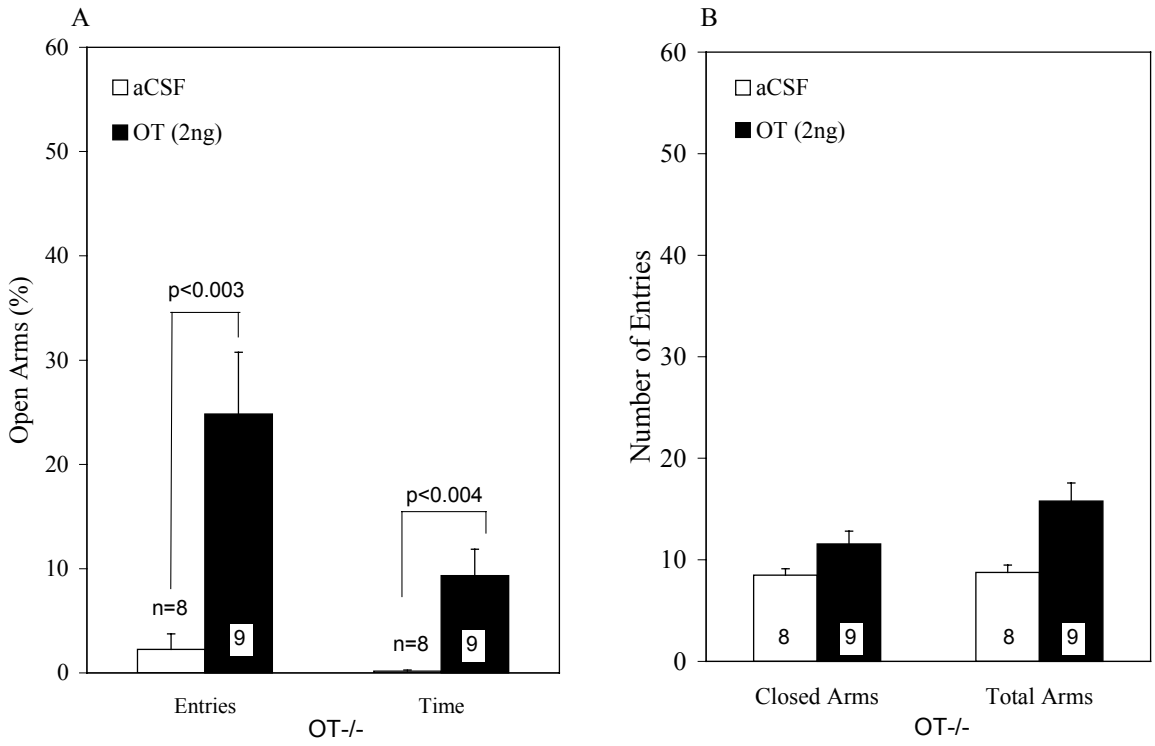

Figure 19. Behavior of Female Oxytocin Deficient Mice in the Elevated Plus Maze Following Administration of Synthetic Oxytocin.

(A) Administration of synthetic oxytocin (OT, 2ng) into the lateral ventricles of oxytocin deficient (OT-/-) mice enhanced the percentage of entries ($p < 0.003$, 2 tailed *t*-test) and time spent ($p < 0.004$, 2 tailed *t*-test) in the open arms of the plus maze compared to OT-/- mice that received aCSF icv. (B) The number of closed arm or total arm entries did not differ between genotype. The number of mice per group is located in or above the data bars. Figure was reprinted with permission from reference [123], Copyright 2004, The Endocrine Society.

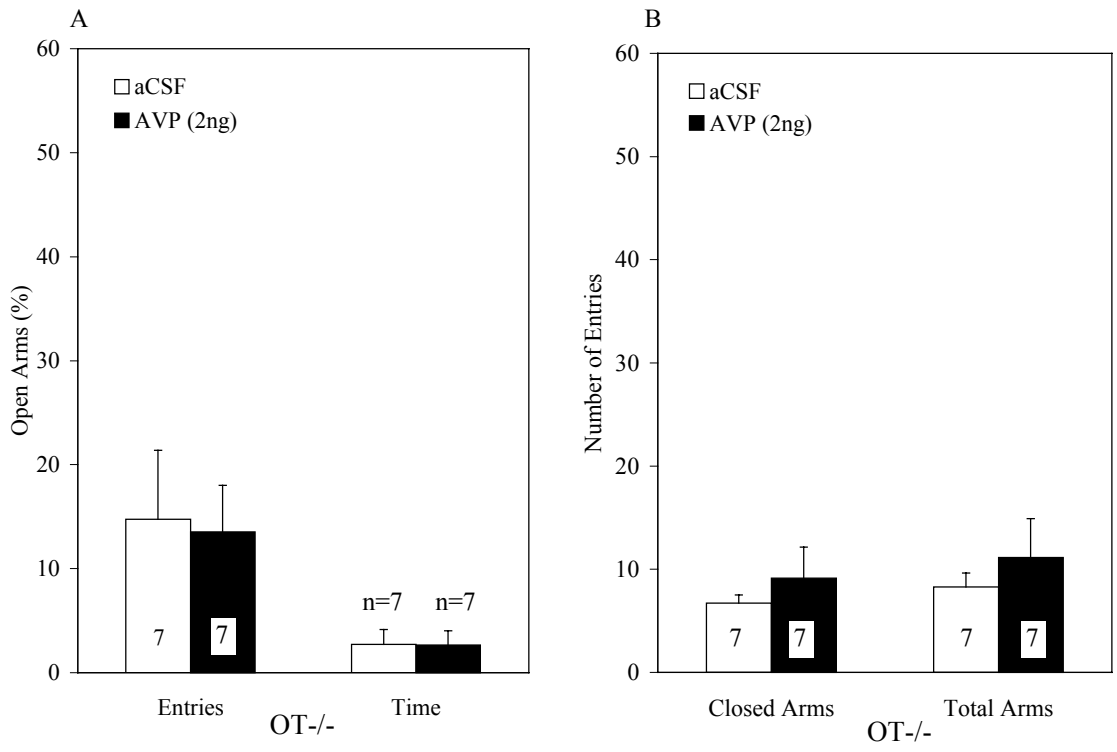

Figure 20. Behavior of Female Oxytocin Deficient Mice in the Elevated Plus Maze Following Administration of Synthetic Vasopressin.

(A) Administration of arginine vasopressin (AVP, 2ng) into the lateral ventricles of oxytocin deficient (OT-/-) mice did not alter the percentage of entries ($p > 0.05$, 2 tailed *t*-test) or time spent ($p > 0.05$, 2 tailed *t*-test) in the open arms of the plus-maze compared to artificial cerebrospinal fluid treated OT-/- mice. (B) Closed arm and total arm entries were not significantly different between female OT+/+ and OT-/- mice. The number of mice per group is located in or above the data bars. Figure was reprinted with permission from reference [123], Copyright 2004, The Endocrine Society.

Experiment 4. Behavior of Female OT-/- Mice in the Elevated Plus Maze Following Administration of an Oxytocin Antagonist Prior to Oxytocin

Central administration of 100ng Atosiban prior to 2ng OT infusion into the lateral ventricles of female OT-/- mice prevented the anxiolytic effects of OT. OT-/- mice treated with Atosiban prior to OT made the same percentage of entries ($p = 0.90$) and spent the same amount of time ($p = 0.72$) in the open arms of the EPM as OT-/- mice infused with aCSF (Figure 21). Overall activity in the closed and total arm entries was not different between mice receiving aCSF and Atosiban followed by OT.

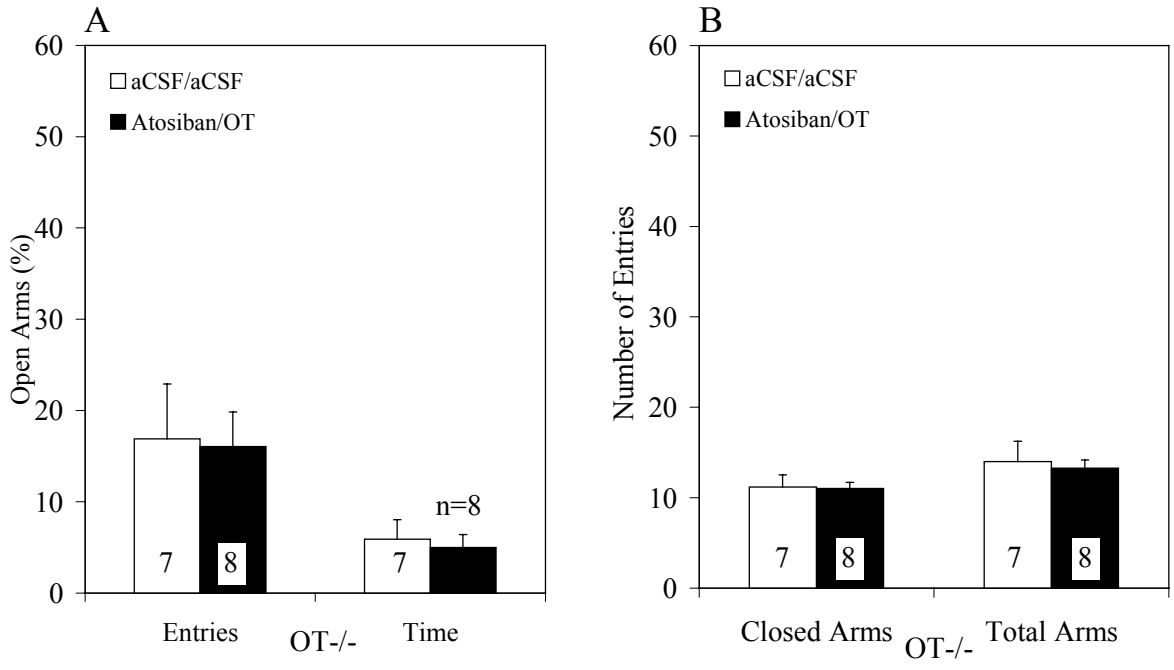

Figure 21. Behavior of Female Oxytocin Deficient Mice in the Elevated Plus Maze After Administration of an Oxytocin Antagonist Followed by Oxytocin.

(A) Oxytocin deficient (OT-/-) mice treated with Atosiban (an oxytocin antagonist, 100ng) prior to oxytocin (OT, 2ng) made the same percentage of entries and spent the same amount of time in the open arms of the elevated plus maze as OT-/- mice treated with artifical cerebrospinal fluid (aCSF). (B) Closed arm and total arm entries were not significantly different between female OT-/- aCSF/aCSF treated mice compared to Atosiban/OT treated mice. The number of mice per group is located in or above the data bars. Figure was reprinted with permission from reference [123], Copyright 2004, The Endocrine Society.

Experiment 5. Behavior of Male OT+/+ and OT-/- Mice in the Elevated Plus Maze

Male OT-/- mice displayed less anxiety-like behavior than OT+/+ mice in the plus maze (Figure 22). The percentage of entries ($p < 0.007$) and time spent ($p < 0.004$) in the open arms of the maze was greater in OT-/- mice than OT+/+ mice. The increase in anxiety-like behavior was not due to altered locomotor activity, as overall activity in the closed and total arm entries were not different between genotype (Figure 22).

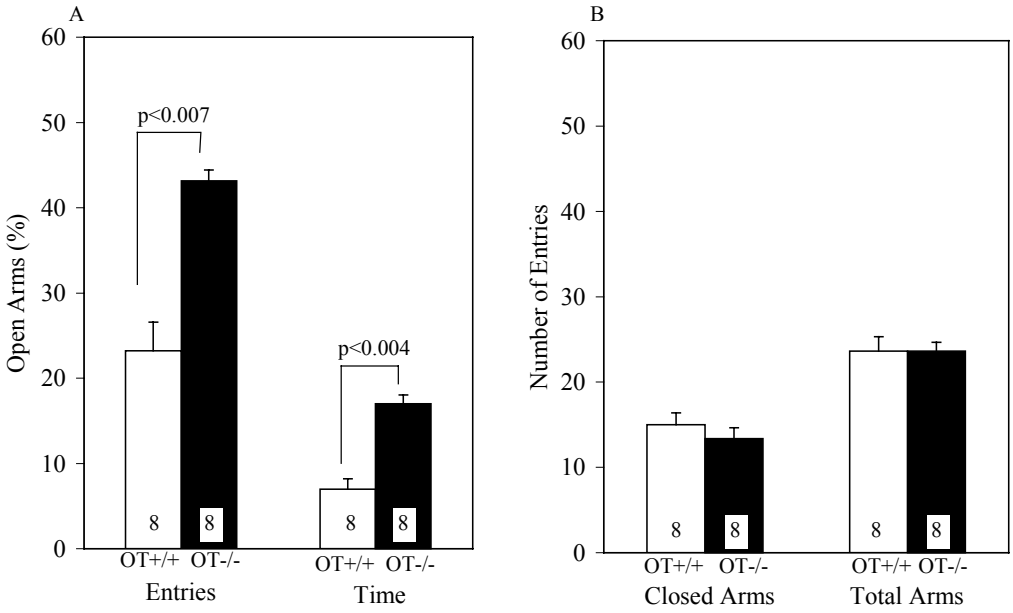

Figure 22. Behavior of Male Wildtype and Oxytocin Deficient Mice in the Elevated Plus Maze.

Behavior was observed and scored in male oxytocin deficient (OT-/-) and wildtype (OT+/+) mice in the elevated plus-maze. (A) The percentage of entries ($p < 0.007$, 2 tailed *t*-test) and time spent ($p < 0.004$, 2 tailed *t*-test) in the open arms of the maze was more in OT-/- mice than OT+/+ mice. (B) The number of closed arm or total arm entries did not differ between genotype. The number of mice per group is located in the data bars.

C. Discussion

This study evaluated the role of OT in modulating anxiety behavior. Because OT is believed to be anxiolytic, it was hypothesized that OT-/- mice would display increased anxiety-related behavior compared to wildtype mice. These experiments demonstrate that female OT-/- mice display greater anxiety-related behavior in the EPM, which is attributed to the absence of functional OT pathways.

Female OT-/- mice spent less time in the open arms of the EPM, an index of anxiety-related behavior, compared to female OT+/+ mice. This observation is consistent with the concept that activation of oxytocinergic neurons and the subsequent release of OT reduces the amount of anxiety-related behavior observed in a novel environment. Thus, female OT-/- mice, which lack the ability to synthesize and release OT, displayed increased anxiety-related behavior.

Intracerebroventricular administration of OT into the lateral ventricles of female OT-/- mice reduced anxiety-related behavior. Furthermore, the findings of this study are consistent with data showing decreased anxiety-related behavior in female rats [211] and mice [129] administered OT centrally and tested in the EPM. In addition, the decrease in anxiety-related behavior in OT-/- mice is OT dependent. Central administration of an equivalent dose of AVP, a peptide closely related to OT, did not alter anxiety-related behavior. This observation suggests that the OT receptor must be activated for OT to exert its anxiolytic affect.

Similarly, central administration of an OT receptor antagonist into the lateral ventricles of female OT+/+ mice decreased the number of open arm entries in the EPM, demonstrating that blockade of endogenous OT with an OT receptor antagonist increased anxiety-related behavior. This is the first time that blockade of endogenous OT in the mouse has been shown to increase anxiety-related behavior in the EPM. Furthermore, infusion of the same OT receptor antagonist into the lateral ventricles of female OT-/- mice prior to administration of synthetic OT blocked the anxiolytic affect of OT in OT-/- female mice, supporting that the anxiolytic affect of OT is mediated via the OT

receptor. The findings in female mice are similar to those of female rats, which displayed an increase in anxiety-related behavior in the EPM following administration of an OT receptor antagonist [139]. Thus, the anxiolytic effect of OT in C57BL/6 mice is mediated by the OT receptor.

A minor concern with this study is that female mice of both genotypes displayed differences in baseline entries and time spent in the open arms of the EPM in *Experiments 1, 2, 3* and *4*. Differences in the experimental design of each study may account for the variations in the baseline behavioral response. Differences in the baseline behavioral response may be due to some mice undergoing cannulation surgeries and receiving central administration of vasopressin, Atosiban, and/or oxytocin. Because of the expected variation in anxiety-related behavior, separate control groups were included for each individual study. Therefore, baseline EPM entries and time spent in the open arms should not be compared between studies.

Male OT-/- mice were tested using the same behavioral task, the EPM. Surprisingly, unlike female OT-/- mice, male OT-/- mice displayed decreased anxiety-like behavior in the EPM and the open field compared to male OT+/+ mice. Winslow and colleagues also reported decreased anxiety-related behavior in male OT-/- mice compared to male OT+/+ mice in the EPM [212]. Furthermore, in male rats tested in the EPM, infusion of an OT receptor antagonist decreased anxiety-related behavior in the EPM [139]. This study and those of others do not support an anxiolytic role for OT in male rats or mice tested in the EPM.

Areas of the brain involved in anxiety processing, such as the BNST, amygdala and mPOA are hormone responsive. OT mRNA expression [150,185] and OT receptor binding [221] are facilitated by estradiol. Furthermore, regions of high specific binding in the male rat brain, such as the BNST and the central amygdala, display low OT binding in the mouse brain [86]. Differences in OT receptor function and in turn neuronal activation during anxiety result in sex and genotype differences. The interaction between gonadal hormones and OT function in response to anxiety can be evaluated by testing male and female OT+/+ and OT-/- mice with the elevated plus maze in the

presence or absence of gonadal hormones. A similar study has been performed by McCarthy *et al*. [129]. Estrogen-primed ovariectomized mice that were systemically administered OT displayed decreased anxiety-like behavior in the EPM compared to mice receiving estrogen alone, OT alone, or neither estrogen nor OT [129]. In addition, central administration of OT into the lateral ventricles of estrogen-primed ovariectomized mice also resulted in decreased anxiety-related behavior in the EPM compared to ovariectomized that received OT without estrogen [129]. Therefore, the anxiolytic role of OT may be dependent upon the presence of estradiol and/or progesterone.

In summary, these findings indicate that oxytocinergic pathways play an anxiolytic role in female mice tested in the EPM. The absence of normally functioning OT systems in an OT deficient mouse results in increased anxiety-related behavior. The enhanced anxiety was reversed by central administration of exogenous OT. Blockade of endogenous OT receptors via central administration of an OT receptor antagonist to OT+/+ female mice resulted in increased anxiety-related behavior in the EPM. In addition, infusion of an OT receptor antagonist prior to administration of exogenous OT in OT-/- female mice inhibited the anxiolytic effects of OT. These findings suggest that genetic, gonadal steroid, and/or pharmacological interruption of OT may inhibit activation of OT receptors in brain regions involved in the anxiety response.

V. Forebrain Activation of the OT Deficient Mouse in Response to Stress and Anxiety

Results of this chapter have been published in part [3,124].

Neural circuits that coordinate the stress and anxiety response to a stimulus include projections from the amygdala, lateral septum, and hippocampus to the medial parvocellular paraventricular nucleus of the hypothalamus (PVN; the site of neurons that release corticotrophin releasing hormone) via a neural circuit that involves synapses in the bed nucleus of the stria terminalis (BNST) and the medial preoptic area of the hypothalamus (mPOA) [23,73,77,161]. OT immunoreactive neurons originating in the PVN project to the BNST [33,82], and a few OT immunoreactive fibers have been identified in the amygdala [33,82]. In addition, the projections of OT neurons correspond to the location of OT receptors in the limbic system and include the BNST [85], CeA [85], MeA [86], lateral septum [63,85,86], mPOA [63], and hippocampus [85,86]. OT and its receptor are located in brain areas that modulate the hypothalamic pituitary adrenal (HPA) axis response to stress and anxiety. Although a number of these areas participate in regulating both anxiety behavior and HPA axis activity to stressful stimuli, it is not known whether OT modulates the actions of these brain areas during the stress and anxiety response.

Many studies have explored the central pathways mediating stress and anxiety by mapping neuronal activation using the proto-oncogene protein product Fos, a marker of neuronal activation. The Fos gene is expressed immediately (within minutes) in response to extracellular stimuli and plays an important role in signal transduction and transcription regulation in cells. In 1988 it was discovered that neurons also express Fos when stimulated [170] and in 1989 the first application of Fos to stress studies was made [26]. Studies have demonstrated forebrain patterns of Fos expression in response to various stress and anxiety models, such as restraint [6,26,36,81], swim stress [36], foot shock [156], and anxiety [45]. Therefore, to define potential OT-sensitive stress and anxiety circuitry in the forebrain, the effect of OT on stress- and anxiety-induced Fos activation was examined in male

and female mice exposed to shaker stress, cholecystokinin administration, or elevated plus maze exposure. The brain areas examined for Fos activation were limited to limbic stress pathways, specifically the CeA, MeA, BNST, and mPOA (Figure 23). These brain areas were chosen because they contain OT neurons and receptors. In addition, the amygdala plays a critical role in modulating the stress response. The amygdala has very few direct projections to the PVN. Therefore, the amygdala projects to the BNST and mPOA, which are PVN-projecting brain areas. Therefore, it is likely that OT mediates the stress and anxiety responses through this pathway.

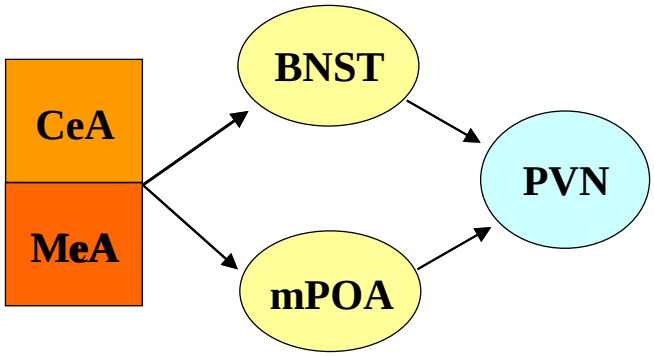

Figure 23. Stress-Related Brain Pathways Examined for Fos Activation.

Bed nucleus of the stria terminalis (BNST), central nucleus of the amygdala (CeA), medial nucleus of the amygdala (MeA), medial preoptic area (mPOA), paraventricular nucleus of the hypothalamus (PVN).

A. Experimental Design

Experiment 1. Shaker Stress Induced Fos Expression in Stress-Related Forebrain Pathways

The purpose of this experiment was to evaluate genotypic differences in Fos expression in hypothalamic and forebrain areas associated with the stress response to psychogenic stress. Naive female and male OT+/+ and OT-/- mice were exposed to shaker stress for 10 min (n=3 of each sex) and returned to their home cages. Sixty to seventy-five minutes later mice were anesthetized, perfused, and brains were harvested for immunocytochemistry. Control mice (n=3 of each sex), left undisturbed in their home cage, were perfused at the same time.

Experiment 2. Cholecystokinin Induced Fos Expression in Stress-Related Forebrain Pathways

The purpose of this experiment was to evaluate genotypic differences in Fos expression in hypothalamic and forebrain areas associated with the stress response to a physical/systemic stressor. Naive female and male OT+/+ and OT-/- mice were administered 10μg/kg CCK, intraperitoneally (n=3 of each sex) or the same volume of saline (n=3 of each sex). Sixty to seventy-five minutes later mice were anesthetized, perfused, and brains were harvested for immunocytochemistry.

Experiment 3. Elevated Plus Maze Exposure Induced Fos Expression in Anxiety-Related Forebrain Pathways

The purpose of this experiment was to evaluate genotype differences in Fos expression in hypothalamic and forebrain areas associated with anxiety-related behavior. Naïve OT+/+ and OT-/- female and male mice were placed in the elevated plus maze for 5 minutes and returned to their home cages. Approximately one-hour later mice were anesthetized, perfused, and brains were harvested for immunocytochemistry. Control mice (n=3 of each sex) that were not exposed to the EPM were also perfused at the same time.

B. Results

Experiment 1. Shaker Stress Induced Fos Expression in Stress-Related Forebrain Pathways

Ten minutes of shaker stress robustly activated Fos in the PVN of female OT+/+ and OT-/- mice compared to control mice. As expected, similar numbers of OT-immunoreactive neurons were counted in the PVN of control OT+/+ mice (99.69 $\pm$ 3.90 OT cells per section) and in OT+/+ mice exposed to shaker stress (92.14 $\pm$ 12.53 OT cells per section; $p > 0.05$). OT immunoreactive neurons were not observed in OT-/- mice (Figure 24 A and B). Shaker stress activated Fos in a small, but significant subset of OT positive magno- and/or parvocellular PVN neurons in OT+/+ mice ($p < 0.007$; Figure 24 F). Fos activation did not increase in the SON of either OT+/+ or OT-/- mice after shaker stress exposure (Figure 24 G and H).

Control (OT+/+, 111.6±3.2 cells per section; OT-/- 89.6±9.1 cells per section) and shaker stress exposed mice (OT+/+, 97.27+1.7 cells per section; OT-/- 97.0+18.0 cells per section) of both genotype displayed similar number of AVP immunoreactive neurons in the PVN of the hypothalamus (ANOVA, $F_{(1,8)}$=1.186, $p > 0.05$). In contrast to stressor-induced activation of OT neurons in OT +/+ mice, AVP-positive PVN neurons were not activated after shaker stress in mice of either genotype (Figure 25 A-F). In addition, Fos activation was specific to the PVN and did not increase in the SON of OT+/+ and OT-/- mice exposed to shaker stress. (Figure 25 G and H)

Control (OT+/+, 116±1 CRH cells per section; OT-/-, 108.96±2.29 CRH cells per section) and shaker stress exposed mice (OT+/+, 124.17+7.62 CRH cells per section; OT-/-, 126.97±12.99) did not display genotypic differences in the amount of CRH-immunoreactive cells in the PVN of the hypothalamus (ANOVA, $F_{(1,6)} = 3.884$, $p > 0.05$). Significantly greater numbers of CRH-positive PVN neurons were activated to express Fos in OT+/+ (ANOVA, $F_{(1,3)} = 62.25$, $p < 0.004$) and OT-/- mice (ANOVA, $F_{(1,3)} = 44.81$, $p < 0.001$) after shaker stress compared to activation in control mice (Figure 26). The number of CRH neurons expressing Fos after shaker stress was not different in OT-/- mice compared to OT+/+ mice (ANOVA, $F_{(1,8)} = 6.72$, $p = 0.06$; Figure 26).

Female control OT+/+ and OT-/- mice displayed similar amounts of Fos immunoreactivity in the BNST, MeA, CeA, and mPOA. Ten minutes of shaker stress significantly increased the number of Fos-positive neurons in each of the forebrain regions evaluated compared to control mice of both genotypes. However, a statistically significant effect of genotype on stress-induced Fos expression was observed in the MeA but not in the other forebrain regions (Figure 27). Stress-induced activation of MeA was lower in OT-/- mice compared to activation in OT+/+ mice exposed to shaker stress (ANOVA, $F_{(1,8)}$ =9.379, $p < 0.02$; Figure 27).

Like female mice, similar numbers of OT-immunoreactive neurons were counted in the PVN of male control OT+/+ mice (101.33 ± 13.03 OT cells per section) and in OT+/+ mice exposed to shaker stress (102.03 ± 10.73 OT cells per section; $p > 0.05$). OT-immunoreactive neurons were not

observed in OT-/- mice. Ten minutes of shaker stress also activated Fos in the PVN of male mice of both genotype compared to Fos activation in control mice. Shaker stress activated Fos in a small subset of OT positive magno- and/or parvocellular neurons of the PVN ($p < 0.001$; Figure 28). Fos activation did not increase in the SON in either male OT+/+ or OT-/- mice after shaker stress.

Control (OT+/+, 114.36±8.34 cells per section; OT-/- 96.86±9.28 cells per section) and shaker stress exposed mice (OT+/+, 123.80±23.4 cells per section; OT-/- 130.55±25.15 cells per section) of both genotype displayed similar number of AVP immunoreactive neurons in the PVN of the hypothalamus (ANOVA, $F_{(1,8)}=0.086$, $p > 0.05$). Similar to female mice, AVP-positive neurons of the PVN and SON were not activated in male mice.

Control (OT+/+, 76.03 ± 8.15 CRH cells per section; OT-/-, 69.00 ± 4.00 CRH cells per section) and shaker stress exposed male mice (OT+/+, 75.00 ± 5.00 CRH cells per section; OT-/-, 86.94 ± 5.53 CRH cells per section) did not display genotypic differences in the amount of CRH-immunoreactive cells in the PVN of the hypothalamus (ANOVA, $F_{(1,6)} = 0.131$, $p > 0.05$). Shaker stress activated Fos in CRH neurons of the PVN in male OT+/+ (ANOVA, $F_{(1,3)} = 2021.62$, $p < 0.0001$) and OT-/- mice (ANOVA, $F_{(1,3)} = 172.44$, $p < 0.001$) compared to control mice (Figure 29). The number of CRH neurons expressing Fos after shaker stress was not different in OT-/- mice compared to OT+/+ mice (ANOVA, $F_{(1,6)} = 0.85$, $p >0.05$).

Based on the findings of Fos activation in the forebrains of female mice exposed to shaker stress, Fos was quantified in the MeA of male mice. Compared to control mice of both genotypes, shaker stress significantly activated Fos in the MeA (ANOVA, $F_{(1,8)} = 31.009$, $p < 0.0005$) of male mice. Unlike female mice, the number of Fos immunoreactive cells was not different in the MeA of male OT-/- and OT+/+ mice exposed to shaker stress (ANOVA, $F_{(1,8)} = 1.970$, $p > 0.05$).

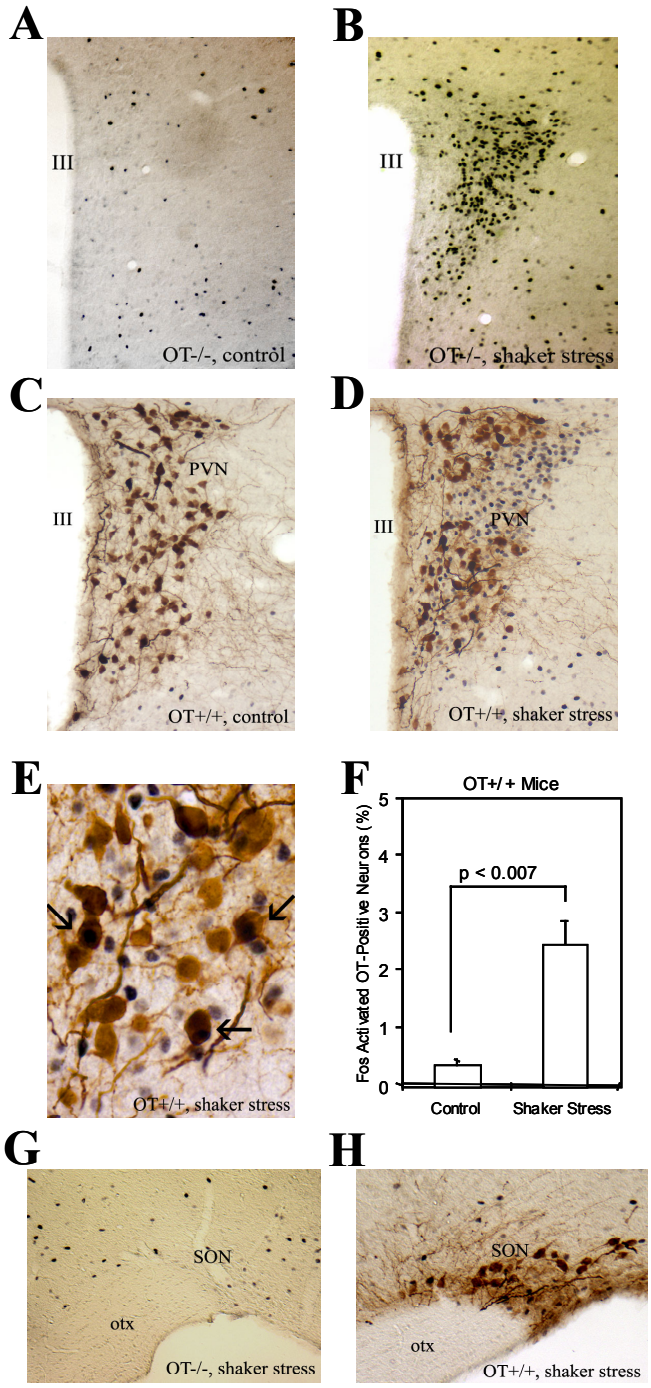

Figure 24. Shaker Stress-Induced Fos Activation in Oxytocin Neurons of the Hypothalamus of Female Mice

Color photomicrographs illustrating Fos immunostaining (blue-black nuclei) in the paraventricular nucleus (PVN) of control OT-/- (A) and OT+/+ (C) mice and OT-/- (B) and OT+/+ (D) mice exposed to shaker stress. Tissue sections are double-labeled for Fos and oxytocin (OT, brown cells). (E) Arrows indicate cells that are double-labeled for Fos and OT. (F) Bar graph depicting the percent of Fos positive OT neurons within the PVN. Exposure to shaker significantly increased Fos expression in OT positive neurons of the PVN. However, exposure to shaker stress did not result in increased Fos immunostaining in the supraoptic nucleus (SON) of OT-/- (G) or OT+/+ mice (H). III, third ventricle; otx, optic tract. Figure was reprinted with permission from reference [124].

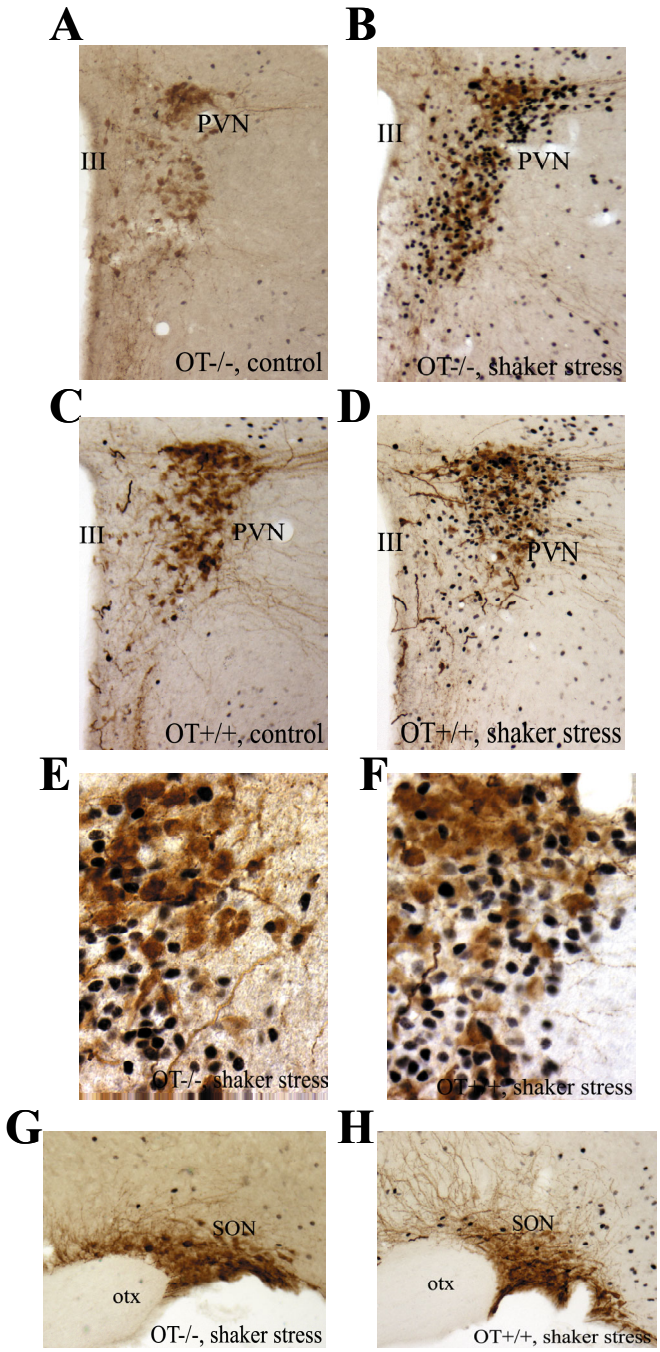

Figure 25. Shaker Stress-Induced Fos Activation in Vasopressin Neurons of the Hypothalamus of Female Mice

Color photomicrographs illustrating Fos immunostaining (blue-black nuclei) in the paraventricular nucleus (PVN) of control OT-/- (A) and OT+/+ (C) mice and OT-/- (B) and OT+/+ (D) mice exposed to shaker stress. Tissue sections are double-labeled for Fos and arginine vasopressin (AVP, brown cells). Exposure to shaker did not increase Fos expression in AVP positive neurons of the PVN of OT-/- (E) or OT+/+ mice (F). In addition, exposure to shaker stress did not result in increased Fos immunostaining in the supraoptic nucleus (SON) of OT-/- (G) or OT+/+ mice (H). III, third ventricle; otx, optic tract. Figure was reprinted with permission from reference [124].

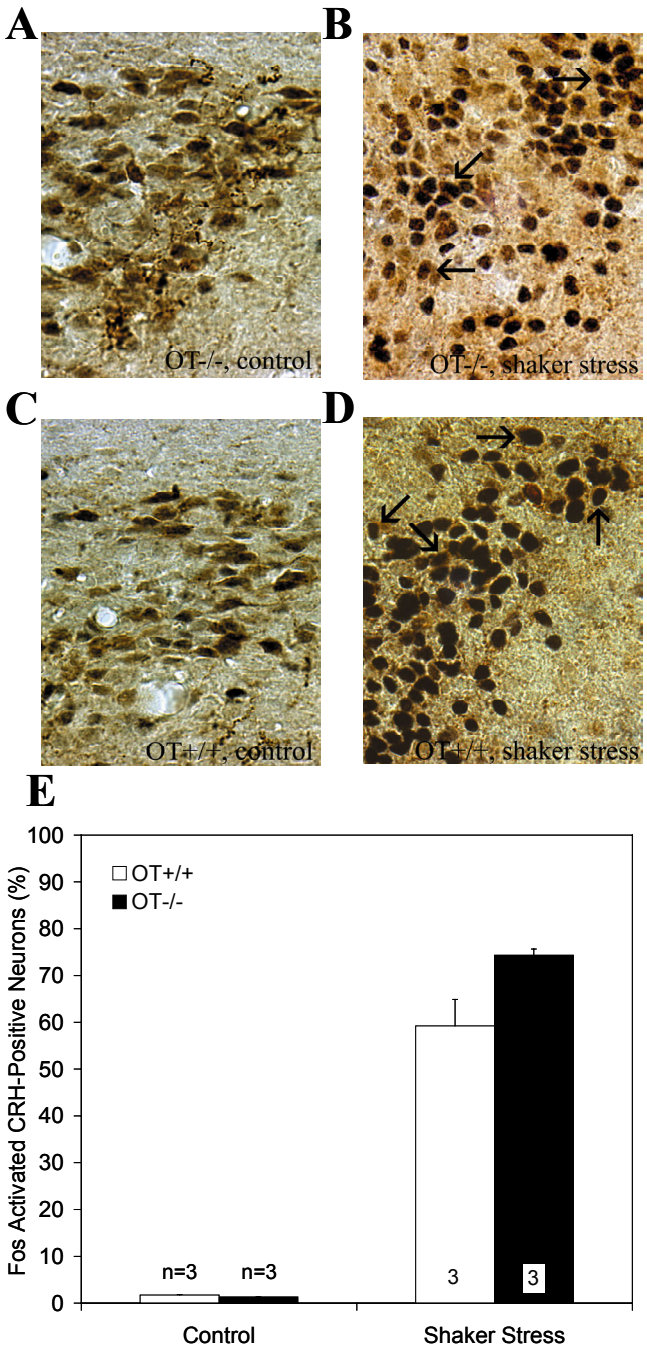

Figure 26. Shaker Stress-Induced Fos Activation in Corticotropin Releasing Hormone Neurons of the Hypothalamus of Female Mice.

Color photomicrographs illustrating Fos immunostaining (blue-black nuclei) in the paraventricular nucleus (PVN) of control OT-/- (A) and OT+/+ (C) mice and OT-/- (B) and OT+/+ (D) mice exposed to shaker stress. Tissue sections are double-labeled for Fos and corticotropin releasing hormone (CRH, brown cells). Arrows indicate cells that are double-labeled for Fos and CRH in OT-/- (B) and OT+/+ mice (D). (E) Bar graph depicting the percent of Fos positive CRH neurons within the PVN. Exposure to shaker significantly increased Fos expression in CRH positive neurons of the PVN in OT+/+ and OT-/- mice (ANOVA, $F_{(1,3)} = 62.25$, $p<0.004$). There was not a genotypic difference in the number of Fos positive CRH neurons in response to shaker stress (ANOVA, $F_{(1,8)} = 6.72$, $p = 0.06$). The number of mice per group is located in or above the data bars. Figure was reprinted with permission from reference [124].

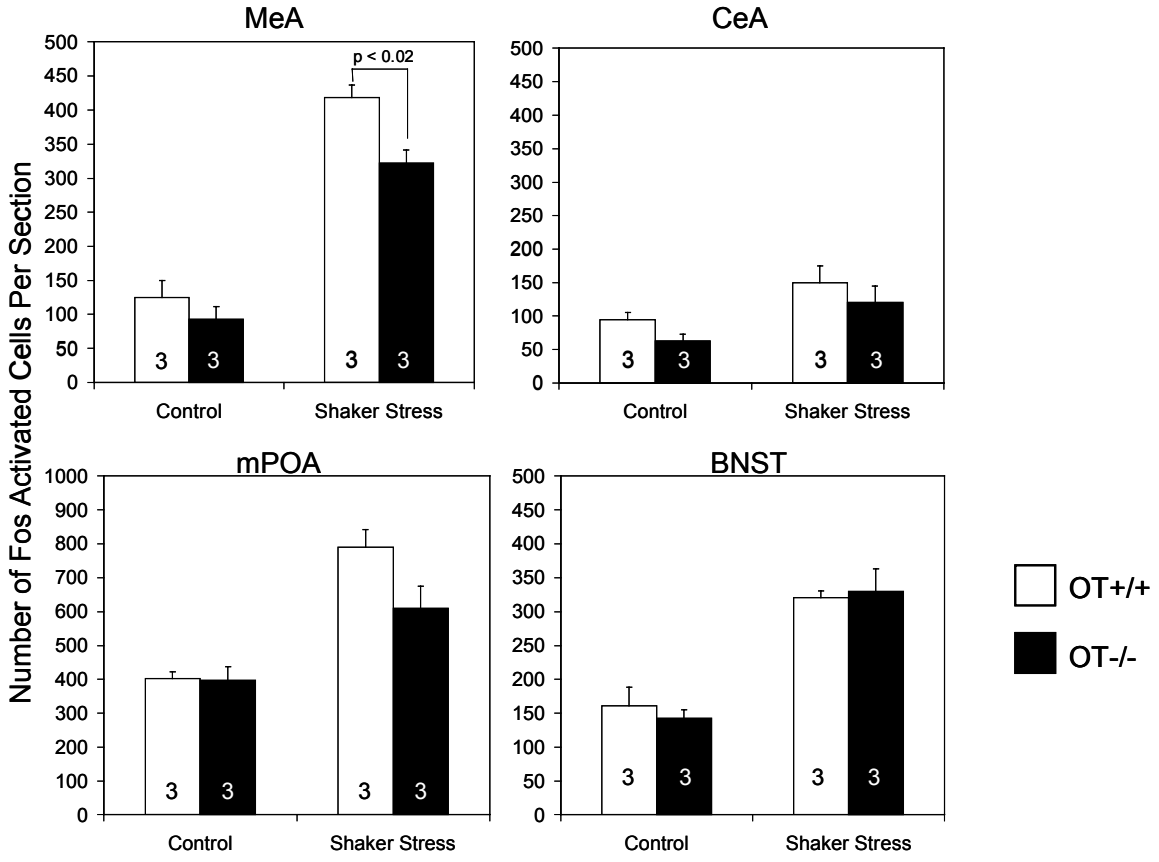

Figure 27. Shaker Stress Induced Neural Activation of the Limbic Forebrain of Female Mice

Shaker stress significantly activated Fos in the bed nucleus of the stria terminalis (BNST; ANOVA, $F_{(1,8)} = 54.75$, $p < 0.0001$), medial (MeA; ANOVA, $F_{(1,8)} = 159.97$, $p < 0.0001$) and central (CeA; ANONA, $F_{(1,8)} = 9.11$, $p < 0.02$) nuclei of the amygdala, and medial preoptic area (mPOA; ANOVA, $F_{(1,8)} = 39.74$, $p < 0.0002$). Shaker stress significantly activated Fos in all the brain areas evaluated compared to control mice of both genotypes. The number of Fos immunoreactive cells was lower in the MeA of OT-/- mice than OT+/+ mice exposed to shaker stress. The number of mice per group is located in the data bars. Portions of this figure were reprinted with permission from reference [124].

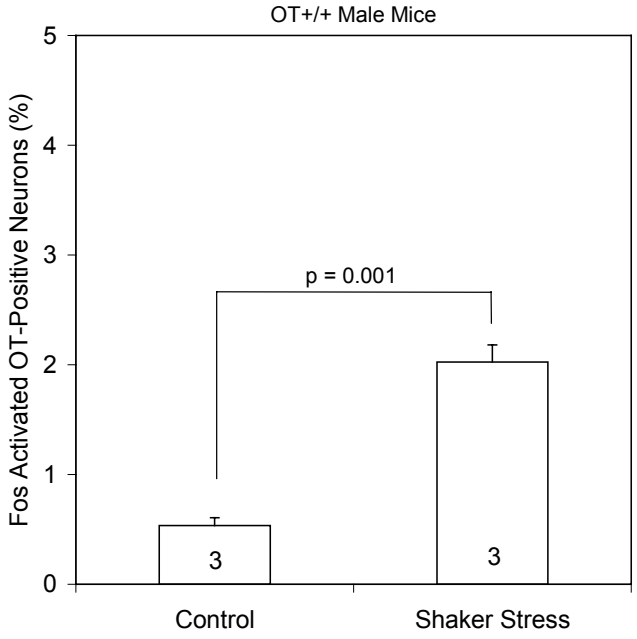

Figure 28. Fos Activation of Oxytocin Immunoreactive Neurons in the Paraventricular Nucleus of the Hypothalamus of Wildtype Male Mice.

Exposure to shaker stress significantly increased Fos expression in oxytocin (OT) positive neurons of the paraventricular nucleus of the hypothalamus PVN of wildtype (OT+/+) mice ($p < 0.001$). The number of mice per group is located in the data bars.

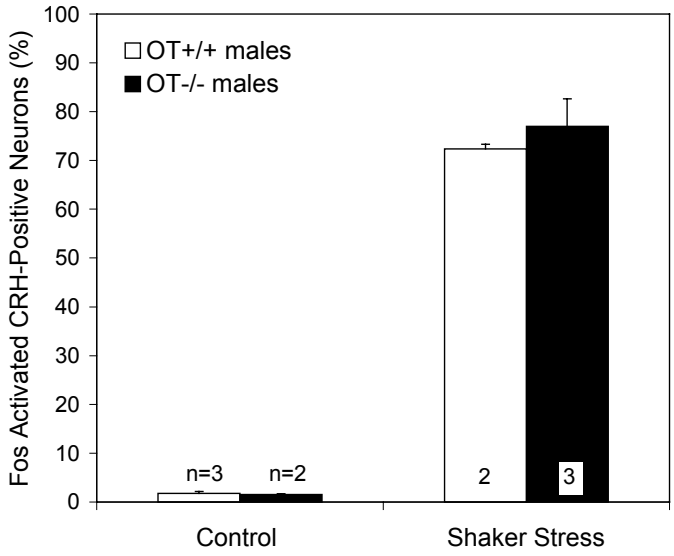

Figure 29. Fos Activation of Corticotropin Releasing Hormone Immunoreactive Neurons in the Paraventricular Nucleus of the Hypothalamus of Male Mice

Exposure to shaker stress significantly increased Fos expression in corticotropin releasing hormone neurons (CRH) positive neurons of the paraventricular nucleus of the hypothalamus (PVN) of wildtype (OT+/+; ANOVA, $F_{(1,3)} = 65.89$, $p < 0.0001$) and OT deficient (OT-/-; ANOVA, $F_{(1,3)} = 108.55$, $p < 0.002$) compared to control mice. There was not a genotypic difference in the number of Fos positive CRH neurons in response to shaker stress. The number of mice per group is located in or above the data bars.

Experiment 2. Cholecystokinin Induced Fos Expression in Stress-Related Forebrain Pathways

Peripheral administration of CCK activated Fos in OT positive magno and/or parvocellular neurons of the PVN of OT+/+ mice compared to saline treated controls ($p < 0.009$; Figure 30). Similar numbers of OT-immunoreactive neurons were counted in the PVN of control OT+/+ mice (85.52+8.19 cells per section) and in OT+/+ mice administered CCK (74.31±1.26 cells per section; $p < 0.05$). OT immunoreactive neurons were not observed in OT-/- mice.

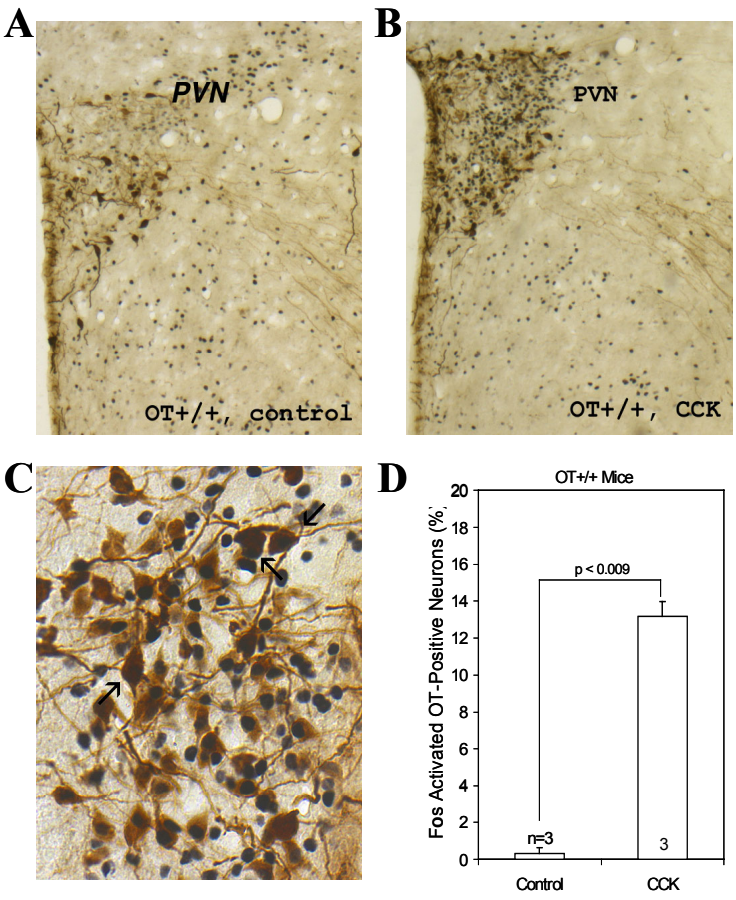

Figure 30. Cholecystokinin Induced Fos Activation in Oxytocin Neurons of the Paraventricular Nucleus of Wildtype Male Mice

Color photomicrographs illustrating Fos immunostaining (blue-black nuclei) in the paraventricular nucleus of the hypothalamus (PVN) of control (A) and cholecystokinin-administered (CCK; B) OT+/+ mice. (C) Arrows indicate cells that are double-labeled for Fos and OT in OT+/+ mice administered CCK. (D) Bar graph depicting the percent of Fos positive OT neurons within the PVN ($p < 0.009$). The number of mice per group is located in or above the data bars.

Control (OT+/+, 112.67±3.34 cells per section, OT-/-, 112±4 cells per section) and CCK-administered mice (OT+/+, 104.28±6.75 cells per section, OT-/-, 105,27±0.82 cells per section) of both genotype displayed similar numbers of AVP-immunoreactive neurons in the PVN of the hypothalamus. Furthermore, CCK administration did not activate AVP-positive neurons of the PVN (Figure 31).

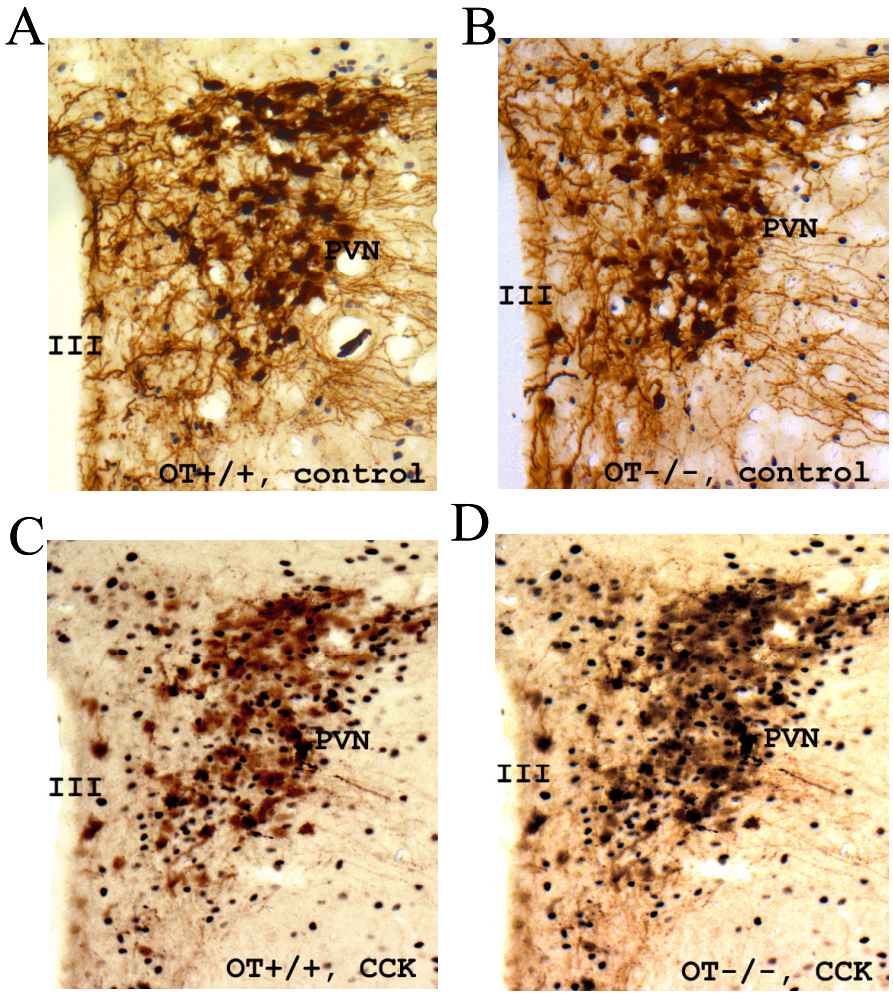

Figure 31. Cholecystokinin Induced Fos Activation in Vasopressin Neurons of the Hypothalamus of Male Mice

Color photomicrographs illustrating Fos immunostaining (blue-black nuclei) in the paraventricular nucleus (PVN) of control OT-/- (A) and OT+/+ (B) mice and OT-/- and (C) OT-/- and OT+/+ mice administered cholecystokinin (CCK) (D). Tissue sections are double labeled for Fos and arginine vasopressin (AVP; brown cells). CCK administration did not result in increased Fos expression in AVP positive neurons of the PVN of OT+/+ or OT-/- mice.

Control (61.67±2.03 cells per section) and CCK-administered mice (74.75±6.38) did not display treatment differences in the amount of CRH-immunoreactive cells in the PVN of the hypothalamus (ANOVA, $F_{(1,3)}=0.49$, $p > 0.05$). Mice that received CCK administration displayed increased Fos activation in CRH-positive neurons of the PVN mice compared to control mice (ANOVA, $F_{(1,3)}=58.72$, $p < 0.005$). However, it was not possible to determine whether there were genotypic differences in CRH neuron activation in response to CCK administration in this study.

Qualitative assessment of the PVN of female OT-/- and OT+/+ mice revealed similar findings to those of male mice. CCK-administration activated Fos in OT positive magnocellular and parvocellular neurons of the PVN of OT+/+ mice compared to saline treated controls. Furthermore, CCK administration activated AVP- and CRH-positive neurons in the PVN of mice of both genotypes. However, genotypic differences in the activation of CRH or AVP neurons in response to CCK administration were not observed in female mice. Peripheral administration of CCK to female mice significantly activated Fos in the BNST (ANOVA, $F_{(1,6)}=90.32$, $p<0.0001$), CeA (ANOVA, $F_{(1,6)}=135.9$, $p<0.0001$), and mPOA (ANOVA, $F_{(1,6)}=42.73$, $p<0.0006$), but not the MeA ($p > 0.05$) compared to control mice of both genotypes (Figure 32). However, female OT+/+ and OT-/- mice displayed similar amounts of Fos activation in the BNST, CeA, and mPOA following CCK administration.

Peripheral administration of CCK to male mice also resulted in increased Fos expression in the BNST (ANOVA, $F_{(1,6)}=9.91$, $p<0.02$) and the CeA (ANOVA, $F_{(1,7)}=40.54$, $p<0.02$), but not the MeA or the mPOA (Figure 33). Male OT+/+ and OT-/- mice displayed similar numbers of Fos immunoreactive cells in the BNST and the CeA following CCK administration.

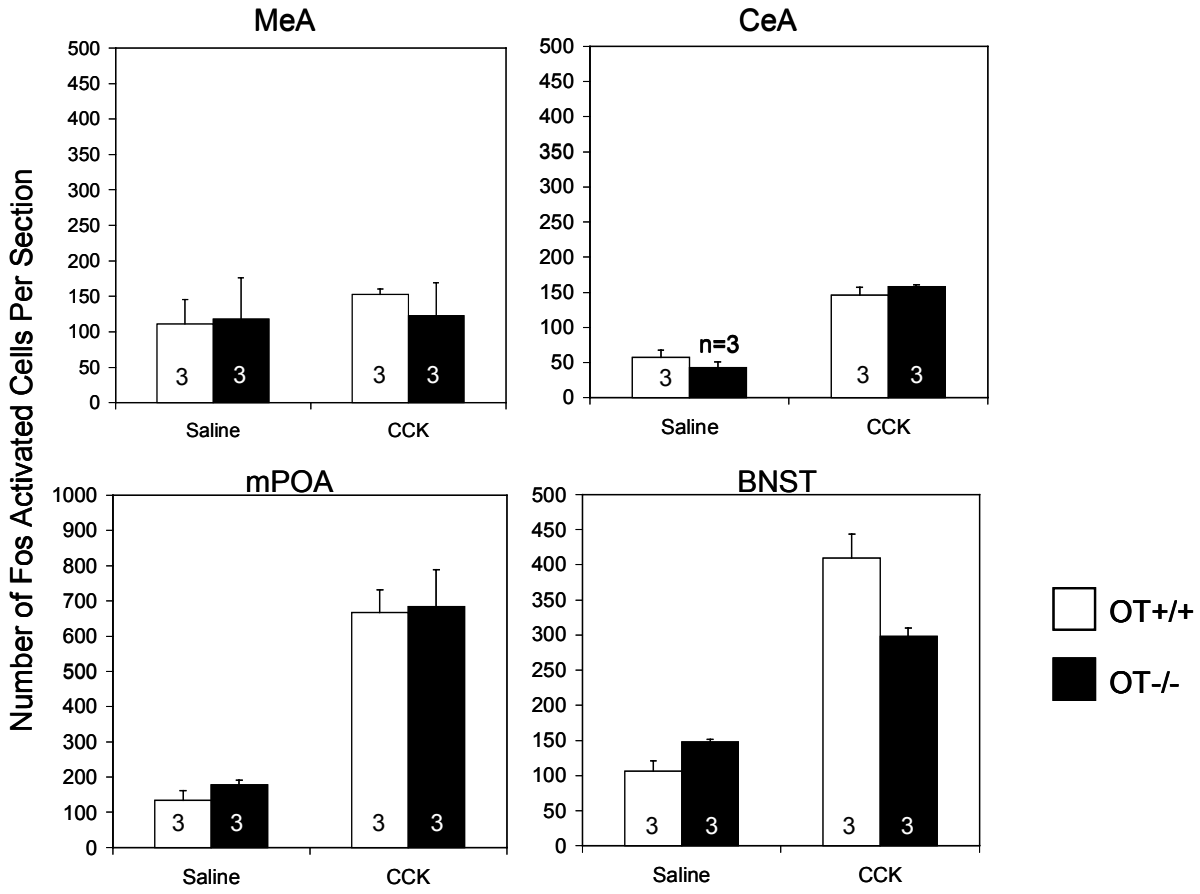

Figure 32. Cholecystokinin Induced Neural Activation of the Limbic Forebrain of Female Mice

Cholecystokinin administration significantly activated Fos in the bed nucleus of the stria terminalis (BNST; ANOVA, $F_{(1,6)}$=90.32, $p < 0.0001$), central nucleus of the amygdala (CeA; ANOVA, $F_{(1,6)}$=135.9, $p < 0.0001$), and medial preoptic area (mPOA; ANOVA, $F_{(1,6)}$=42.73, $p < 0.0006$), but not the medial nucleus of the amygdala (MeA; $p>0.05$). However, female OT+/+ and OT-/- mice displayed similar amounts of Fos activation in the BNST, CeA, and mPOA following CCK administration. The number of mice per group is located in the data bars.

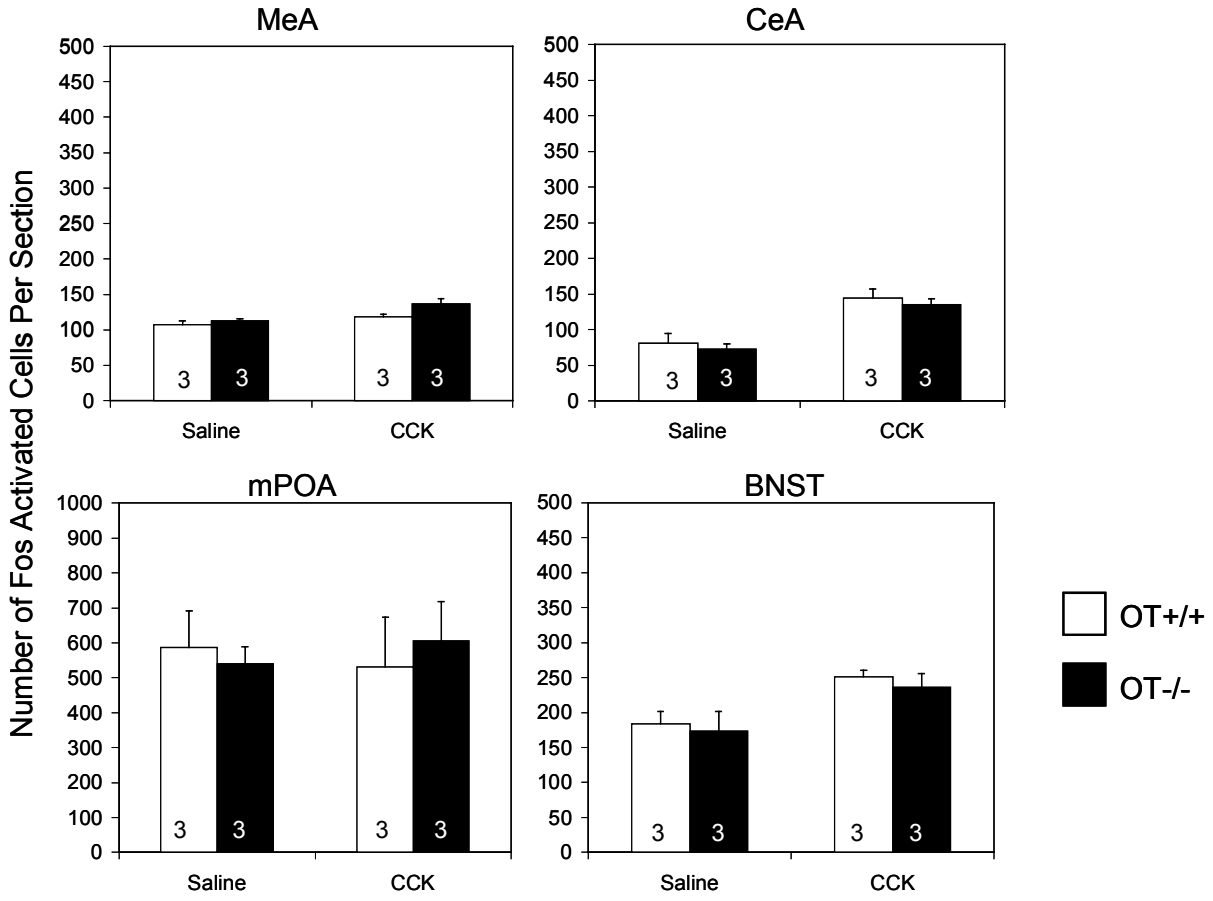

Figure 33. Cholecystokinin Induced Neural Activation of the Limbic Forebrain in Male Mice

Cholecystokinin administration significantly activated Fos in the bed nucleus of the stria terminalis (BNST; ANOVA, $F_{(1,6)}=9.91$, $p < 0.02$) and central nucleus of the amygdala (CeA; ANOVA, $F_{(1,6)}=40.54$, $p < 0.02$), but not the medial preoptic area (mPOA; $p > 0.05$) or the medial nucleus of the amygdala (MeA; $p>0.05$). Male OT+/+ and OT-/- mice displayed similar amounts of Fos activation in the BNST and CeA following CCK administration. The number of mice per group is located in the data bars.

Experiment 3. Elevated Plus Maze Induced Fos Expression in Anxiety-Related Forebrain Pathways

Exposure to the elevated plus maze (EPM) for five minutes did not activate neurons within the PVN of male or female OT+/+ or OT-/- mice compared to control mice. However, exposure to the EPM activated Fos in anxiety-related brain areas of male and female OT+/+ and OT-/- mice. These areas include, the BNST (ANOVA, $F_{(1,8)}=16.49$, $p < 0.004$), MeA (ANOVA, $F_{(1,8)}= 76.10$, $p < 0.0001$), and CeA (ANOVA, $F_{(1,8)}=8.71$, $p < 0.02$), but not the mPOA ($p > 0.05$; Figure 34). In addition, female mice exposed to the elevated plus maze expressed a genotypic difference in the number of Fos immunoreactive cells in the MeA (ANOVA, $F_{(1,8)}=5.38$, $p<0.05$; Figure 34). A *post hoc* analysis revealed that OT-/- mice displayed greater numbers of Fos immunoreactive cells in the MeA compared to OT+/+ mice ($p < 0.005$).

Based on the findings of Fos activation in the forebrains of female mice exposed to the EPM, Fos was quantified in the MeA of male mice. Compared to control mice of both genotypes, EPM significantly activated Fos in the MeA of male mice (ANOVA, $F_{(1,6)}=8.38$, $p < 0.03$; Figure 35). Unlike female mice, the number of Fos immunoreactive cells was not different in the MeA of male OT-/- and OT+/+ mice exposed to the EPM (ANOVA, $F_{(1,6)}=0.183$, $p>0.05$).

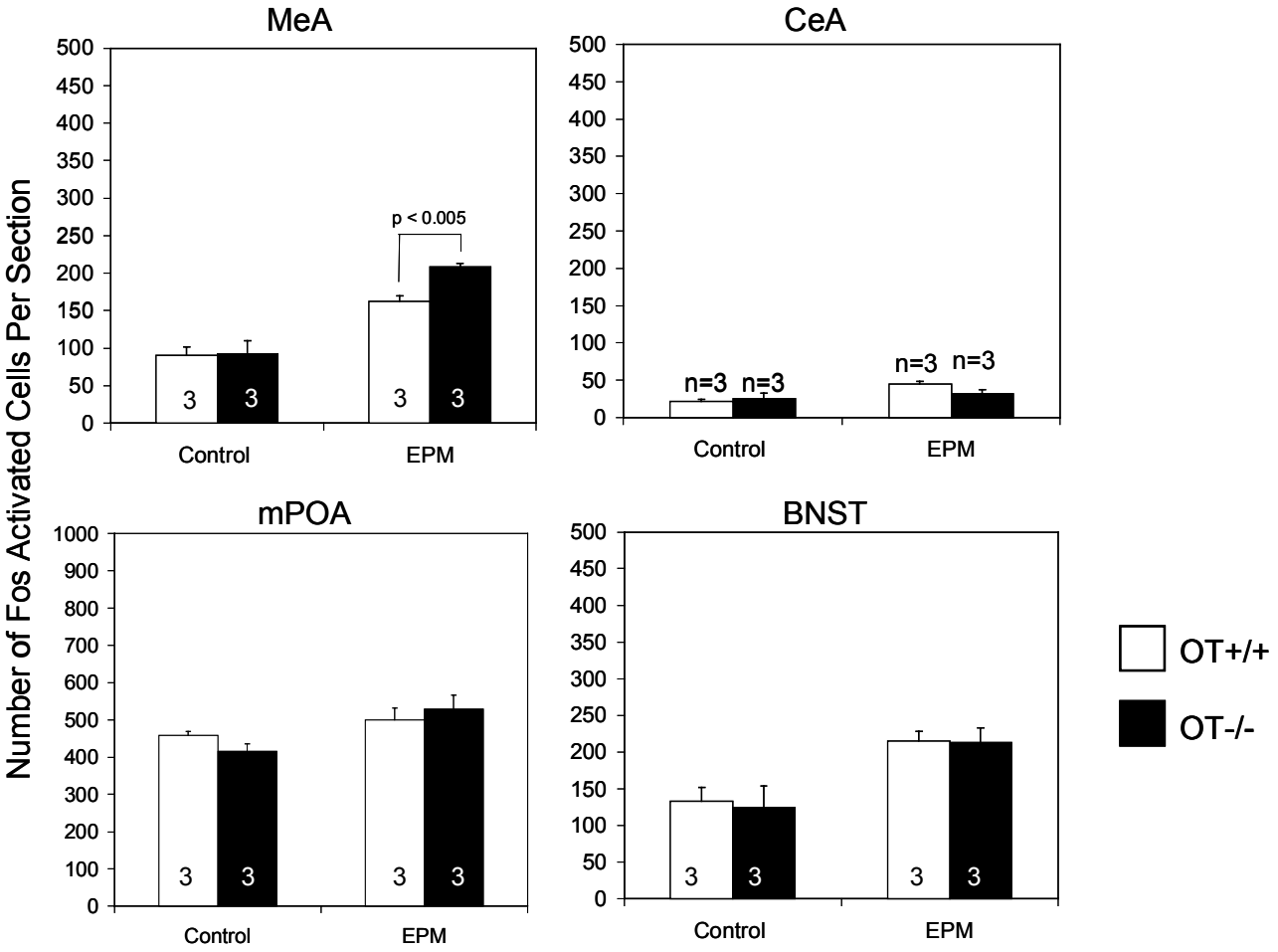

Figure 34. Elevated Plus Maze Induced Neural Activation of the Limbic Forebrain in Female Mice

Exposure to the elevated plus maze activated Fos in the bed nucleus of the stria terminalis (BNST; ANOVA, $F_{(1,8)}$=16.49, $p < 0.004$), medial (MeA; ANOVA, $F_{(1,8)}$=76.10, $p<0.0001$) and central nucleus of the amygdala (CeA; ANOVA, $F_{(1,8)}$=8.71, $p<0.02$), but not the medial preoptic nucleus (mPOA) compared to control female mice. The number of Fos immunoreactive cells was greater in the MeA of female OT-/- mice than OT+/+ mice exposed to the elevated plus maze. The number of mice per group is located in the data bars.

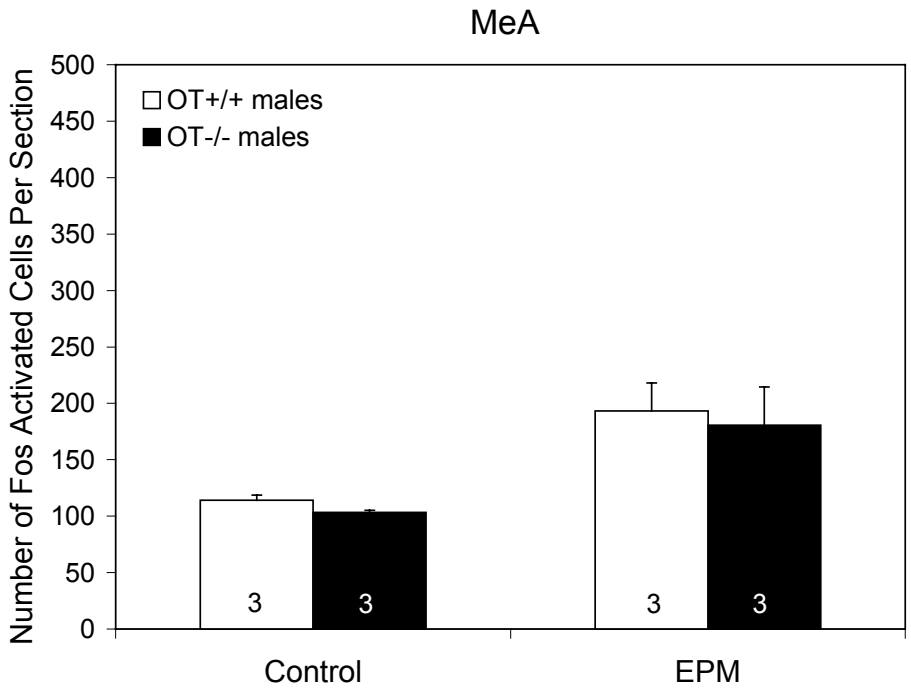

Figure 35. Elevated Plus Maze Induced Neural Activation of the Medial Amygdala of Male Mice

Exposure to the elevated plus maze activated Fos in the medial nucleus of the amygdala (MeA; ANOVA, $F_{(1,6)}=8.38$, $p < 0.03$). However, unlike female mice, the number of Fos immunoreactive cells in the MeA of male mice did not differ between genotype. The number of mice per group is located in the data bars.

C. Discussion

The pattern of neuronal activation after shaker stress, CCK administration, or EPM exposure was determined by mapping Fos immunoreactivity in select hypothalamic and forebrain regions of OT+/+ and OT-/- mice. This study demonstrates that the absence of OT results in altered Fos activation of the PVN and/or the MeA, which correlates with genotypic differences in the corticosterone response to psychogenic stress and anxiety-related behavior. Specifically, diminished activation of medial amygdala neurons and a trend towards enhanced activation of CRH immunoreactive neurons of the PVN in female OT-/- versus OT+/+ mice suggests a neural correlate for the genotypic difference observed in the corticosterone response to shaker stress. Moreover, enhanced activation of MeA neurons in female OT-/- versus OT+/+ mice suggests a neural correlate for the genotypic difference anxiety-related behavior observed in the EPM. This study supports recent reports suggesting that the MeA may be a target for OT actions in mice [54,129,143] or rats [210].

Shaker stress, a psychogenic stressor, has been reported to release corticosterone and OT, but not AVP, within the PVN and into the plasma of rats [69,142]. This study confirms the findings in the rat using an OT deficient mouse model. Shaker stress releases corticosterone in mice as it does in rats (refer to Chapter III) and activates Fos expression within the PVN of both genotypes. Shaker stress activates Fos in OT-positive neurons of the PVN of male and female OT+/+ mice, but not OT-/- mice. Moreover AVP-positive hypothalamic neurons were not activated by shaker stress in mice of either genotype, consistent with the finding in rats that shaker stress selectively releases OT, but not AVP [142]. AVP within the PVN acts synergistically with CRH to stimulate ACTH and in turn corticosterone secretion [5,166]. Therefore enhanced release of AVP is unlikely to account for the greater stress-induced release of corticosterone in OT-/- mice.

To determine whether the lack of OT may alter activation of CRH neurons in the PVN, double immunohistochemistry for Fos and CRH was performed in hypothalamic brain sections of

control and shaker stress exposed mice of both genotypes. Shaker stress activated Fos in CRH-positive neurons in the PVN of male and female mice of both genotypes compared to control mice. In addition, the number of activated CRH-positive neurons of female OT-/- mice tended to be higher (approaching statistical significance, $p = 0.06$) than in OT+/+ mice, although the difference did not reach statistical significance. Recently male OT-/- mice exposed to 4 hours of restraint stress were reported to have greater abundance of CRH mRNA than OT+/+ mice [143]. Unlike *in situ* hybridization histochemistry that is a measure of CRH mRNA expression, double immunohistochemistry for Fos and CRH is an assessment of the activation of CRH-positive neurons. Therefore, these findings are a quantitative assessment of the population of CRH neurons activated in response to shaker stress. These findings may not be reflective of the abundance of CRH mRNA expressed in response to shaker stress. Perhaps it will be possible to identify statistically significant increases in CRH activation if OT-/- mice are administered longer or more intense exposure to platform shaker. However, lack of OT appears to result in increased CRH activation and expression [143] in mice exposed to psychogenic stressors.

Unlike shaker stress, exposure to the EPM, a measure of anxiety-related behavior, did not result in increased Fos expression in the PVN of male or female mice. It is possible that the duration of the stimulus was not long enough (or intense enough) to result in Fos activation of the PVN of male or female mice. This theory is supported by a study performed by Duncan *et al*. [45]. Rats tested in the EPM for 5 minutes did not reveal Fos activation within the PVN compared to unhandled control rats [45]. However, 15 minutes of EPM exposure resulted in increased Fos activation within the PVN of male rats compared to control cohorts [186].

Exposure to shaker stress and the EPM resulted in increased Fos expression in the limbic forebrain of OT-/- and OT+/+ mice. Moreover, shaker stress exposed OT-/- mice displayed decreased Fos activation, while OT-/- mice exposed to the EPM displayed increased Fos activation, in the medial amygdala (MeA) compared to OT+/+ mice. It is not known why, in comparison to

OT+/+ mice, female OT-/- mice display increased Fos activation following EPM exposure versus decreased Fos activation following shaker stress. This discrepancy may be due to differences in the activation of brain areas that project to the MeA, such as the subiculum, hippocampus, and hypothalamus. Further studies examining stress- and anxiety-related brain areas projecting to the MeA would be necessary to confirm this hypothesis. However, the MeA appears to be a target area for the actions of OT. Altered Fos activation in the medial amygdala of OT-/- versus OT+/+ mice was also reported in male mice following 1h restraint stress [143] and a social memory task [54], suggesting differences in forebrain processing in response to these stimuli. Moreover, infusion of synthetic OT into the lateral ventricles of ovariectomized rats exposed to restraint stress reduced stress-induced Fos activation in specific forebrain regions [210]. The OT pathways as well as OT receptors, which have been identified in the limbic forebrain, are in an anatomical position to modulate the HPA axis response to stress. No studies to date have reported OT release within the MeA of mice during stress, although the present results suggests that the medial amygdala in particular appears to be a target area for the actions of OT. A genetic absence of OT alters activation of this nucleus when mice are exposed to psychogenic stressors. The PVN receives little direct input from the medial amygdala, but the MeA sends inhibitory GABAergic projections to the BNST and mPOA in rats [24], which in turn send GABAergic projections to the medial parvocellular PVN [161]. Consequently, activation of the MeA results in activation of the HPA axis via disinhibition of inhibitory limbic forebrain projections to the PVN. The medial amygdala may be one of the brain areas that can account, at least in part, for heightened stress-induced corticosterone response and enhanced anxiety-related behavior in OT-/- mice. Therefore, it is possible that OT modulates forebrain projections leading into the PVN, specifically projections terminating on CRH neurons within the PVN.

CCK administration releases corticosterone (refer to Chapter III) and increases Fos expression within the PVN and SON of both OT+/+ and OT-/- mice. Like shaker stress, CCK

administration activated OT, but not AVP neurons, within the PVN of mice. In addition, CCK administration activated CRH neurons of the PVN in mice, but not to a different degree between genotypes. It was not possible to quantitatively determine statistically significant genotypic differences in Fos activation of CRH-positive neurons because of the few number of tissue sections per mouse. However, a qualitative assessment did not reveal differences in Fos activated CRH neurons. Similar CRH activation in the PVN of OT+/+ and OT-/- mice (as assessed qualitatively) may reflect the comparable corticosterone release in OT+/+ and OT-/- mice. CCK penetrates the blood-brain barrier poorly and the excitation of OT cells appears to be due to peripheral actions. Systemically administered CCK binds to CCK-A receptors of gastrointestinal afferents, resulting in the stimulation of gastric sensory input to the nucleus of the solitary tract and the dorsal motor nucleus of the vagus [127,134,163]. In turn, these hindbrain areas relay the sensory signal to the hypothalamus and forebrain. Previous studies have determined that male OT+/+ and OT-/- mice did not display differences in the amount of Fos activation in the ascending hindbrain areas (i.e., area postrema, nucleus of the solitary tract, and the dorsal motor nucleus of the vagus) that result in PVN activation [122]. Furthermore, CCK administration resulted in similar Fos activation in the CeA and BNST, without activating the MeA of male and female OT+/+ and OT-/-mice. Therefore, OT deficiency does not appear to play a role in the modulation of the CCK-induced stress response.

Unlike in the rat, the magnocellular and parvocellular neurons in the PVN of the mouse cannot be segregated. Therefore, it is difficult to determine if magnocellular, parvocellular, or both subsets of OT neurons of the PVN were activated using immunohistochemistry for Fos and OT. Retrograde tracing using systemic administration of Fluorogold confirmed that parvocellular and magnocellular OT and AVP neurons are intermingled in the PVN of OT+/+ and OT-/- mice (unpublished observations, JA Amico and L Rinaman). Fluorogold labeling applied systemically identifies brain and spinal cord neurons whose axon terminals lie outside the blood-brain barrier, including hypothalamic endocrine neurons [117,131]. Therefore, future studies employing

Fluorogold labeling in addition to dual immunohistochemistry for OT and Fos are necessary to determine which subset of OT neurons of the PVN are activated in response to shaker stress, cholecystokinin administration, and elevated plus maze exposure.

It should be noted that, while double immunohistochemistry is a valuable technique for identifying neuroendocrine cells within the PVN, it may not be sensitive enough to identify the entire population of AVP and/or CRH in the PVN of mice. CRH neurons are located in the medial parvocellular subdivision of the PVN in rats [191]. However, there are limitations to using double immunohistochemistry for Fos and CRH to evaluate genotypic differences in the activation of CRH-positive neurons. It appears that without an adrenalectomy or pre-treatment with colchicine most CRH antibodies only stain for a subset of CRH neurons in the PVN of mice. Removal of circulating steroids in rodents by adrenalectomy is a potent stimulus to CRH neurons of the PVN, which respond to adrenalectomy with increased CRH mRNA and peptide [100,180,215]. Colchicine is another experimental tool that is traditionally used to enhance the number of immunoreactive cells in the central nervous system [39]. Colchicine blocks neuronal transport of both peptides and mRNA [39,105]. However, colchicine has been demonstrated to induce *fos* expression in CRH neurons of the PVN [26] and elevate plasma ACTH and corticosterone concentrations in rats [168]. Both adrenalectomy and colchicine pre-treatment would result in an increased number of CRH-positive cells, but would not allow us to examine the differences in the CRH function of OT+/+ and OT-/- mice in response to shaker stress. Therefore, evaluating Fos activation of CRH-positive neurons may be problematic in determining genotypic differences in CRH function. Furthermore, AVP neurons that co-express CRH and respond to stress are also located in the medial parvocellular subdivision of the PVN in rats [191]. AVP expression in this subset of neurons is not easily detected with immunocytochemistry alone. Therefore it is necessary to enhance AVP expression via adrenalectomy [180,181] or administration of colchicine [180,181]. Fos activation was not observed in the AVP immunoreactive neurons of the PVN in either genotype after shaker stress or CCK-

administration but it is not known if all of the AVP neurons in the PVN of mice are capable of being detected by immunohistochemistry. Although immunohistochemistry selectively identified Fos activation in OT, but not AVP, PVN neurons following shaker stress in mice, it is also possible that all of the AVP expressing neurons of the PVN were not identified with this technique.

The hippocampus, which plays a primary role in the inhibition of the HPA axis as well as modulation of the stress and anxiety response, was not evaluated in this study. OT neurons within the PVN project to and OT receptors are located in the hippocampus. However, it is not known if there are differences in Fos activation of the hippocampus OT+/+ and OT-/- mice exposed to shaker stress or EPM exposure. It is possible that the hippocampus, in addition to the MeA, may be a target area for the OT in response to stress and anxiety. Future studies examining activation of the hippocampus in response to stress in the presence and absence of OT are necessary to answer this question.

Corticosterone released from the adrenals in response to stress provides negative feedback to the HPA axis indirectly through the hippocampus and the amygdala. Both the hippocampus and MeA contain corticosteroid receptors [104]. Two corticosteroid receptors have been identified, the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) [132,197]. Corticosterone is able to activate both receptors but corticosterone has a ten-fold higher affinity for MR than for GR [162]. Therefore, the glucocorticoid concentration determines which receptor is activated. Low concentrations of corticosterone are believed to activate MR and higher concentrations are believed to activate GR during stress. The GR has a broad expression pattern, whereas the MR has restricted expression in the neurons of the hippocampus and amygdala [104,162]. In addition, administration of OT results in decreased expression of GR mRNA and increased expression of MR mRNA in the hippocampus of the rat [153]. Therefore, it is possible that OT deficiency alters MC and/or GC function in OT-/- mice.

In summary the absence of normally functioning OT systems in OT-/- mice results in the altered activation of the MeA, and perhaps other regions. The MeA is an important area of the limbic circuitry responsible for processing anxiety and psychogenic stress. Anxiety stimuli and psychogenic stressors requiring higher-order processing of a stimulus, such as shaker stress in rats [69,142], restraint stress in male OT-/- mice [143], or female rats [210] activate limbic forebrain pathways. Exposure to shaker stress and the EPM resulted in altered Fos expression in the MeA forebrain of OT-/- compared to OT+/+ mice. The OT pathways as well as OT receptors, which have been identified in the limbic forebrain, are in an anatomical position to modulate the HPA axis response to stress. The MeA in particular appears to be a target area for the actions of OT. A genetic absence of OT alters activation of this nucleus when mice are exposed to an anxiety stimuli or psychogenic stressors. The medial amygdala may be one of the brain areas that can account, at least in part, for heightened anxiety-related behavior and stress-induced corticosterone response in OT-/- mice. Therefore, it is possible that OT modulates forebrain projections leading into the PVN, specifically projections terminating on CRH neurons within the PVN.

VI. Conclusion

The experimental findings presented in this thesis provide insight into the role of OT in the stress and anxiety response. Pharmacological studies have consistently shown that central OT may be an anxiolytic, as well as play an inhibitory role in the up-regulation of the HPA axis in response to stress in females. Using an OT deficient mouse model, these studies have determined that the inhibition of anxiety and the HPA axis in response to psychogenic stressors is dependent upon the presence of OT.

OT is responsible for the inhibition of corticosterone release in response to psychogenic stressors. The genotypic differences in the corticosterone response to shaker stress are not due to altered activation of the HPA axis. Firstly, OT+/+ and OT-/- mice have similar diurnal plasma corticosterone concentrations over a 24h period. Secondly, intracerebroventricular administration of CRH resulted in similar corticosterone release in both genotypes. Lastly, a genotypic difference in corticosterone release is dependent upon the type of stressor administered to OT+/+ and OT-/- mice. For instance, shaker stress elicited a genotype difference in the corticosterone response but CCK administration resulted in similar increases in corticosterone between OT+/+ and OT-/- male mice. Therefore, it appears as if the differences in HPA axis activation in response to certain stressors are due to differences in neuronal activation up-stream of the HPA axis, possibly at the level of the medial nucleus of the amygdala.

OT also plays an anxiolytic role. Female OT deficient mice displayed enhanced anxiety-related behavior compared to wildtype mice. Administration of an OT antagonist to OT+/+ mice mimicked the anxiogenic behavior of OT-/- mice, while administration of OT into the lateral ventricles attenuated enhanced anxiety-related behavior in OT-/- mice. Therefore, the anxiolytic role of OT is dependent upon binding at the OT receptor.

Many studies have suggested that ovarian hormones, specifically estradiol, facilitate OT binding and enhance oxytocinergic actions. Although similar genotypic differences in shaker stress-induced corticosterone release were not observed in male mice, the genotypic difference in the corticosterone response of female mice was not dependent upon the estrous cycle. Similarly, male OT-/- mice displayed decreased anxiety-related behavior compared to OT+/+ mice. This finding opposes the increased anxiety behavior evaluated in female mice. However, preliminary studies suggest that ovariectomizing female OT+/+ and OT-/- mice eliminates the ability of OT to inhibit the corticosterone response to psychogenic stress and anxiety-related behavior. Therefore, it appears that the presence of estradiol enhances the ability of OT to inhibit the HPA axis and anxiogenic behavior.

To date, there is no direct evidence that OT is released into the MeA after stress. However, OT receptors are located in the MeA, suggesting a role for OT in the limbic system. Psychogenic stressors result in Fos activation of the MeA and OT neurons of the PVN. In addition, stimulation of the MeA activates the HPA axis, while excitotoxic lesions of the MeA result in the suppression of HPA axis activation in response to psychogenic stress. The findings of these studies suggest that the MeA may be responsible for altered neuroendocrine response to shaker stress and anxiety response detected in OT-/- mice. Exposure to shaker stress and the EPM resulted in increased Fos expression in the limbic forebrain of OT+/+ and OT-/- mice. Shaker stress exposure resulted in decreased Fos activation, while EPM exposure resulted in increased Fos activation in the MeA of OT-/- mice compared to OT+/+ mice. The deletion of OT alters the activation of the MeA when mice are exposed to psychogenic stressors. The MeA may be partly responsible for the increased anxiety and heightened corticosterone response in OT-/- mice. Therefore, it is possible that OT modulates MeA projections, which are relayed through the BNST and mPOA of the hypothalamus, to CRH neurons in the medial parvocellular PVN of the hypothalamus.

The findings presented in this thesis provide insight into the role of OT in the stress and anxiety response. The experiments in this thesis have determined that OT inhibits activation of the

HPA axis in response to psychogenic stress, as well as inhibiting the anxiety response in female mice. Moreover, it appears that OT acts at the level of the MeA to inhibit anxiety behavior and the HPA axis response to psychogenic stress.

Future Directions

Stress Response of Wildtype and Oxytocin Deficient Mice Exposed to Different Stressors

Examining the stress response of OT+/+ and OT-/- mice that have been exposed to an expanded repertoire of stressors would be valuable in further exploring the hypothesis of oxytocin mediating stress-induced forebrain activation versus hindbrain activation. Increasing the number of psychogenic stressors, preferably stressors that have been shown to activate the MeA as well as OT neurons, would be interesting to determine whether genotypic differences in the corticosterone response to stress only occur following psychogenic stress. It would also be interesting to more fully explore a number of physical stressors to determine if stress-induced hindbrain activation results in genotypic differences in corticosterone release similar to that found following shaker stress.

Central Administration of Oxytocin to Oxytocin Deficient Mice Exposed to Shaker Stress

In addition to expanding the number and type of stressors, it would support the current hypothesis to prove that central administration of oxytocin is capable of attenuating the enhanced stress response of OT-/- mice compared to OT+/+ mice. Administration of OT into the lateral ventricles of OT-/- mice exposed to EPM attenuated the enhanced anxiety-like behavior in comparison to OT+/+ mice. It is expected that similar studies administering OT into the lateral ventricles of OT-/- mice exposed to shaker stress would attenuate the enhanced corticosterone response of OT-/- mice. This study would be useful in confirming that the presence of OT is necessary to attenuate the corticosterone response to shaker stress.

Evaluating Gender Differences in Oxytocin Deficient Mice in Response to Stress and Anxiety

At this time it is not known why male and female mice respond differently to shaker stress and elevated plus maze exposure. Exploring the sex differences between male and female mice

exposed to shaker stress and the EPM may be useful in evaluating the interaction between OT and gonadal hormones. The HPA axis is sensitive to gonadal steroids. Pretreatment of estrogen to ovariectomized female rats enhances corticosterone secretion following stress [25,55,202]. In contrast, testosterone administration inhibits HPA activation in response to stress [201,203]. OT mRNA expression [150,185] and OT receptor binding [221] are facilitated by estradiol, and areas of the brain involved in the stress response, such as the amygdala, BNST, and the mPOA are hormone responsive. Pilot studies conducted comparing ovariectomized OT+/+ and OT-/- mice to intact OT+/+ and OT-/- mice implicated that estrogen and/or progesterone interact with OT to attenuate the corticosterone response to shaker stress, as well as reduce anxiety-related behavior in the EPM. It appears that removing gonadal steroids in female mice eliminates the ability of OT to attenuate the corticosterone response to stress and reduce anxiety-like behavior. These preliminary findings support studies conducted by McCarthy *et al*. reporting that administration of OT to ovariectomized mice was not able to inhibit anxiogenic behavior in the EPM without the administration of estrogen [129]. The number of mice included in these studies was too few to include the findings in this thesis. However, these pilot studies provide an interesting starting-point to examine the gender differences in the stress and anxiety response of OT-/- mice. Examining the role of OT in the stress and anxiety responses of ovariectomized and gonadectomized OT+/+ and OT-/- mice, followed by the replacement of gonadal steroids would provide incite into the gender differences of the stress-induced corticosterone response and anxiety-related behavior of OT-/- mice.

Expanding the Methods of Analysis Used to Examine Activation of the Central Nervous System

While double immunohistochemistry is a valuable technique for identifying neuroendocrine cells within the PVN, it may not be sensitive enough to identify the entire population of AVP and/or CRH in the PVN of mice. Unlike OT and AVP neurons, CRH neurons (and AVP-containing CRH neurons) do not contain a ready releasable pool of peptide making the neurons difficult to identify

with immunohistochemistry. Therefore, it may be helpful to also process and evaluate tissue using *in situ* hybridization for mRNA or hnRNA of OT, AVP, and/or CRH.

The Role of the Hippocampus in the Stress Response of Oxytocin Deficient Mice

The hippocampus, also plays a primary role in the inhibition of the HPA axis as well as modulation of the stress and anxiety response, was not evaluated in this study. OT neurons within the PVN project to and OT receptors are located in the hippocampus. As discussed in Chapter V, it is not known if there are differences in Fos activation of the hippocampus OT+/+ and OT-/- mice exposed to shaker stress or EPM exposure. It is possible that the hippocampus, in addition to the MeA, may be a target area for the OT in response to stress and anxiety. Future studies examining Fos activation of the hippocampus in response to stress in the presence and absence of OT are necessary to answer this question.

The Function of Corticosteroid Receptors in Oxytocin Deficient Mice

Corticosterone released from the adrenals in response to stress provides negative feedback to the HPA axis indirectly through the hippocampus and the amygdala. Both the hippocampus and MeA contain corticosteroid receptors [104]. As discussed in Chapter V, corticosterone is able to activate both receptors but corticosterone has a ten-fold higher affinity for mineralocorticoid receptors (MR) than for glucocorticoid receptors (GR) [162]. Low concentrations of corticosterone are believed to activate MR and higher concentrations are believed to activate GR during stress. Furthermore, administration of OT results in decreased expression of GR mRNA and increased expression of MR mRNA in the hippocampus of the rat [153]. It is not known if the enhanced corticosterone release of OT-/- mice in response to stress alters the binding affinity or number of GR and/or MR. Therefore, evaluating the number and binding affinity of MC and GC receptors in the amygdala and hippocampus of mice of both genotypes would provide information on how oxytocin deficiency may alter MC and/or GC receptor function in OT-/- mice.

Health Consequences Due to Enhanced Glucocorticoid Response to Stress in Oxytocin Deficient Mice

Oxytocin deficient mice also provide a unique animal model to evaluate the short and long-term health consequences in response to enhanced HPA activation. Many organs and physiological systems are sensitive to glucocorticoids. Glucocorticoids influence cardiovascular tone, immunity, metabolism, neural function, and behavior. During an acute response to stress glucocorticoids influence the increase blood pressure and cardiac output [173], inhibit synthesis and release of cytokines and other mediators that promote immune and inflammatory reactions [208], mobilize energy stores and increase circulating glucose [174], and enhance synaptic plasticity [151] and neuronal excitability [12,92] in the hippocampus leading to enhanced learning and memory. However, it is not known whether enhanced corticosterone response to an acute stress is advantageous or detrimental to an organism. Therefore, the increased corticosterone release of OT-/- mice compared to OT+/+ in response to certain stressors may be helpful, resulting in improved mobilization, or harmful, resulting in the "over-stimulation" of the physiological and central response to stress.

VII. References

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