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A Psychological Dimension of Stress Selectively Alters Serotonin-1A Receptor Function in the Dorsal Raphe Nucleus

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by

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A Psychological Dimension of Stress Selectively Alters Serotonin-1A Receptor Function in the Dorsal Raphe Nucleus.

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Acute, traumatic, stress is an experiential factor that can predispose an individual to the development of psychiatric disorders such as depression and post-traumatic stress disorder. However, not all individuals react similarly to traumatic events. This variability has led to the study of the components that can mitigate the unwanted consequences of stress. One component that potently modulates the consequences of stress is stressor controllability. Indeed, uncontrollable, but not controllable, stress produces a variety of behaviors that have been termed anxiety- or depression-like.

The studies herein elucidate a mechanism that could explain the behavioral differences following uncontrollable and controllable stress. We found that uncontrollable stress functionally impairs serotonin-1A receptors in the dorsal raphe nucleus, while controllable stress does not. Furthermore, this protective effect of control was mediated by the medial prefrontal cortex. Finally, an initial experience with controllable stress prevented subsequent uncontrollable stress-induced impairments of serotonin-1A receptor function. These results indicate that activation of the medial prefrontal cortex produces both short- and long-term resistance to some of the consequences of stress.

To Dad

Acknowledgements

First, I must thank my family for their unflagging support. The love and camaraderie that we share is extraordinary and I carry these warm sentiments with me daily as inspiration. I would especially like to thank my mom. I have never met anyone who embodies resilience as fully as she does. No matter the challenge Mom has always been a pillar of stability for her children and an exemplar of strength and courage.

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Contents

Figures

Chapter 1:

General Introduction

General Overview

Acute, traumatic, events can have a profound impact on the psychological well being of an individual. Indeed, following a traumatic event, many individuals develop psychiatric conditions such as post-traumatic stress disorder (PTSD) (Heim and Nemeroff, 2009), substance use disorders (Sinha, 2008), and major depression (Nemeroff and Vale, 2005). The manifestation of these psychiatric disorders following aversive environmental events, or simply "stress", has led to the realization that stress can serve as a potent predisposing factor in the etiology of mental illness. However, individual reactions to stress are variable. In fact, some individuals maintain healthy levels of psychological functioning and never develop psychiatric disorders following traumatic events (Tang and Fox, 2001; Solomon and Mikulincer, 2006; Fincham et al., 2009; Wingo et al., 2010). This phenomenon suggests the questions, "what produces resilience?" or "are some stressors more harmful than others?" and "can a previous experience with stress protect from later stress?".

One broad, single, conclusion will not likely answer all of the above questions. However, researchers are beginning to dissect some of the critical components of the stress experience that can lead to vulnerability or resilience. An examination of the critical components of the stress experience that mitigate the psychological consequences of stress has great

clinical relevance as it may provide novel behavioral therapies or suggest pharmaceutical medications to produce stress-resistance. This dissertation informs such an effort by investigating a single, critical, component that potently modulates the stress experience, the psychological dimension of stressor controllability. By manipulating stressor controllability we have found neural mechanisms that lead to both vulnerability and resilience to the consequences of stress.

Isolating the dimension of controllability in the laboratory is accomplished by using what has been termed the triadic design (Maier and Seligman, 1976; Weiss et al., 1981). Typically rodents are used for these studies and electric shock is delivered to the rodent's tail, serving as the stressor. In the triadic design, one subject is allowed to terminate each of a series of unsignaled tail shocks by performing an operant escape response, turning a small wheel located on the front wall of the chamber. This subject has behavioral control of the stressor and therefore, this treatment is termed controllable stress (escapable stress, ES). Another subject is placed in a similar wheel-turn box, but is yoked to the ES subject rather than having the ability to terminate the tail shocks. Each tail shock is terminated whenever the ES subject turns the wheel. This treatment is called uncontrollable stress (inescapable stress, IS). Therefore, the ES and IS subjects receive the same number, intensity, pattern, and duration of shock. The only difference

between these two subjects is the element of controllability. A third subject remains in the colony room as a homecage (HC) control subject.

Subsequent shuttlebox escape learning was the first behavior found to be sensitive to the element of prior stressor controllability. In this behavioral paradigm animals must learn to escape foot shock in a shuttlebox. Subjects that had received ES the day before shuttlebox testing learned to escape the foot shock similarly to HC, but the IS subjects failed to learn to escape the foot shock. This is the so-termed "learned helplessness" effect (Seligman and Maier, 1967). A number of other behavioral changes have been observed to occur following IS, but not ES. These can be broadly classified as anxiety- or depression-like, including exaggerated fear conditioning, reduced social exploration, potentiated drug reward, and exaggerated shock-elicited freezing (Maier and Watkins, 2005). Importantly, in the stressor controllability paradigm, behavioral testing occurs in a completely different environment than that in which tail shock was administered. As such, the behavioral outcomes following IS are thought to be trans-situational and not elicited by contextual cues. Lastly, the behavioral effects of IS are typically observed in a window spanning 24-72 hr following IS exposure (Glazer and Weiss, 1976; Jackson et al., 1978; Grau et al., 1981; Weiss et al., 1981; Weiss JM, 1981; Maier, 1990), but see (Will et al., 1998); however, these effects can be extended past 72 hr if the animal is periodically re-exposed to the IS environment (Maier, 2001).

A comment on the use of electric shock as the aversive stimulus in the stressor controllability paradigm is in order. The manipulation of stressor controllability requires the use of an aversive stimulus that can be initiated and terminated by the experimenter with millisecond temporal specificity so that subjects with and without control receive physically identical events. Other stressors that might be used such as tail-suspension, restraint, social defeat, etc., do not have this characteristic. Electric shock is used because it is the only aversive stimulus whose onset and offset can be manipulated with high temporal specificity and also motivates animals to perform operant responses to terminate/escape the aversive stimulus.

Stress Responsive Brain Regions

Because IS and ES are physically identical but only IS leads to anxiety- and depression-like behavioral changes, there has been great interest in determining how IS produces these behaviors (Maier and Watkins, 2005). A general strategy for finding the mechanisms underlying IS behaviors has been to uncover a system that is preferentially activated only during IS.

Indeed, several monoamine systems have been investigated in the stressor controllability paradigm. A number of studies have found that IS, but not ES, reduced norepinephrine (NE) content in a number of brain regions including the hypothalamus, brain stem, and frontal cortex (Weiss et

al., 1970; Weiss et al., 1981). The primary source of the stress-induced NE is thought to originate from the locus coeruleus (LC), a brainstem structure that contains the greatest number of NE cells in the central nervous system (Swanson and Hartman, 1975). IS-induced reduction of NE content is thought to be the result of increased NE turnover during the stress experience (Weiss et al., 1981). Weiss and colleagues hypothesized that increased NE turnover by IS, later produced a sensitization of NE cells by reducing the sensitivity of noradrenergic α-2 receptors in the LC (Simson et al., 1986). Since α-2 receptors are expressed on the soma and dendrites of NE cells in the LC (Young and Kuhar, 1980) and agonism at this receptor causes increased K+ conductance (Andrade and Aghajanian, 1985), α-2 receptors are thought to be autoregulatory and in control of LC neuron firing. As such, reduced levels of α-2 receptors in the LC would produce a sensitized NE response and this sensitized NE response was hypothesized to be responsible for behavioral outcomes following IS (Weiss et al., 1994).

However, perhaps at odds with the work of Weiss and colleagues is the finding that both ES and IS produced similar activation of NE cells in the LC (McDevitt et al., 2009). This similar activation of LC NE cells suggests that if the behavioral effects of IS are dependent upon the NE system, then the critical stress-induced changes in the NE system are likely occurring at axon terminals rather than the cell bodies. Indeed, the development of IS behaviors can be modulated by manipulations within terminal regions of LC

efferents. For example, one brain region that receives NE efferents from the LC is the dorsal raphe nucleus (DRN) (Peyron et al., 1996); the LC is thought to provide the majority of NE afferents to the DRN (Clement et al., 1992). Indeed when the α-1 receptor antagonist benoxathian was microinjected into the DRN immediately before stress, the shuttlebox escape failure normally observed 24 hr after IS was blocked (Grahn et al., 2002). This pharmacological prevention of the shuttlebox escape failure suggests that modulation of the serotonin (5-hydroxytryptamine, 5-HT) system may have a critical role in the development of IS-induced behaviors as α-1 receptor agonism depolarizes 5-HT cells in the DRN (Trulson and Crisp, 1984; Aghajanian, 1985).

The role of excitatory amino acids has also been investigated in the stressor controllability paradigm. Pharmacological manipulations that blocked N-methyl-D-aspartate (NMDA) receptors in the DRN prevented the development of IS-induced behaviors when administered immediately before stress exposure (Grahn et al., 2000). One structure that provides excitatory amino acid input to the DRN is the lateral habenula (Kalen et al., 1985). The lateral habenula sends aspartate-containing projections to the DRN that excite 5-HT cells and increase extracellular 5-HT in DRN projection regions (Kalen et al., 1985; Kalen et al., 1986; Kalen et al., 1989). Lesioning the lateral habenula blocked IS-induced shuttlebox escape failure as well as stress-induced elevations of extracellular 5-HT in the DRN (Amat et al.,

2001). These results suggested that excitatory amino acid release during stress was critical to the development of IS-induced behaviors and that the DRN may have served as a site of integration for the organism's response to aversive stimuli.

Neuroendocrine peptides and steroids have also been investigated in the stressor controllability paradigm. One steroid, corticosterone (cortisol in humans) is secreted as an end-product of an activated hypothalamus-pituitary-adrenal (HPA) axis. However, when plasma levels of corticosterone were measured using a shock protocol that produces behavioral differences between ES and IS subjects, ES and IS evoked the same corticosterone response (Maier et al., 1986). Additionally, the timecourse of corticosterone levels were also similar between ES and IS groups. Additionally, plasma levels of adrenocorticotropic hormone (ACTH) were measured because ACTH is another component of the HPA axis response. As with corticosterone, the stress-induced levels and timecourse of plasma ACTH were identical between ES and IS. Because ES and IS produces similar levels of corticosterone and ACTH in the blood, these HPA products are not thought to be a component of the stress experience that produces the behavioral differences between ES and IS.

Related to stress-induced activation of the HPA axis, is the secretion of the neuropeptide corticotropin releasing hormone (CRH). CRH is involved in the complex constellation of sympathetic, neuroendocrine, and behavioral

responses to stress (Dunn and Berridge, 1990; Heinrichs and Koob, 2004) and is secreted in hypothalamic (Lowry and Moore, 2006) as well as extrahypothalamic brain regions (Gray and Magnuson, 1992; Liang et al., 1992; Lee and Davis, 1997). Interestingly, the DRN receives CRH afferents (Sakanaka et al., 1987) and also expresses CRH1 and CRH2 receptors, although CRH2 is expressed at a higher density (Chalmers et al., 1995; Day et al., 2004). As such, agonism of the CRH2 receptor increases both DRN 5-HT cell firing and extracellular 5-HT in projection regions of the DRN (Lowry et al., 2000; Amat et al., 2004).

Indeed, application of the CRH2 receptor agonist, urocortin II, into the DRN, in the absence of tail shock, reproduced behaviors associated with IS (Hammack et al., 2003). Additionally, if the CRH2 receptor antagonist anti-sauvagine-30 was microinjected into the DRN immediately before IS, the usual IS-induced behaviors were blocked (Hammack et al., 2003). Although CRH has a clear role in the mediation of stress-induced behaviors and is both necessary and sufficient to produce IS-behaviors, the brain region responsible for CRH secretion has not been identified. However, the bed nucleus of the stria terminalis (BNST) is a likely candidate as it is CRH-immunoreactive (Peyron et al., 1998) and lesions in this region block IS-induced behaviors (Hammack et al., 2004).

The studies reviewed thus far have concentrated on regions of the brain that become activated during aversive events. Many of the monoamine

and endocrine systems described are equally activated during both ES and IS. However, this equal activation of brain regions between ES and IS subjects does not illuminate a critical brain structure(s) that may be preferentially involved during IS, but not ES. However, many of the monoamine and endocrine systems described earlier shared a similar attribute, namely that they excited 5-HT cells in the DRN. Since many of the brain regions that respond to stress can activate 5-HT cells in the DRN, it is possible that the DRN may serve as an integration site for the organism's response to stress. For this reason activation of 5-HT cells in the DRN was studied in the stressor controllability paradigm.

Stressor Controllability, Serotonin, and the Dorsal Raphe Nucleus

The DRN is a brainstem structure that contains approximately 30,000 cells and is only one portion of a larger raphe complex (Hornung, 2010). The DRN expresses many different neurotransmitter cell types including dopamine, GABA, and glutamate (Michelsen et al., 2007). However, serotonergic neurons are the most prominently expressed cell type in the DRN, comprising 33-66% of the total cell population in the DRN (Vertes and Crane, 1997). These serotonergic cells project to several brain regions including the motor cortex, amygdala, dorsal periaqueductal gray (dPAG), the medial prefrontal cortex (mPFC), hippocampus, and the superior

colliculus (Lowry et al., 2008a). Because the DRN gives rise to serotonergic efferents that terminate in a variety of brain regions, 5-HT has consequently been implicated in several different behaviors such as appetitive, pain, sexual, anxiety, sleep, aggressive, and reward (Mendelson, 1992; Rueter et al., 1997; Miczek et al., 2002; Abrams JK, 2005; Kranz et al., 2010). Additionally, the DRN receives afferents from many structures including the LC, BNST, hypothalamus, amygdala, mPFC, and lateral habenula (Lowry et al., 2008a). Many of these structures projecting to the DRN have been labeled anxiety- or stress-sensitive brain regions. So, although the DRN is relatively small in size, the diversity of DRN afferents and efferents provide the possibility for integration and production of many different behaviors.

Behaviors following traumatic or aversive experiences are most relevant to this thesis. Indeed, others have documented increased extracellular levels of 5-HT following aversive stimuli such as tail pinch, restraint, and cold water stress (Rueter et al., 1997). Although many of the aversive stimuli tested only moderately increased extracellular 5-HT, these findings presented the possibility that IS may also activate DRN 5-HT cells.

To investigate whether IS and ES modulate extracellular 5-HT, *in vivo* microdialysis within the DRN was performed during stress. Microdialysis probes were placed in the DRN rather than projection regions to ensure that extracellular increases of 5-HT were specific to the DRN and not other serotonergic raphe structures. Additionally, extracellular 5-HT in the DRN is

thought to closely resemble extracellular 5-HT within projection regions of the DRN as 5-HT is released from the soma and dendrites of 5-HT cells (Tao R, 2000). Several findings from Maier and colleagues have demonstrated that exposure to IS, but not ES, potently elevated extracellular 5-HT in the DRN, upwards to 300% above baseline, throughout the stress session (Maswood et al., 1998; Amat et al., 2001; Amat et al., 2005). Additionally, extracellular 5-HT in projection regions of the DRN, such as the mPFC (Bland et al., 2003), the ventral hippocampus (Amat et al., 1998b), and the basolateral amygdala (BLA) (Amat et al., 1998a), was elevated during IS, but not ES, as well.

Increases of extracellular 5-HT presumably reflect greater activation of 5-HT cells in the DRN. This possibility was confirmed using immunohistochemistry (IHC). To identify the specific activation of 5-HT cells in the DRN, midbrain slices were double-labeled for the protein product of the immediate early gene c-*fos* and 5-HT. Indeed, IS, but not ES, produced a significantly elevated number of cells in the DRN double-labeled for Fos and 5-HT, 2 hours following stress (Grahn et al., 1999). Notably, this effect was specific to the mid and caudal subregions of the DRN. These findings using IHC and *in vivo* microdialysis demonstrate that IS, but not ES, selectively activates DRN 5-HT cells. However, the behavioral differences that are sensitive to stressor controllability are not tested until 24 hr after

stress, so the role of DRN 5-HT was also investigated 24 hr after stress exposure.

As described above, poor shuttlebox escape learning was the first behavior discovered to be sensitive to stressor controllability (Seligman and Maier, 1967), where only IS animals failed to escape in the shuttlebox. Since the foot shocks administered in the shuttlebox are unsignaled, the behaviors elicited in this paradigm, such as running, jumping, and biting, are unconditioned responses. These unconditioned responses have been termed circa-strike defensive behaviors (Fanselow et al., 1988). One interpretation of IS-produced failure to escape was an inhibition of circa-strike defensive behaviors. Therefore, the brain structures involved in circa-strike defensive behaviors were investigated to begin to understand the mechanisms involved in IS-induced shuttlebox escape failure.

Previous studies characterizing the necessary neural components for circa-strike defensive behaviors indicate that the dPAG is a critical structure (De Oca et al., 1998). Indeed, pharmacological and electrical stimulation of the dPAG produces circa-strike behaviors (Bandler, 1988). Furthermore, application of 5-HT_2 receptor agonists in the dPAG can blunt these running and jumping defensive behaviors (Schutz et al., 1985). Interestingly, the dPAG receives serotonergic projections from the DRN (Steinbusch and Nieuwenhuys, 1983). The above anatomy and pharmacology suggest that

perhaps DRN 5-HT cell activation could be responsible for the shuttlebox escape failure in observed in IS subjects.

To investigate the role of DRN 5-HT in IS-induced behaviors, Maier and colleagues did a number of pharmacological and lesion studies that targeted 5-HT cells in the DRN. Initially, Maier et al. demonstrated that activation of DRN 5-HT cells during IS was necessary to produce shuttlebox escape failure 24 hr later. Additionally, they showed that activation of DRN 5-HT cells during shuttlebox escape testing was necessary to produce the IS shuttlebox escape failure as well (Maier et al., 1995a). Lastly, Maier et al. demonstrated that simple activation of 5-HT cells in the DRN, in the absence of tail shock, was sufficient to produce shuttlebox escape failure (Maier et al., 1995b).

These findings illuminate a number of critical concepts important to unraveling the neural processes responsible for IS-induced behavioral outcomes. First, the hyperactivation of DRN 5-HT cells during IS, as measured by IHC and *in vivo* microdialysis, is not an epiphenomenon; it is necessary to produce shuttlebox escape failure in IS subjects. Second, these results demonstrate that activation of 5-HT cells in the DRN is necessary and sufficient to produce behaviors associated with IS exposure. Lastly, these results indicate that two processes are involved in the generation of IS-induced behaviors. The first phase is exposure to IS, and it requires activation of DRN 5-HT cells; this is termed the *induction* phase. The second

critical process is during behavioral testing, and it also requires activation of DRN 5-HT cells; this is termed the *expression* phase.

Although two necessary processes are likely critical in the genesis of IS behavioral outcomes, one question remained unknown: why is DRN 5-HT cell activation necessary during the induction phase to produce IS behaviors? Maier and colleagues hypothesized that activation of DRN 5-HT cells during tail shock was necessary because the hyperactivation of DRN 5-HT cells produced by IS sensitized those 5-HT cells (Maier et al., 1994; Maier et al., 1995a). So now, 24 hours later, during shuttlebox testing, the unsignaled foot shock produced a sensitized or increased amount of extracellular 5-HT in projection regions of DRN. This IS-specific sensitized 5-HT response was hypothesized to be the critical event that produced the shuttlebox escape failure.

Maier and colleagues investigated whether exposure to IS indeed produced a sensitized 5-HT response 24 hr after IS. Microdialysis probes were aimed at the BLA and two foot shocks were given 24 hr following IS, ES, or HC (Amat et al., 1998a). Two foot shocks did not produce a large increase of BLA extracellular 5-HT in HC. However, following IS, but not ES, foot shock-induced extracellular 5-HT in the BLA was sensitized. This finding indicated that previous IS had sensitized 5-HT cells. This sensitization of 5-HT cells was later shown in other behaviors sensitive to stressor controllability, including social exploration (Christianson et al., 2010).

Importantly, this sensitized 5-HT response is necessary to produce the behavioral outcomes associated with IS (Bland et al., 2004; Christianson et al., 2010). Here, again, the sensitized 5-HT response in IS subjects does not appear to be an epiphenomenon.

The 5-HT$_{1A}$ Receptor and Inescapable Stress

The literature reviewed above discussing the relationship between activation of 5-HT cells in the DRN and the behavioral consequences of IS suggest that IS sensitizes 5-HT cells in the DRN, and this sensitization is necessary to produce the behavioral consequences of IS. As mentioned earlier, two phases, an induction and expression phase, are thought to be critical.

Given (i) the necessity of DRN 5-HT cell activation during IS and (ii) the large amounts of extracellular DRN 5-HT during IS, it was hypothesized that the IS experience somehow compromised the usual autoregulatory or negative feedback mechanisms critical for tapering 5-HT cell activation in the DRN. This is the induction phase and its consequence is a sensitization of DRN 5-HT cells. Also, since DRN 5-HT cells must be activated during behavioral testing, it was hypothesized that the behaviors, such as shuttlebox escape learning, tested were activating the sensitized DRN 5-HT cells to produce IS-like behaviors. This is the expression phase and it is

characterized by activation of sensitized DRN 5-HT cells and the concurrent potentiation of extracellular 5-HT in projection regions of the DRN. Since ES and IS subjects showed differential extracellular DRN 5-HT during stress, we focused on this dissociation as a locus for determining the mechanisms involved in 5-HT cell sensitization following IS.

The literature was reviewed to determine the consequences of increases of extracellular 5-HT. Several studies demonstrated that pharmacologically induced increases in 5-HT or direct application of 5-HT receptor agonists produced adaptations to the serotonin-1A receptor (5-HT_{1A}-R) (Beer et al., 1990; van Huizen et al., 1993; Harrington et al., 1994; Riad et al., 2004). Generally speaking, large amounts of 5-HT_{1A}-R agonism can produce a "desensitization" or "downregulation" of 5-HT_{1A}-Rs. It should be mentioned here that throughout this thesis the term "desensitization" is used to describe the functional impairment of the receptor. Although this term can denote an uncoupling of a receptor to a G-protein (Li et al., 1996; Clark et al., 1999; Hensler, 2002), here it will be used as shorthand for "functional desensitization" or "receptor adaptation".

Desensitization of 5-HT_{1A}-Rs can have a significant consequence on the excitability of a 5-HT cell because the 5-HT_{1A}-R is included in a family of receptors that are responsible for regulating the excitability of 5-HT cells; these are called autoreceptors (Stamford et al., 2000). The 5-HT_{1A}-R is expressed on the soma and dendrites of 5-HT cells in the DRN (Sotelo et al.,

1990). The 5-HT_{1A}-R is a G_i-protein coupled receptor, that upon agonist binding, inhibits 5-HT cell firing (Sprouse and Aghajanian, 1987; Innis et al., 1988) and decreases extracellular 5-HT in DRN projection regions (Casanovas et al., 1997). Therefore, a functional desensitization of DRN 5-HT_{1A}-Rs could produce sensitized DRN 5-HT cells (Beer et al., 1990).

Moreover, application of selective 5-HT_{1A}-R agonists into the DRN reduces anxiety in a number of behavioral tests including the elevated plus maze (File and Gonzalez, 1996) and social interaction (Picazo et al., 1995). Together, these behavioral and neurochemical findings support the notion that excessive DRN 5-HT cell activation is anxiogenic and responsible for many of the behavioral outcomes of IS. The idea that excessive 5-HT can produce anxiety in an organism will be further explored in this thesis.

Since the 5-HT_{1A}-R has an autoregulatory role and can be functionally desensitized following excessive stimulation with 5-HT, we hypothesized that IS was desensitizing DRN 5-HT_{1A}-Rs. The diagram in Figure 1.1 further illustrates this hypothesis. The experiments presented in Chapter 2 were designed to determine whether excessive DRN 5-HT during stress functionally desensitizes 5-HT_{1A}-Rs.

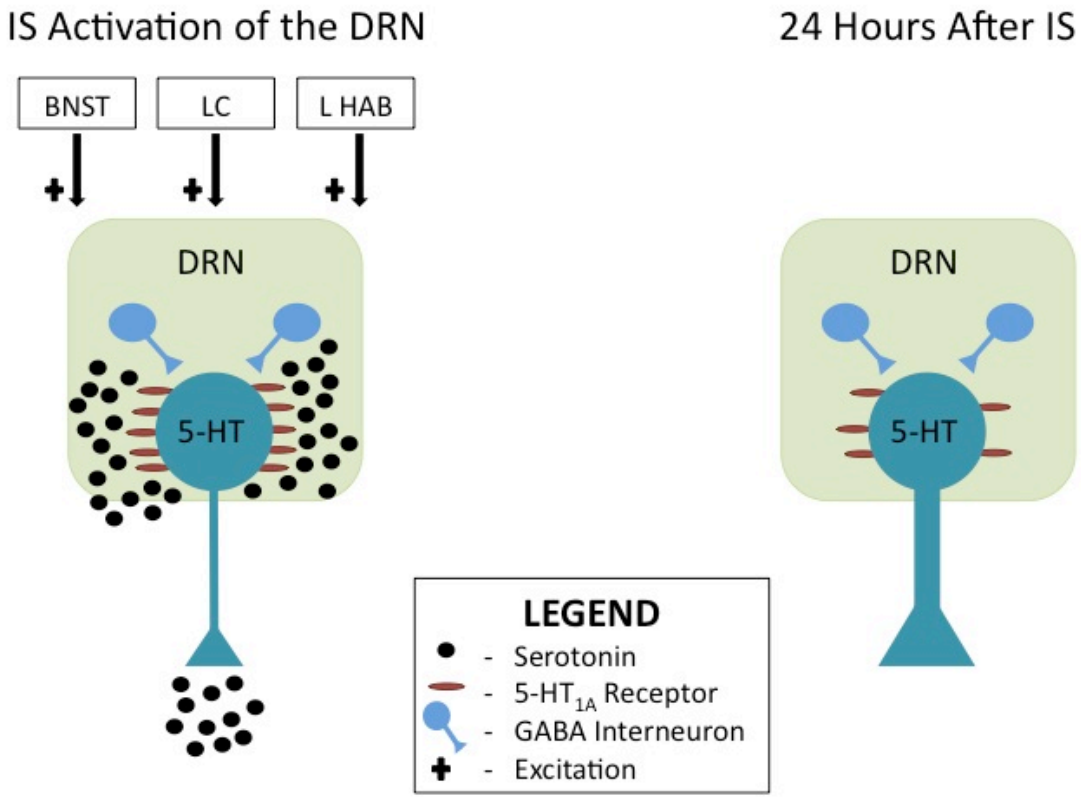

Figure 1.1. Brain regions that become activated during inescapable stress (IS) leads to activation of serotonin (5-HT) cells in the dorsal raphe nucleus (DRN) and causes sensitization of 5-HT cells in the DRN. IS activates several brain regions that produce increases of extracellular 5-HT in the DRN, including the bed nucleus of the stria terminalis (BNST), the locus coeruleus (LC), and the lateral habenula (L HAB). The consequence of prolonged elevated DRN 5-HT is a desensitization of 5-HT_{1A} inhibitory autoreceptors. A desensitization of 5-HT_{1A} receptors is thought to reduce autoregulation of 5-HT cell activation and consequently produce sensitization of DRN 5-HT cells.

The Medial Prefrontal Cortex and Stressor Controllability

So far, I have emphasized the behavioral, molecular, and neurochemical consequences of IS but have yet to discuss how control prevents these outcomes. It is remarkable that ES subjects behaviorally resemble HC controls since ES subjects receive the same shock protocol as do IS subjects. This behavioral phenomenology suggests that perhaps during ES there is an active neural process that is producing resistance to shock-induced outcomes. Given (i) that pharmacological inhibition of DRN 5-HT cells blocks IS-induced behaviors and (ii) IS, but not ES, produces excessive amounts of extracellular 5-HT in the DRN, one explanation for ES-resistance to shock-induced behaviors is that ES does not produce prolonged excessive amounts of DRN 5-HT. Therefore, it is possible that inhibition of DRN 5-HT cells is the active neural process during ES.

Before one can determine the neural mechanisms involved during ES, an understanding of the criteria that must be satisfied for an event to be controllable should be discussed. This discussion will help to narrow the possible brain regions involved in behavioral control by establishing a set of criteria necessary for mediating the protective effects of control. To learn whether it can control an event, an organism must be sensitive to two conditional probabilities. One is the probability of reinforcement given a

response has occurred. The other is reinforcement given the absence of a response. Control is defined by the conjoint variation between these two probabilities. If the two probabilities are equal the organism has no control, if they are unequal the organism has control. So an organism could only detect control by being sensitive to variations in a Cartesian space formed by these two conditional probabilities.

Thus, in order to be directly sensitive to control, a structure must, at minimum, receive somatomotor afferents providing information as to whether a response has occurred (e.g. turning a wheel) and somatosensory afferents to provide information as to whether reinforcement (e.g. the termination of shock) has or has not occurred. As mentioned earlier, DRN 5-HT cells are hyperactivated during IS, but not ES. However, the absence of 5-HT cell activation in the DRN during ES does not necessarily mean the DRN is processing the critical contingencies learned during ES. Since the DRN is a relatively small brainstem structure and does not receive direct somatomotor and somatosensory afferents (Lowry et al., 2008a), it is likely "downstream" of other structures that mediate the effects of control. Therefore, during ES, another structure likely receives the relevant contingency information and in-turn regulates DRN serotonergic cells.

Brain structures that receive a variety of somatomotor and somatosensory afferents and contain diverse efferents are likely candidates responsible for coordinating complex behaviors. Indeed, the learning of

action-outcome contingencies, temporal organization of events, and working memory all depend upon activation of the prefrontal cortex (Fuster, 1985; Goldman-Rakic, 1995; Miller and Cohen, 2001; Ostlund and Balleine, 2005; Hoover and Vertes, 2007). The mPFC is a cortical structure that satisfies the criteria necessary for detecting control and additionally the mPFC also contains pyramidal cell efferents that terminate in the DRN (Sesack et al., 1989; Peyron et al., 1998; Vertes, 2004).

If activation of DRN 5-HT cells is necessary for the behavioral outcomes of IS, then a structure that inhibits DRN 5-HT cell activation is a likely candidate brain region necessary for the ES phenomenon. Anatomical studies have determined that excitatory pyramidal efferents of the mPFC preferentially synapse onto GABA interneurons in the DRN (Jankowski and Sesack, 2004). These GABA interneurons then synapse onto the soma and dendrites of 5-HT cells (Wang et al., 1992). Therefore, activation of mPFC pyramidal cell efferents would inhibit DRN 5-HT cell activation via activation of inhibitory GABA interneurons. Indeed, DRN 5-HT cells express receptors for GABA (Gao et al., 1993; Wirtshafter and Sheppard, 2001) and pharmacological methods antagonizing DRN $GABA_A$ receptors attenuates mPFC-induced inhibition of DRN 5-HT cell firing (Varga et al., 2001). Electrophysiological studies have confirmed the pharmacology and histology described above. Indeed, electrical stimulation of the ventral regions of the mPFC reduces DRN 5-HT cell firing (Hajos et al., 1998).

Therefore, the necessity of mPFC activation was investigated in the stressor controllability paradigm. The hypothesis tested was that inactivation of mPFC output during ES would produce large and prolonged extracellular levels of DRN 5-HT. Again, this hyperactivation of DRN 5-HT cells during stress would presumably sensitize DRN 5-HT cells, which is the necessary event to produce behaviors associated with IS. What Amat et al. (2005) found was that inactivation of the mPFC during ES produced escape failure in the shuttlebox, similar to that of IS subjects. Correlated with the behavioral phenomenon, inhibition of the mPFC during ES also produced large, sustained levels of DRN 5-HT and increased cellular activation as measured by IHC. Again, all of these measures were similar to IS subjects. Therefore, when the mPFC cannot be activated during ES, all of the stress-buffering effects of behavioral control were abolished and therefore ES subjects now neurochemically and behaviorally resembled IS subjects.

What remains unknown is whether inactivation of the mPFC during ES also produces desensitization of 5-HT_{1A}-R in the DRN. This thesis will explore this possibility with the general hypothesis that if a particular stress protocol produces behaviors identical to the behaviors produced by IS alone, then DRN 5-HT_{1A}-Rs are likely functionally desensitized.

The Medial Prefrontal Cortex and Behavioral Immunization

Before the role of the mPFC was isolated in the stressor controllability paradigm, many researchers believed IS to be the "active" experimental treatment and ES to be the "passive" experimental treatment. As such, a majority of stressor controllability research was focused on IS. This view was adopted primarily because only IS produced behavioral outcomes different from HC treatment. However, given the most recent findings (Amat et al., 2005; Christianson et al., 2009; Rozeske et al., 2009), it has become clear that ES is the "active" condition and IS is the "passive" condition. That is, activation of the mPFC during stress overcomes the normal neurochemical and behavioral consequences produced by tail shock stress; this is what is meant by the "active" component of ES.

Since ES subjects behaviorally resemble HC controls, ES could be thought of as resilience or resistance training in the face of aversive events. That is, learning the contingencies involved in stressor controllability produces resistance to the normal stress-induced behavioral outcomes of tail shock. Because ES is an operant (i.e. active) learning process, and learning produces persistent memories by strengthening synaptic signaling in the brain, it became relevant to determine whether ES could produce long-term resistance to the effects of stress as a consequence of the synaptic strengthening that occurred during ES.

Importantly, the hypothesis here is not that ES would necessarily form a "memory" specific for wheel turning. Rather, the memory is thought to be

more general. ES is hypothesized to produce synaptic strengthening by co-activating the cells responsible for signaling aversive stimuli and the cells responsible for detecting behavioral control over aversive stimuli. Consequently, what is learned during ES, and the "memory" that ES produces, is that the cells that detect control inhibit the cells that are activated by aversive stimuli. This relationship of two cell populations being "tied" can most simplistically be regarded as a Hebbian process and thus produces synaptic strengthening between the two cell populations. Therefore, if memory in its most reduced form is the strengthening of synapses, then ES could be framed as an experience that produces a memory for stress-resistance or resilience.

Since ES subjects behaviorally resemble HC, if we want to probe for the long-term effects of ES, a subsequent challenge must be presented to ES and HC to reveal any long-term consequences of the ES or HC treatments. One such paradigm would involve giving a subject ES, waiting 24 hr (or even 1 week), and then administering IS. A HC subject who is given IS, will behaviorally resemble an IS subject, obviously. However, if the previous experience with ES has produced long-term stress-resistance or resilience, the normal behavioral outcomes of IS will not be observed. Indeed, this paradigm has been tested and termed "behavioral immunization" (Seligman and Maier, 1967; Williams and Maier, 1977).

When a subject has received ES some time before IS, the neurochemical and behavioral outcomes of IS are blocked; these animals are now stress-resistant. Behavioral immunization has been demonstrated neurochemically by measuring extracellular 5-HT in the DRN; indeed, ES prior to a subsequent IS, blocked the normal IS-induced prolonged elevations of DRN 5-HT (Amat et al., 2006). Behavioral immunization also blocked the normal IS-induced shuttlebox escape failure (Amat et al., 2006) and shock-elicited freezing (Amat et al., 2008).

To observe behavioral immunization, the mPFC must be activated during the initial ES experience and during the subsequent IS experience (Amat et al., 2006). Intuitively we can make sense of the necessity of mPFC activation during the initial ES exposure because this is necessary for the acute effects of ES, as previously discussed (Amat et al., 2005). However, since inactivation of the mPFC during subsequent IS blocked the expression of behavioral immunization, it was hypothesized that during subsequent uncontrollable stressors the subject has the "illusion of control" because the mPFC would not normally be activated during IS (Amat et al., 2008). Additionally, simple activation of the mPFC in the absence of tail shock did not produce behavioral immunization to subsequent IS (Amat et al., 2008), so although mPFC activation was necessary, it was not sufficient for behavioral immunization. It is for these reasons that behavioral immunization is thought to involve a coincidence or "tied" relationship

between activation of the mPFC and aversive stimulation, regardless of the aversive event being controllable or uncontrollable.

As mentioned earlier, if a particular paradigm produces the behavioral effects of IS, we would hypothesize that 5-HT_{1A}-Rs in the DRN have become desensitized. Conversely, with behavioral immunization, if a behavioral experience blocks the behavioral outcomes of IS, we would hypothesize that DRN 5-HT_{1A}-Rs are as sensitive as those in the DRN of HC controls. Since an initial experience with ES prevents the subsequent IS-induced hyperactivation of DRN 5-HT cells, we would expect that behavioral immunization is preventing the desensitization of DRN 5-HT_{1A}-Rs. This will be examined below.

Introduction Conclusions and Thesis Organization

The stressor controllability paradigm is a useful paradigm to study the psychological variable of control rather than stress *per se*. Our knowledge of the circuits responsible for the effects of ES and IS has grown much richer throughout the past 20 years. Indeed, IS potently activates 5-HT cells in the DRN and activation of the mPFC is intimately involved in blocking this effect of IS. Although several studies have found behavioral and neurochemical differences between ES and IS subjects, surprisingly, no study has attempted to identify the mechanism responsible for the production of IS-

induced behaviors. Up to this point it is known that to observe the behavioral effects of IS the DRN must be activated during both stress and behavioral testing, but it is unknown why. This thesis seeks to enrich our understanding of the mechanisms responsible for producing the behavioral effects of IS by providing a hypothesis and evidence that satisfies the necessary conditions for expression of IS behavioral outcomes.

In Chapter 2, I will provide evidence that IS desensitizes DRN 5-HT_{1A}-Rs. These experiments will use an *ex vivo* electrophysiology approach: subjects will receive ES, IS, or HC and then 24 hr later *in vitro* single unit recordings of DRN cell firing will be recorded. The initial experiments provided will determine the consequences of ES and IS on the sensitivity of DRN 5-HT_{1A}-Rs. Subsequent experiments will then determine whether stress protocols known to block or produce the behavioral effects of IS, also block or produce desensitization of DRN 5-HT_{1A}-Rs, respectively.

We will provide evidence that only IS desensitizes DRN 5-HT_{1A}-Rs and that the IS-induced desensitization of DRN 5-HT_{1A}-Rs follows the behavioral timecourse of IS. Furthermore, we will show that activation of the mPFC during stress is necessary to prevent stress-induced desensitization of DRN 5-HT_{1A}-Rs. Lastly, we will show that behavioral immunization prevents IS-induced desensitization of DRN 5-HT_{1A}-Rs.

Chapter 2:

Uncontrollable, but not Controllable, Stress Produces a Functional Desensitization of 5-HT_{1A} Receptors in the Dorsal Raphe Nucleus.

Robert R. Rozeske, Andrew K. Evans, Linda R. Watkins, Christopher A. Lowry, and Steven F. Maier

Abstract

Uncontrollable stressors produce behavioral changes that do not occur if the organism can exercise behavioral control over the stressor. Previous studies suggest that the behavioral consequences of uncontrollable stress are dependent on hypersensitivity of serotonergic neurons in the dorsal raphe nucleus (DRN), but the mechanisms involved have not been determined. We used *ex vivo* single unit recording in rat brain slices to test the hypothesis that the effects of uncontrollable stress are produced by desensitization of 5-HT_{1A} receptor-mediated autoinhibition of DRN serotonergic neuronal firing rates. These studies revealed that uncontrollable, but not controllable, tailshock impaired serotonin-mediated inhibition of DRN neuronal firing. This same effect occurred if the specific 5-HT_{1A} receptor agonist ipsapirone was used. Furthermore, temporary inactivation of the medial prefrontal cortex (mPFC) with the $GABA_A$ receptor agonist muscimol, which eliminates the protective effects of control on behavior, led even controllable stress to now produce functional desensitization of DRN 5-HT_{1A} receptors. Additionally, behavioral immunization, an experience with controllable stress before uncontrollable stress that prevents the behavioral outcomes of uncontrollable stress, also blocked functional desensitization of DRN 5-HT_{1A} receptors by uncontrollable stress. Thus, treatments that prevent controllable stress from being protective led to functional desensitization 5-HT_{1A} receptors, and treatments that block the behavioral effects of

uncontrollable stress also blocked 5-HT_{1A} receptor desensitization. These data suggest that uncontrollable stressors produce a functional desensitization of somatodendritic 5-HT_{1A} autoreceptors in the DRN, and that this desensitization is responsible for the behavioral consequences of uncontrollable stress.

Introduction

The degree of behavioral control that an organism has over a stressor critically determines the behavioral and neurochemical consequences of that stressor. Behavioral control is typically studied in a paradigm in which one subject can behaviorally terminate tailshocks (escapable shock, ES), while another yoked subject cannot (inescapable shock, IS). Many behaviors occur following IS (impaired escape learning, exaggerated anxiety, etc.), that do not occur after ES, despite identical shock delivery (Maier and Watkins, 2005). This phenomenon is termed "learned helplessness" (Maier and Seligman, 1976).

Considerable effort has been devoted to understand why IS and ES produce different behavioral outcomes. Alterations in dorsal raphe nucleus (DRN) serotonergic (5-HT) functioning are clearly involved. IS, relative to ES, intensely activates DRN 5-HT neurons (Grahn et al., 1999), leading to large accumulations of extracellular 5-HT within the DRN (Amat et al., 2005) and projection regions (Bland et al., 2003). This activation sensitizes DRN 5-HT neurons for 24-72 hr. Now, after IS, stimulation of 5-HT neurons, as occurs during behavioral testing, releases exaggerated amounts of 5-HT in projection regions (Amat et al., 1998a). Finally, exaggerated 5-HT in projection regions appears responsible for IS behaviors since (i) inhibition of DRN activation during either behavioral testing or IS blocks and prevents, respectively, the expression of IS-induced behaviors (Maier et al., 1995a);

(ii) blocking 5-HT receptors in projection regions prevents IS-induced behaviors (Christianson et al., 2010); and (iii) pharmacological activation of DRN 5-HT neurons in the absence of IS produces IS-induced behaviors (Maier et al., 1995b).

Clearly, sensitization of DRN 5-HT neurons is essential in this process. However, the mechanism(s) by which IS sensitizes these neurons is unknown. One possibility is that IS desensitizes DRN inhibitory autoreceptors. The 5-HT_{1A} receptor (5-HT_{1A}-R) is a likely candidate. In the DRN, 5-HT_{1A}-Rs are somatodendritically expressed and, upon activation, inhibit 5-HT cell firing (Sprouse and Aghajanian, 1987) and release (Bonvento et al., 1992) by opening K+ channels (Williams et al., 1988; Penington et al., 1993). Indeed, reduced 5-HT_{1A}-R expression is associated with excessive 5-HT release in DRN projection regions upon stimulation (Richardson-Jones et al., 2010). Furthermore, DRN 5-HT_{1A}-Rs are desensitized by excessive 5-HT (Le Poul et al., 1995). As noted above, IS produces large and prolonged elevations of extracellular 5-HT within the DRN. Thus, the conditions for 5-HT_{1A}-R desensitization are present.

Here we investigate whether stressor controllability alters DRN 5-HT_{1A}-R function. We do this by measuring single unit activity of 5-HT cells in midbrain slices following exposure to stress. We explore causality between DRN 5-HT_{1A}-R desensitization and IS-produced behaviors by using treatments known to (i) eliminate the protective effects of ES and (ii) block

the behavioral effects of IS. Since inactivation of the medial prefrontal cortex (mPFC) during ES eliminates its protective effects on behavior (Amat et al., 2005), then mPFC inactivation during ES should lead ES to desensitize 5-HT_{1A}-Rs. Conversely, since prior exposure to ES blocks the behavioral effects of IS (Amat et al., 2006), then prior ES should also prevent IS-induced 5-HT_{1A}-R desensitization. The experiments below investigate these hypotheses.

Materials & Methods

Subjects. Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing 275-325 g were used in all experiments. Rats were singly housed and maintained on a 12 hr light/dark cycle (lights on at 07:00 and off at 19:00) with food and water provided *ad libitum*. Rats were allowed at least 7 days to acclimate in the colony room before any surgery or experimentation. All procedures were approved by the Institutional Animal Use and Care Committee of the University of Colorado-Boulder.

Surgery and Cannulation. Rats were anesthetized with isoflurane (Webster Veterinary, Sterling, MA, USA) and a 26-gauge dual guide cannula (Plastics One, Roanoke, VA, USA), 1 mm center-to-center distance, was implanted. The tips of the cannulae were directed 1 mm above the boarder of the infralimbic (IL) and prelimbic (PL) subregions of the mPFC (+2.7 mm rostral, −3.3 mm ventral from the dura mater, 0.5 mm relative to midline) (Paxinos and Watson, 1998). Rats were allowed to recover at least 7 day before experimentation.

Microinjection. Microinjections were delivered with a Kopf Instruments Model 5000 microinjector (Kopf Instruments, Tujunga, CA, USA). A dual 33-gauge microinjector tip (Plastics One) was connected to two 10 μl microsyringes with polyethylene 50 tubing (Becton, Dickinson and Company, Sparks, MD,

USA). Rats were gently wrapped in a towel and the microinjector tip was inserted into the cannula guides, extending 1 mm beyond the cannula tip. Microinjections were given over the course of 1 min and remained in the cannula for 2 min to ensure drug diffusion. Rats received either 0.5 μl of 50 ng of muscimol (Sigma, St. Louis, MO, USA) or 0.9% saline. Microinjections were considered successful if following removal from the guide cannula fluid was readily dispensable from injector tips.

Stressor Controllability. Rats were placed in clear Plexiglas boxes (11 x 14 x 17 cm) containing a wheel at the front and a Plexiglas rod extending from the rear. The rat's tails were taped to the Plexiglas rods and two copper electrodes were affixed to the tail, augmented with electrode paste. Electric shocks were delivered to the rats with a Precision-Regulated Animal Shocker and Graphic State 3.0 software (Coulbourn Instruments, Allentown, PA, USA). The shock protocol consisted of 80 shocks separated by an average inter-trial interval of 60 s. Shock intensity increased every 30 min (1.0 mA, 1.3 mA, 1.6 mA) to maintain reliable escape behavior. Shocks were given in yoked rat pairs (ES and IS); therefore, the shock terminated for both ES and IS rats when the ES rat performed the required operant escape response. The required response at the beginning of the stress session was a ¼ turn of the wheel. The response requirement increased to a ½ wheel-turn following three consecutive trials of ¼ wheel-turns that were performed within 5 sec.

Subsequently, the response requirement increased by 50% provided the previous response requirement was performed within 5 sec. The maximum response requirement was 4 full wheel-turns. Notably, the only difference between shock groups was that ES rats controlled the termination of shock. Rats that received no stress were left in their homecages as homecage controls (HC). Rats in the timecourse experiment received 100 tailshocks at an intensity of 1.0 mA in a Plexiglas restraint tube (17.5 cm in length and 7 cm in diameter) with an average inter-trial-interval of 60 s.

Rats in the "behavioral immunization" experiment were assigned to 1 of 3 treatment groups. The behavioral immunization group (ES/IS) received ES on Day 1 as described above, and 24 hr later on Day 2 received IS. IS on Day 2 consisted of 100 tailshocks in a restraint tube at an intensity of 1.0 mA with an average inter-trial interval of 60 s. The IS treatment group (HC/IS) received HC treatment on Day 1 and IS in a restraint tube on Day 2. Lastly, an unstressed group (HC/HC) remained in their homecages on Days 1 and 2. A treatment group that received IS on both Day 1 and Day 2 (IS/IS group) was not included as previous comparisons to the HC/IS group revealed no differences (Amat et al., 2006). In all experiments, midbrain slices were taken 24 hr following the last shock treatment.

Brain Slice Preparation. Under sodium pentobarbital (60 mg/kg, i.p.), rats were decapitated and the brain was rapidly removed, blocked coronally,

rostral to the DRN, and affixed to a vibratome stage (DSK Microslicer; DTK-1000; Dosaka EM, Kyoto, Japan) with cyanoacrylate glue. Brains were immersed in cold (4 ^{o}C) artificial cerebrospinal fluid (aCSF) containing (in mM): 124 NaCl, 3.25 KCl, 2.4 $MgSO_4$, 2 $CaCl_2$, 1.25 KH_2PO_4, 10 D-glucose, and 26 $NaHCO_3$ bubbled with 95% O_2/5% CO_2 during slicing. The time from decapitation to immersion in aCSF was never more than 150 s. Midbrain slices (450 μm) containing the DRN were collected starting caudally at about −8.76 mm from Bregma (Paxinos and Watson, 1998). Three consecutive sections of the DRN were taken, with the most rostral slice ending approximately at −7.80 mm from Bregma (Paxinos and Watson, 1998). Midbrain slices were placed in a Petri dish containing aCSF (RT) bubbled with 95% O_2/5% CO_2 and allowed to equilibrate for at least 1 hr. Following equilibration, midbrain slices were placed on a sloping liquid-gas interface perfusion chamber for recording. The slope was lined with a double-layer of lens tissue paper underneath the slice and tissue paper flanking the four sides of the slice to maintain hydration. Slices in the chamber were heated at 35 $\pm$ 1 ^{o}C and perfused with oxygenated aCSF containing 3 μM of phenylephrine hydrochloride (an α_1 adrenergic agonist) at a flow rate of 750 μl per min. Phenylephrine hydrochloride was added to increase 5-HT neuronal firing rates to levels observed *in vivo* (VanderMaelen and Aghajanian, 1983) because noradrenergic afferents to the DRN are severed during slicing. Since the strategy of the experiments below was to assess

feedback inhibition of baseline 5-HT cell firing rates, restoration of spontaneous 5-HT firing rates with an α_1 adrenergic agonist was essential.

Extracellular Recording. Extracellular recordings were made with borosilicate glass pipettes filled with aCSF. The glass electrode was connected to an alternating current differential preamplifier (x1000) and visualized in a window discriminator. Units were screened for characteristics consistent with a serotonergic phenotype (VanderMaelen and Aghajanian, 1983). All cells were preferentially sampled from the mid-rostrocaudal to caudal (~ −7.80 mm to −8.30 mm, relative to Bregma) dorsomedial DRN, as this region has several efferents to anxiety- and fear-related structures (Lowry et al., 2008b). Once isolated, activity of a unit was recorded with Spike2 software (version 5.05, Cambridge Electronics Design, Cambridge, UK) for 5 min to assess the baseline firing rate. Slices were then perfused with 50 μM of 5-HT for 2 min. Units that were reversibly inhibited by 5-HT and expressed characteristics consistent with the 5-HT cell phenotype, such as long duration biphasic or triphasic action potentials, a regular firing pattern, and a firing rate approximately ranging from 0.5 Hz to 2.8 Hz (Jacobs and Fornal, 1991), were deemed putative 5-HT cells. Following recovery from application of 50 μM 5-HT, a variety of drugs were applied to the slice and changes in firing rate were calculated.

Drugs. For mPFC microinjections, muscimol (Sigma) was dissolved in 0.9% saline according to required dose. The 5-HT_{1A}-R antagonist WAY 100635 (N-cond ethyl]-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride) and 5-hydroxytryptamine hydrochloride were purchased from Sigma and aliquoted with aCSF. The 5-HT_{1A}-R agonist ipsapirone (2-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-1,2-benzis othiazol-3(2H)-one-1,1-dioxide) was purchased from Tocris Bioscience (Ellisville, MO, USA) and aliquoted in dimethyl sulfoxide (Calbiochem, San Diego, CA, USA). Importantly, ipsapirone is a selective 5-HT_{1A}-R agonist in the DRN while a partial agonist in other brain regions (Glaser et al., 1985; Dong et al., 1997). Unless otherwise noted, all drugs used for *ex vivo* extracellular recordings were applied for 2 min and dissolved in aCSF containing phenylephrine hydrochloride (Sigma) during slice application.

Analysis of Firing Rates and Responses. Unless otherwise noted, the dependent measure used throughout all experiments was 'percent inhibition'. Percent inhibition was calculated by determining the mean firing rate during the 2 min prior to drug application, 'mean baseline'. The mean firing rate during drug perfusion was calculated from the time of drug application until maximal inhibition of firing, the 'mean drug firing rate'. Therefore, the percent inhibition was calculated as (1 – (mean drug firing rate/mean baseline)) x 100. All drugs were applied for 2 min unless

otherwise noted. Stress-induced alterations of baseline firing rates were analyzed by comparing the mean baseline firing rate 2 min prior to application of 50 μM 5-HT, as 50 μM 5-HT was applied to all cells as a test of a serotonergic phenotype.

Histology. To verify cannula location, brains were frozen by placement over dry ice and then stored at −80 °C. Coronal slices measuring 40 μm in thickness were taken throughout the frontal cortex and mounted on gelatin-coated glass slides. Sections were stained with cresyl violet and examined for cannula tracts under light microscopy. Rats with cannula tips outside of IL or PL subregions were excluded from analysis.

Statistics. All data are expressed as mean ± SEM. In experiments assessing dose-response, stress was a between-subjects variable and drug dose was a within-subjects variable. Percent inhibitions of firing rate were analyzed as independent observations using an unpaired *t*-test, one-way ANOVA, or two-way ANOVA, as appropriate. Statistically significant main effects were followed by either Dunnett's Multiple Comparison test or Fisher's Protected Least Significant Difference *post hoc* analysis (two-tailed $\alpha = 0.05$).

Results

Figure 2.1 shows cannula placements within the mPFC for Experiment 5 and the region of the DRN where 5-HT cell recordings were preferentially taken throughout all experiments. All cannulated subjects had microinjector tips terminating in either the IL or PL regions of the mPFC.

Experiment 1: Effect of stressor controllability on serotonin-mediated inhibition of DRN 5-HT cell firing

Rats received either ES, IS, or HC treatment and were sacrificed 24 hr later for extracellular single unit recording. To determine if IS functionally desensitized 5-HT_{1A}-Rs in the DRN the endogenous ligand for the 5-HT_{1A}-R, 5-HT, was used. Varying doses of 5-HT (1, 20, 25, 50, and 100 μM) were applied for 2 min and percent inhibition was calculated. As shown in Figures 2.2 and 2.3, serotonin-mediated inhibition of DRN 5-HT cell firing was impaired following IS, but not following ES. This result was confirmed by a two-way ANOVA revealing significant main effects of *stress* ($F_{(2, 248)}$ = 6.410; $p < 0.01$) and *dose* ($F_{(4, 248)}$ = 38.530; $p < 0.0001$), but no significant interaction. Subsequent *post hoc* comparisons revealed that IS rats were significantly different from ES and HC rats. Importantly, the ES and HC *post hoc* comparison was not significant. Stress-induced changes of baseline

firing rate were analyzed but no significant differences were found ($F_{(2, 58)}$ = 3.008; p > 0.05).

Experiment 2: Role of the 5-HT$_{1A}$-R during serotonin-mediated inhibition of DRN 5-HT cell firing

Although 5-HT is the endogenous ligand for 5-HT receptors, the DRN expresses several 5-HT receptor classes (Barnes and Sharp, 1999). To determine the role of the 5-HT$_{1A}$-R during serotonin-mediated inhibition of DRN cell firing, the 5-HT$_{1A}$-R antagonist WAY 100635 was used. Slices of the midbrain were taken from experimentally naïve rats for extracellular single unit recording. After 5 min of baseline firing rate collection, 100 μM of 5-HT was applied for 2 min and percent inhibition was calculated. After recovery from 100 μM 5-HT, 20 nM of WAY 100635 was applied to the slice for 16 min. Following superfusion with WAY 100635, a cocktail of 20 nM of WAY 100635 and 100 μM of 5-HT was applied to the slice for 2 min each, and percent inhibition was calculated. A similar protocol has been used by others to assess the contribution of the 5-HT$_{1A}$-R during serotonin-mediated inhibition (Fairchild et al., 2003). As shown in Figure 2.4, WAY 100635 blocked serotonin-mediated inhibition of DRN cell firing. An unpaired t-test revealed a significant difference between mean percent inhibition of the 5-HT applications ($t_{(12)}$ = 3.230; p < 0.01).

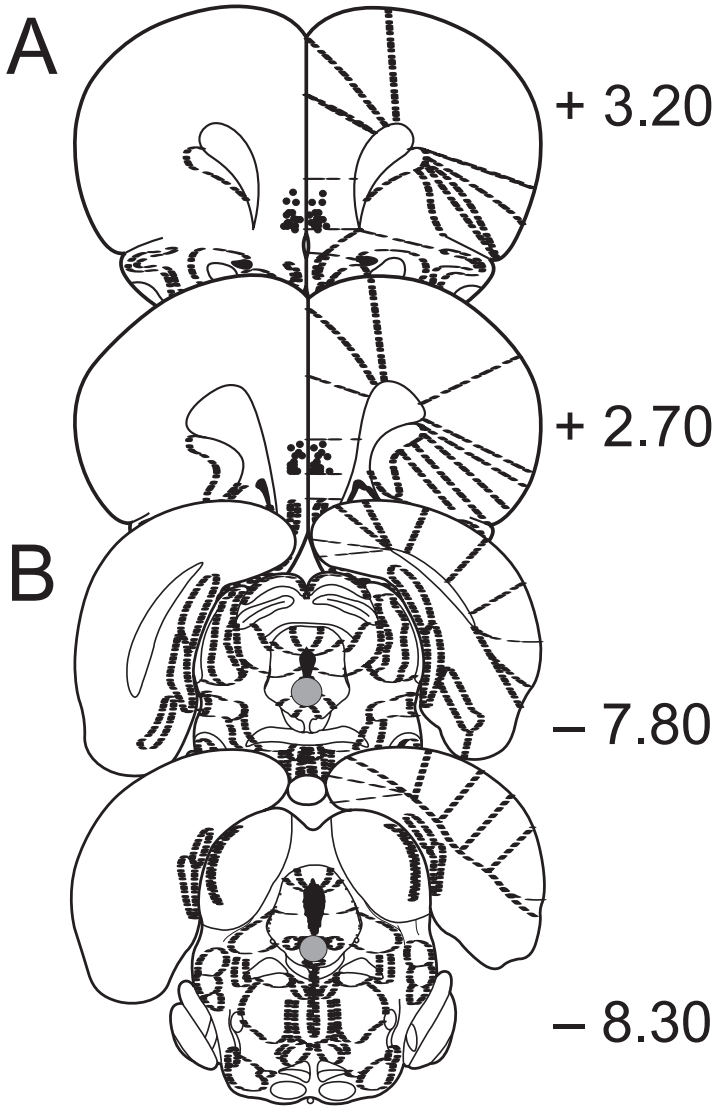

Figure 2.1. A, Placements of microinjection cannulae in the medial prefrontal cortex. Black circles indicate the location of the cannula tips. **B**, Location of single unit extracellular recordings in the dorsal raphe nucleus. The gray-shaded areas indicate where recordings were preferentially taken. Numerals indicate distance from Bregma in millimeters. Anatomical maps adopted from Paxinos and Watson (1998).

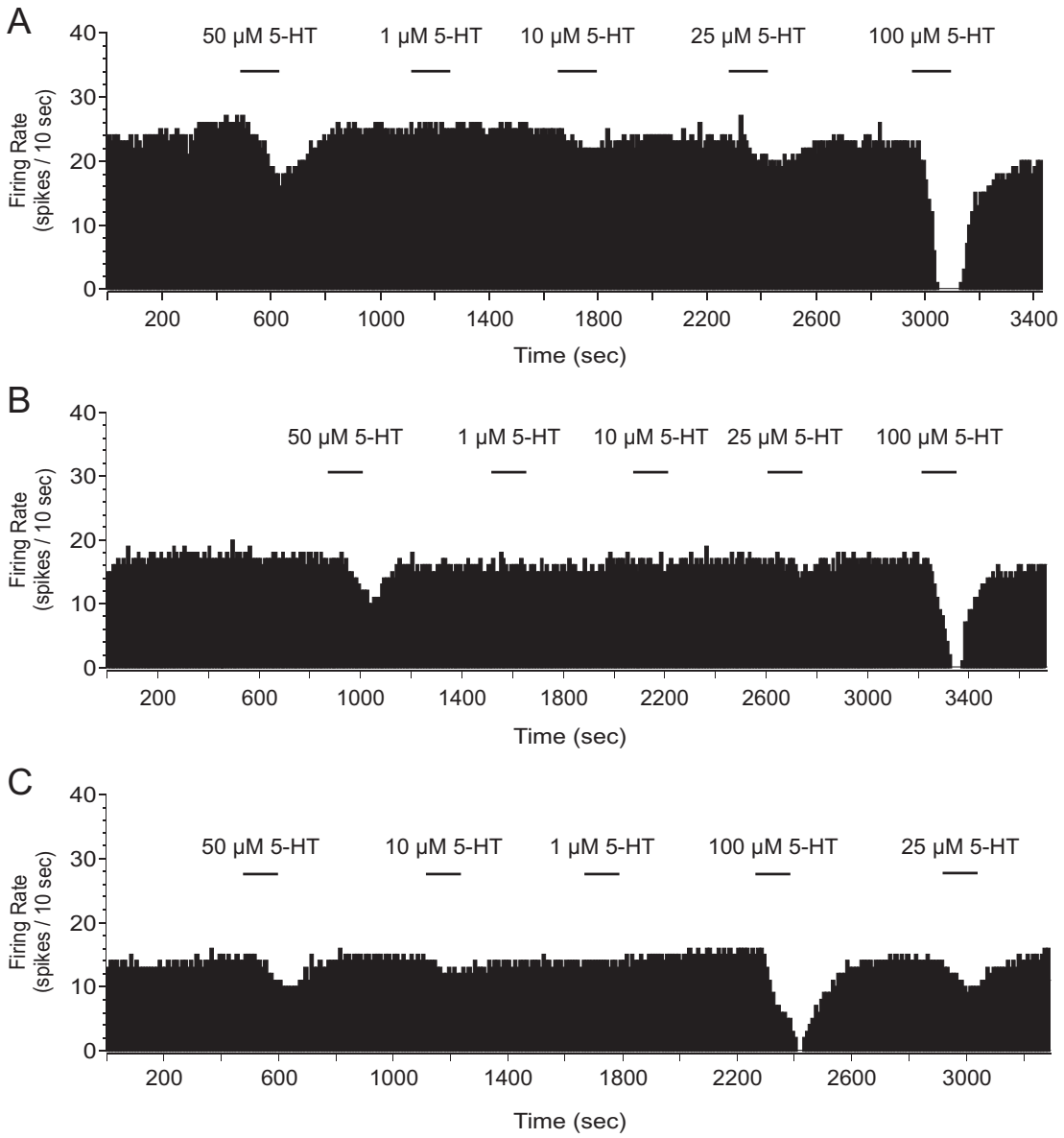

Figure 2.2. Spike frequency histograms from extracellular single unit recording in the dorsal raphe nucleus following homecage control (**A**), escapable stress (**B**), and inescapable stress (**C**). Midbrain slices were taken 24 hr after stress treatment and varying doses of serotonin (5-HT) were applied and percent inhibition was calculated.

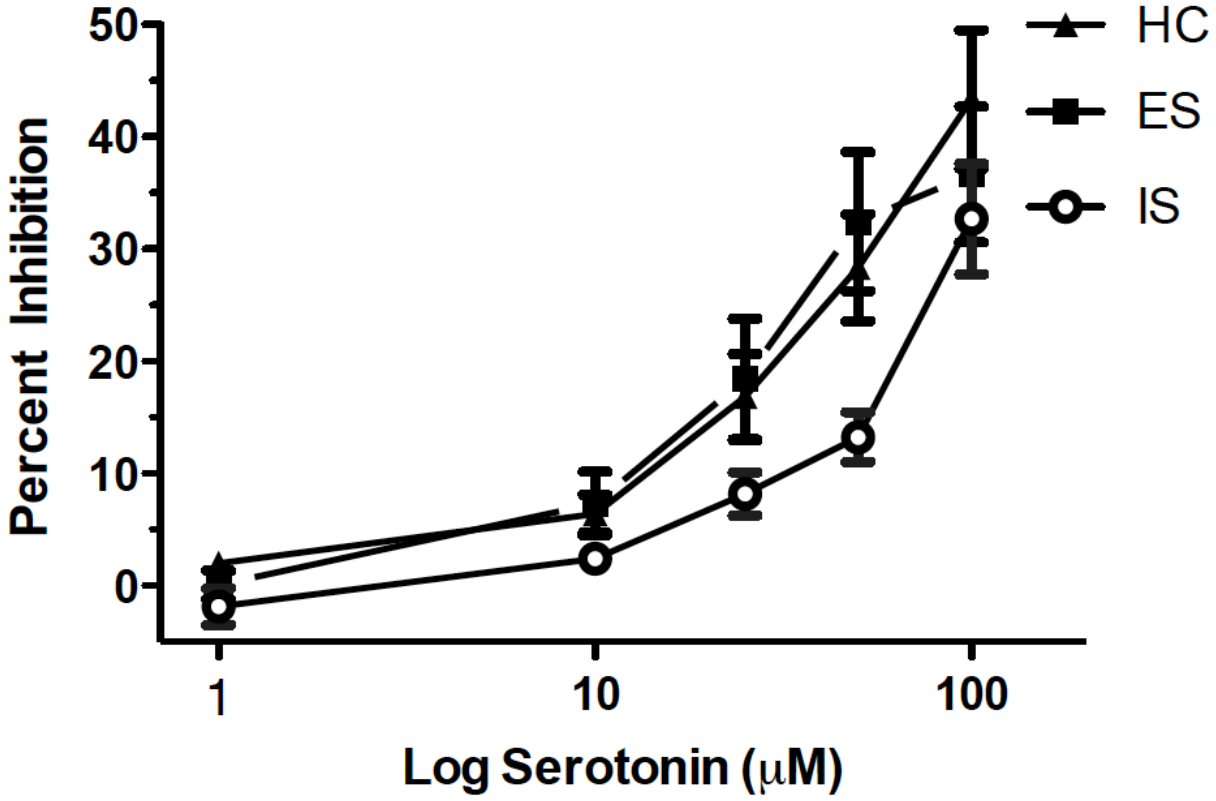

Figure 2.3. Inescapable stress (IS) selectively impairs serotonin-mediated inhibition of dorsal raphe nucleus (DRN) cell firing 24 hr following stress treatment. The graph depicts the effect of stress treatment on serotonin-mediated inhibition of DRN cell firing. Groups are designated as the following: homecage control (HC), closed triangles; escapable stress (ES), closed squares; IS open circles. Data are expressed as mean percent inhibition $\pm$ SEM. Mean percent inhibition of DRN cell firing was significantly different in IS, compared to ES and HC ($p < 0.05$).

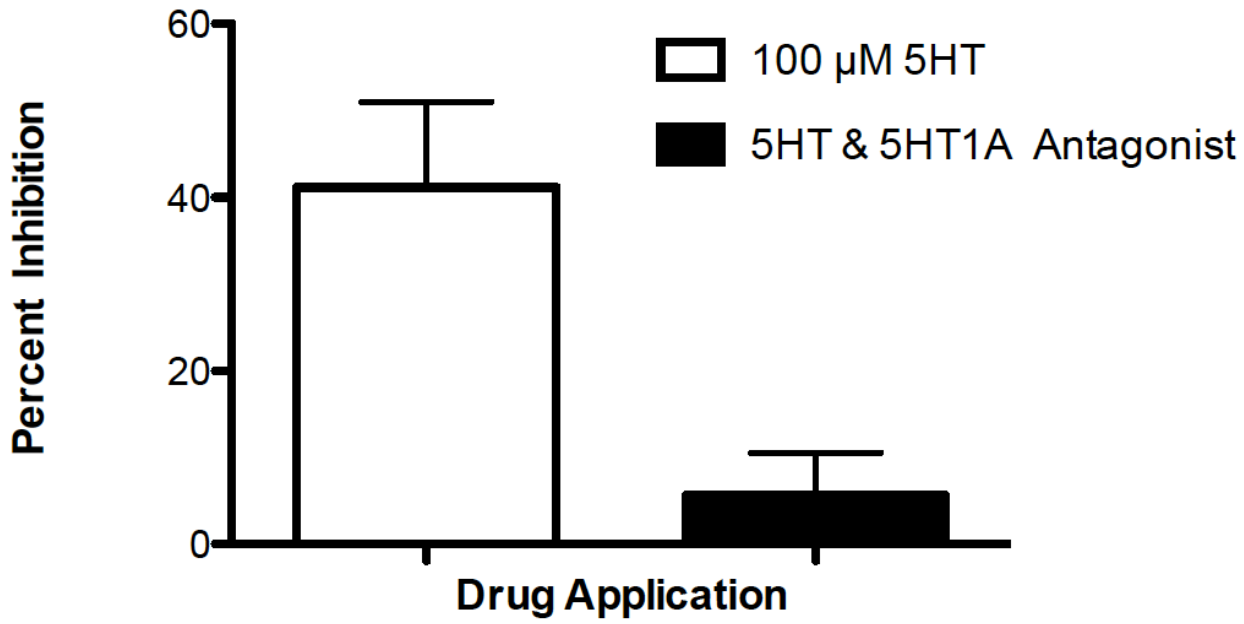

Figure 2.4. Application of 5-HT$_{1A}$ receptor antagonist WAY 100635 attenuates serotonin (5-HT)-mediated inhibition of dorsal raphe nucleus (DRN) cell firing. Data are expressed as mean percent inhibition + SEM. Application of WAY 100635 significantly attenuated serotonin-mediated inhibition of DRN cell firing ($p < 0.05$).

Experiment 3: Effect of inescapable stress on ipsapirone-mediated inhibition of DRN 5-HT cell firing

Rats received either IS or HC treatment and were sacrificed 24 hr later for extracellular single unit recording. Experiment 3 did not contain an ES group as Experiment 1 revealed no significant differences between ES and HC rats. The 5-HT_{1A}-R agonist ipsapirone was applied at varying doses (5, 50, 100, 150, 250, and 500 nM) for 2 min and percent inhibition was calculated. As shown in Figure 2.5, ipsapirone-mediated inhibition was impaired in IS rats at a number of doses. These results were confirmed by a two-way ANOVA. The effect of *stress* ($F_{(1, 161)}$ = 16.32; $p < 0.001$), *dose* ($F_{(5, 161)}$ = 13.76; $p < 0.001$) and interaction of *stress* x *dose* ($F_{(5, 161)}$ = 3.27; $p < 0.01$) were significant. Subsequent *post hoc* comparisons indicated that IS was significantly different from HC. Additionally, *post hoc* analysis of *stress* x *dose* indicated that IS and HC were significantly different at the 150 and 250 nM ipsapirone doses. Again, stress had no significant effect on baseline firing rate ($t_{(68)}$ = 0.8031; $p > 0.05$).

Experiment 4: Timecourse of IS effects on ipsapirone-mediated inhibition of DRN 5-HT cell firing

Rats received IS in a Plexiglas restraint tube or remained in the colony as HC controls. Rats were then sacrificed either immediately following IS (0 Hr), 1 day later (24 Hr), or 1 week later (7 D) for extracellular single unit recording. These time-points were chosen because the behavioral effects of IS are observed between 24-72 hr following IS exposure (Glazer and Weiss, 1976; Grau et al., 1981; Maier, 1990). Additionally, because Experiment 3 revealed a significant difference between IS and HC rats at the 150 nM dose of ipsapirone, only the 150 nM dose was used as it was the lowest dose that produced a maximal difference between IS and HC rats. A one-way ANOVA revealed ($F_{(3, 48)} = 3.977$; $p < 0.05$) a significant stress group effect. A subsequent Dunnett's Multiple Comparison test revealed a significant difference of mean percent inhibition between the IS 24 Hr time-point and HC. The 0 Hr and 7 D time-points were not significantly different from HC.

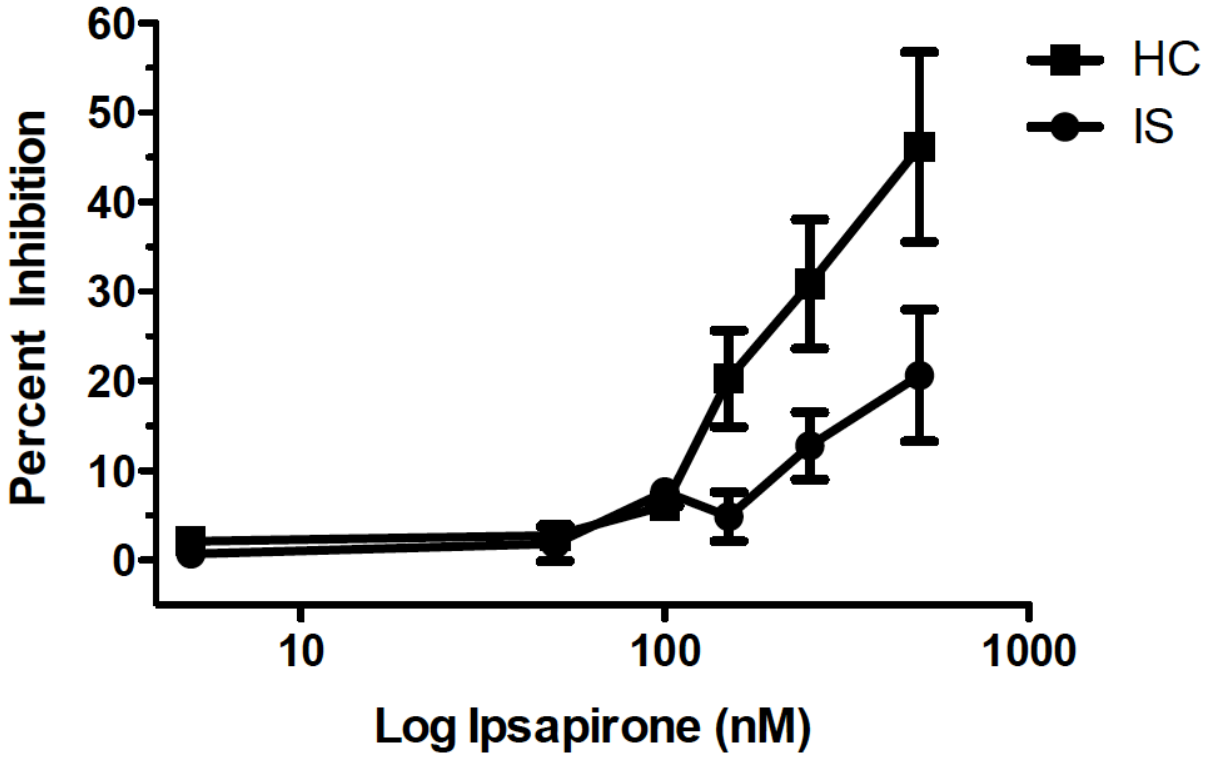

Figure 2.5. Inescapable stress (IS) impairs ipsapirone-mediated inhibition of dorsal raphe nucleus cell firing 24 hr following stress treatment. Groups are expressed as the following: closed squares, homecage control (HC); closed circles, IS. Data are expressed as mean percent inhibition $\pm$ SEM. IS significantly reduced ipsapirone-mediated inhibition of DRN cell firing as compared to HC ($p < 0.001$). Ipsapirone-mediated inhibition was significantly different between IS and HC at the 150 and 250 nM doses ($p < 0.05$).

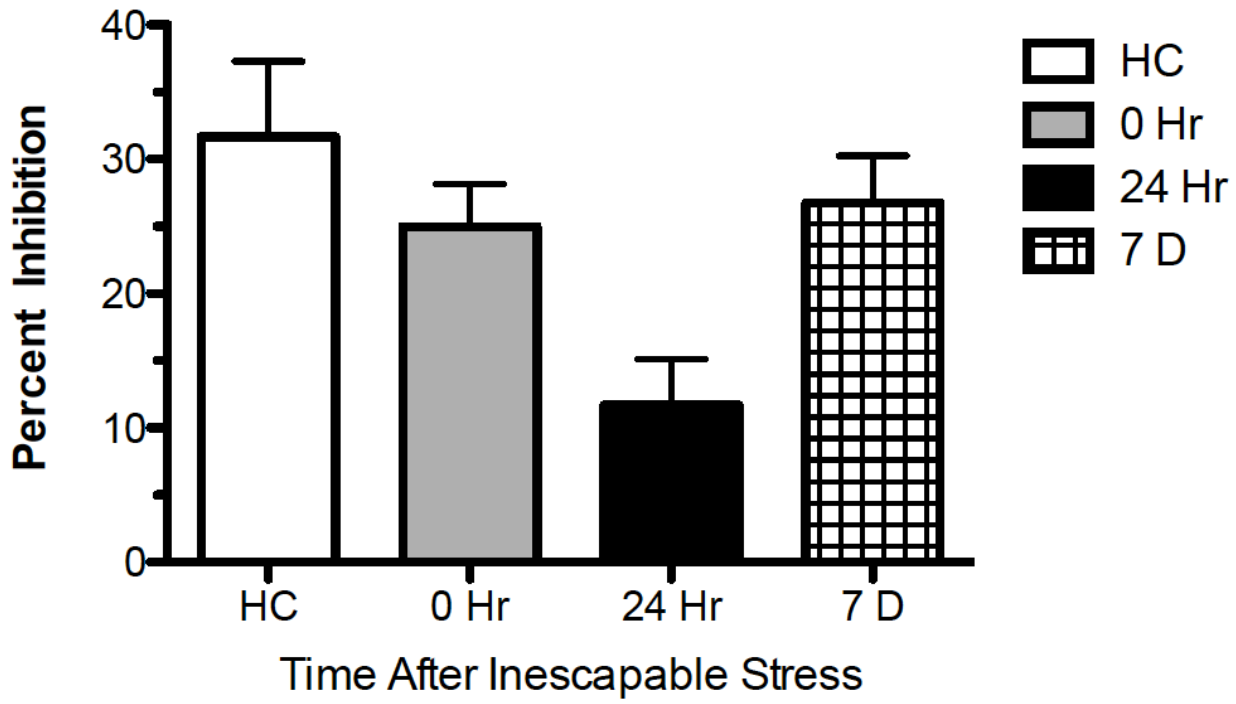

Figure 2.6. Ipsapirone-mediated inhibition of dorsal raphe nucleus serotonin cell firing is selectively impaired 24 Hr following inescapable stress (IS). The white bar indicates non-stressed homecage control (HC), the gray bar indicates IS rats that were sacrificed immediately following IS, the black bar represents rats that were sacrificed 24 Hr after IS, and the patterned bar represents rats that were sacrificed 7 D after IS. The data are expressed as mean percent inhibition + SEM. Mean percent inhibition was significantly different among groups ($p < 0.05$). Dunnett's Multiple Comparison test revealed that only the 24 Hr time-point was significantly different from HC ($p < 0.05$).

Experiment 5: Effect of mPFC inactivation during stress on ipsapirone-mediated inhibition of DRN 5-HT cell firing

Rats received intra-mPFC saline or 50 ng muscimol 45 min before ES, IS, or HC treatment, 24 hr later midbrain slices were taken for single unit recording. Importantly, intra-mPFC muscimol does not interfere with escape learning during ES (Amat et al., 2005). As shown in Figures 2.7 and 2.8, impairment of 5-HT_{1A}-R-mediated inhibition was found in IS rats as compared to HC at 150 nM of ipsapirone. Intra-mPFC muscimol had no effect on IS subjects. However, intra-mPFC muscimol led ES to now impair ipsapirone-mediated inhibition of firing. A two-way ANOVA revealed a significant effect of *stress* ($F_{(2, 57)}$ = 8.08; $p < 0.001$) and *stress* x *muscimol* ($F_{(2, 57)}$ = 4.57; $p < 0.05$) interaction. The effect of *muscimol* ($F_{(1, 57)}$ = 0.26; $p > 0.05$) was not significant. Subsequent *post hoc* comparisons revealed a significant difference between muscimol- and saline-ES rats. Importantly, *post hoc* comparisons also revealed (i) no difference between muscimol-ES and IS groups (ii) no difference between saline-ES and HC groups (iii) a significant difference between muscimol-ES and HC groups (iv) and a significant difference between IS and HC groups. There were no significant differences between groups in baseline firing rate.

Experiment 6: The effect of behavioral immunization on ipsapirone-mediated inhibition of DRN 5-HT cell firing

Since prior studies have shown that an experience with ES before IS (so-called "behavioral immunization") blocks the behavioral and neurochemical consequences of IS (Williams and Maier, 1977; Amat et al., 2006), we tested whether ES prior to IS would block IS-induced functional desensitization of DRN 5-HT_{1A}-Rs. Rats received behavioral immunization or experimental control treatments (see Materials and Methods for detail) and midbrain slices were taken 24 hr later. Again, only the 150 nM dose of ipsapirone was used. As shown in Figures 2.9 and 2.10, IS again reduced ipsapirone-mediated inhibition of cell firing, but this reduction was blocked by prior ES. This conclusion was confirmed by a one-way ANOVA ($F_{(2, 41)}$ = 4.497; $p < 0.05$). Subsequent *post hoc* comparisons revealed that the HC/IS group was significantly different from the ES/IS and HC/HC groups; however, no significant difference was found between the ES/IS and HC/HC groups. Lastly, stress-induced changes in baseline firing rate were analyzed and no significant differences were found ($F_{(2, 41)}$ = 0.06547; $p > 0.05$).

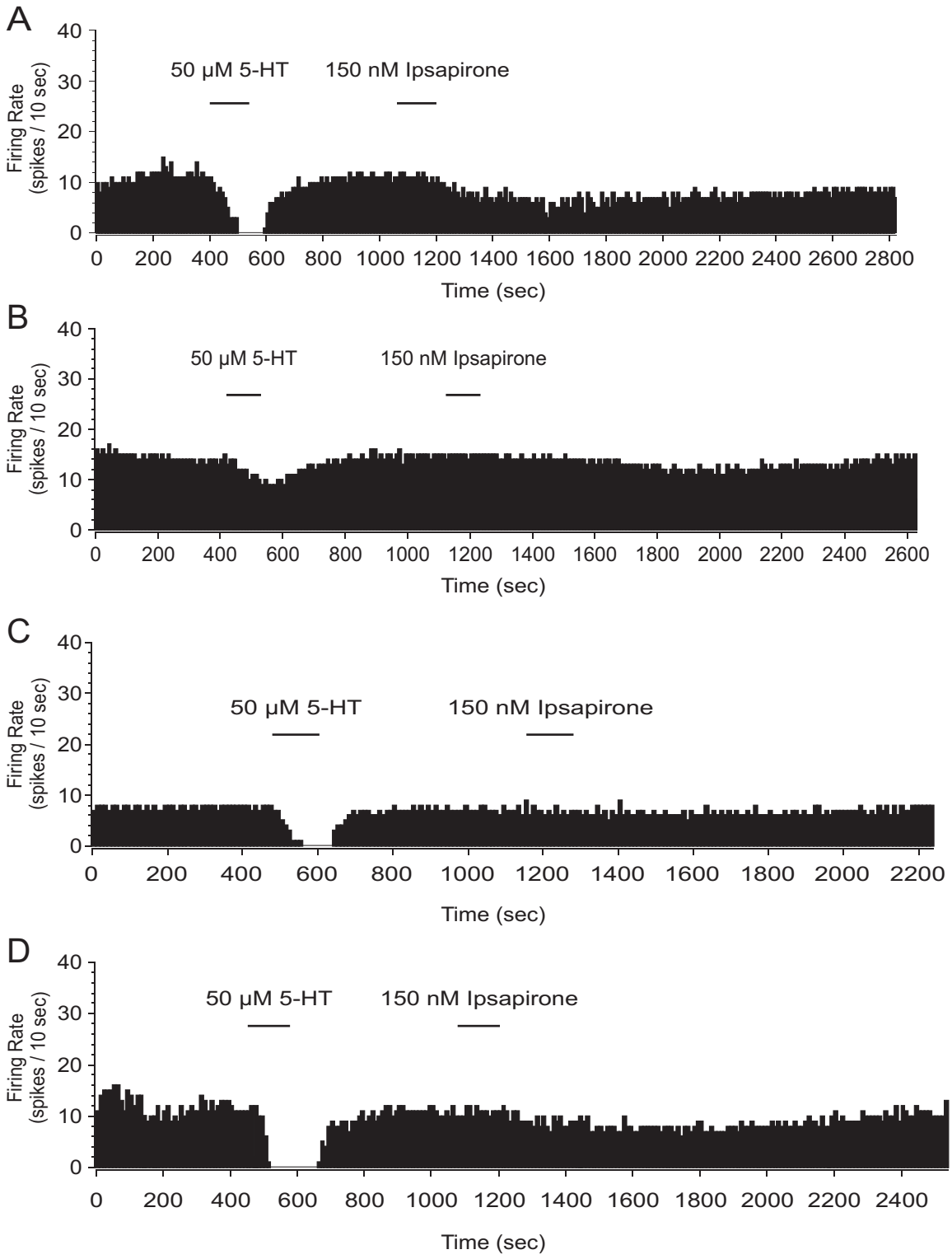

Figure 2.7. Spike frequency histograms from extracellular single unit recording in the dorsal raphe nucleus following intra-medial prefrontal cortex (mPFC) injection of muscimol during homecage control (HC) (**A**), intra-mPFC muscimol during inescapable stress (IS) (**B**), intra-mPFC muscimol during

escapable stress (ES) (**C**), and intra-mPFC saline during ES (**D**). Midbrain slices were taken 24 hr after stress treatment and 50 μM of serotonin (5-HT) was applied followed by 150 nM of ipsapirone and percent inhibition was calculated.

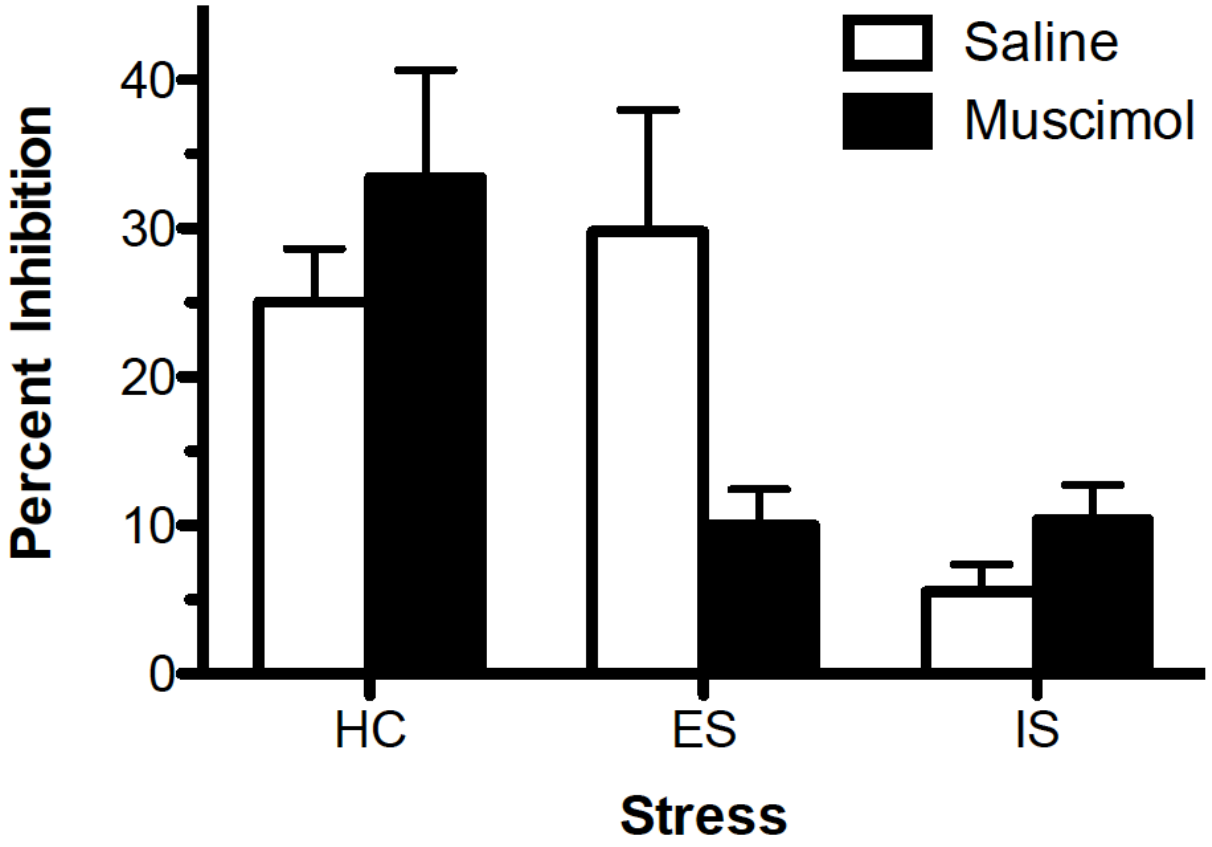

Figure 2.8. A microinjection of muscimol into the medial prefrontal cortex (mPFC) during escapable stress (ES) impairs inhibition of dorsal raphe nucleus (DRN) serotonin (5-HT) cell firing with 150 nM ipsapirone. Open bars indicate rats that received intra-mPFC saline, closed bars indicate rats that received intra-mPFC muscimol microinjections. Data are expressed as mean percent inhibition + SEM. Mean percent inhibition of DRN 5-HT cell firing was significantly different between inescapable stress (IS) and homecage (HC) treatments ($p < 0.05$). Mean percent inhibition of DRN 5-HT cell firing was significantly different between ES rats receiving intra-mPFC muscimol and saline ($p < 0.05$).

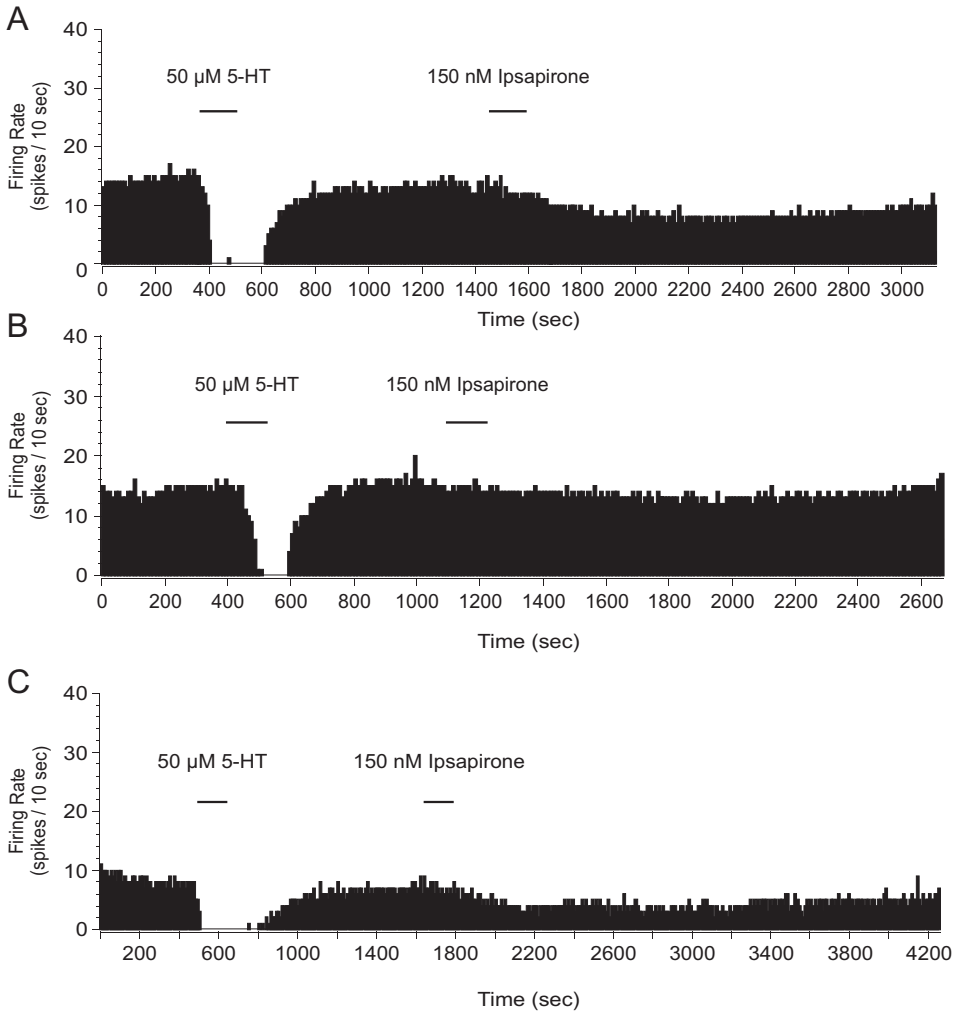

Figure 2.9. Spike frequency histograms from extracellular single unit recording in the dorsal raphe nucleus (DRN) following homecage control (HC/HC) (**A**), homecage control 24 hr before inescapable stress (HC/IS) (**B**), and escapable stress 24 hr before inescapable stress (ES/IS) (**C**). Midbrain slices were taken 24 hr after final stress treatment and 50 μM of serotonin (5-HT) was applied followed by 150 nM of ipsapirone and percent inhibition was calculated.

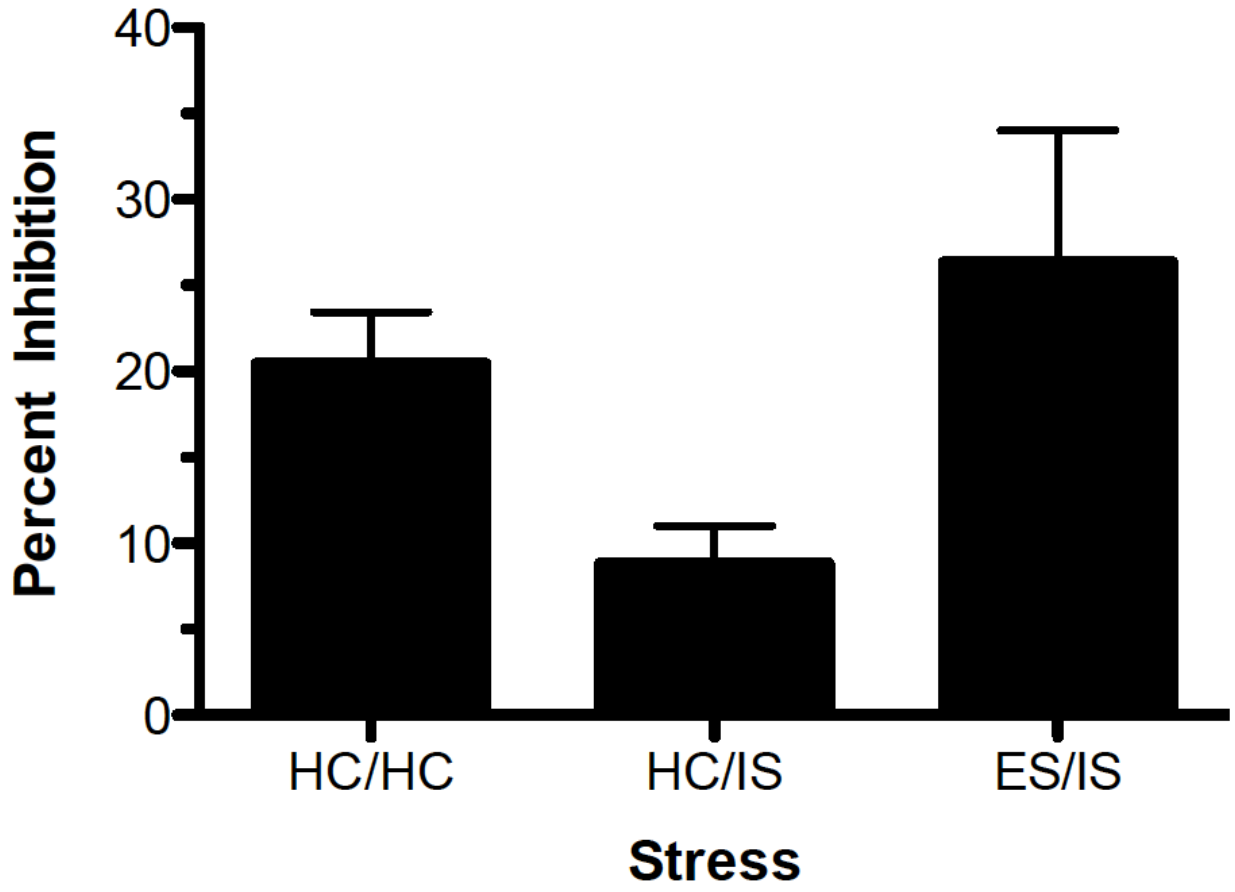

Figure 2.10. Behavioral immunization blocks inescapable stress (IS)-induced impairment of dorsal raphe nucleus (DRN) serotonin (5-HT) cell firing with 150 nM ipsapirone. Data are expressed as mean percent inhibition + SEM. Mean percent inhibition was different among stress groups ($p < 0.05$). Mean percent inhibition of DRN 5-HT cell firing was significantly different in rats that received homecage treatment followed by IS (HC/IS) as compared to rats that received no stress (HC/HC) and behavioral immunization (ES/IS) ($p < 0.05$).

Discussion

Behavioral and neurochemical outcomes following ES and IS are often quite different. In several fear- and anxiety-related tests ES subjects resemble HC controls, while IS subjects exhibit a fear/anxiety phenotype (Maier and Watkins, 2005). Similarly, IS induces several neurochemical changes that do not occur in ES subjects (Maswood et al., 1998). A large body of evidence has shown that IS relative to ES, activates 5-HT neurons in the caudal DRN (Grahn et al., 1999; Amat et al., 2005), resulting in the sensitization of these neurons to subsequent input (Amat et al., 1998a; Bland et al., 2003; Christianson et al., 2010). Moreover, a number of studies have shown that this process is necessary and sufficient to produce typical IS behaviors (Maier et al., 1995b; Maier et al., 1995a; Will et al., 2004; Christianson et al., 2008). However, the mechanism(s) responsible for IS-induced DRN 5-HT sensitization is unexplored. The present experiments examined whether IS, relative to ES, reduces 5-HT feedback inhibition on 5-HT cells, an outcome that would sensitize these cells. Sensitization would occur if IS reduced DRN 5-HT_{1A}-R function. However, reduced 5-HT_{1A}-R function would not, by itself, indicate that this consequence of IS causally mediates IS behaviors.

To investigate the possibility that 5-HT_{1A}-R changes are casual, two strategies were adopted. The first was to determine whether a manipulation that eliminates the protective effects of control on behavior, i.e. a

manipulation that produces IS-like behavior in an ES rat, would now lead ES to also reduce inhibition of DRN cells, as does IS. The second was to determine whether a manipulation known to block the behavioral effects of IS would also block the effects of IS on 5-HT_{1A}-R inhibition of 5-HT activity. This type of co-variation is necessary for implicating a mediational role. There were a variety of methods with which serotonin-mediated inhibition of 5-HT cells could have been studied. *Ex vivo* extracellular single unit recording in the DRN was chosen as it involves a direct assessment of function, and the effect of 5-HT application can be readily examined for its inhibitory effects.

In Experiment 1 we demonstrated that IS, but not ES, impairs serotonin-mediated inhibition of 5-HT cell firing. That is, 5-HT produced dramatically less inhibition of neural activity in rats that had received IS. Although Experiment 1 has limitations because 5-HT is not selective for the 5-HT_{1A}-R, the use of 5-HT rather than a selective 5-HT receptor agonist most closely mimics *in vivo* 5-HT release. For this reason Experiment 2 was designed to determine whether 5-HT inhibits DRN 5-HT cell firing primarily via the 5-HT_{1A}-R. Here, a selective 5-HT_{1A}-R antagonist blocked the inhibitory effects of 5-HT on DRN cell firing, suggesting that 5-HT inhibition is exerted mainly via the 5-HT_{1A}-R. Furthermore, Experiment 3 utilized the 5-HT_{1A}-R agonist ipsapirone to inhibit unit firing. Again, prior IS interfered with the neuronal inhibition. Lastly, the timecourse in Experiment 4

demonstrated that impaired 5-HT_{1A}-mediated inhibitions follow the same timecourse as the behavioral effects of IS. Together these experiments indicate that IS, relative to ES, interferes with serotonin-mediated inhibition of DRN neuronal activity 24 hr later. This effect is likely mediated by reduced 5-HT_{1A}-R function.

As noted above, the occurrence of IS-induced reductions in 5-HT inhibition of DRN activity does not indicate that this change is responsible for the behavioral effects of IS. If this change is causal, then manipulations that block the protective effects of control on behavior should eliminate the lack of effect of ES on DRN 5-HT_{1A}-R sensitivity. Several studies demonstrate that the mPFC inhibits DRN 5-HT activity during ES but not during IS. Thus, inactivation of the mPFC with muscimol during ES eliminates the protective effects of ES. Now, ES produces the same behavioral outcomes as does IS (Amat et al., 2005; Christianson et al., 2009; Rozeske et al., 2009). If reduced 5-HT_{1A}-R sensitivity is causal, then inhibition of the mPFC during the stress treatment should lead both IS and ES to now interfere with DRN unit inhibition. Experiment 5 explored exactly this prediction. Muscimol was microinjected at the IL-PL border as in Amat et al. (2005), and intra-mPFC inhibition during stress led ES to reduce ipsapirone-mediated inhibition to the same degree as IS.

A causal role for reduced 5-HT inhibition of DRN neurons would also predict the converse. Namely, manipulations known to prevent the

behavioral effects of IS should also eliminate the IS-induced impairment of 5-HT_{1A}-R inhibition. Several studies (Williams and Maier, 1977; Amat et al., 2006; Amat et al., 2008) have shown that a prior experience with ES blocks the behavioral effects of subsequent IS, a phenomenon labeled behavioral immunization. As a causal role for reduced inhibition of DRN neurons would predict, prior ES eliminated the IS-induced reduction in ipsapirone-mediated inhibition of unit firing. Using a similar strategy, voluntary exercise was shown to also block the behavioral effects of IS by increasing DRN 5-HT_{1A}-R mRNA (Greenwood et al., 2003). These data together support the idea that reduced 5-HT_{1A}-R function is part of the causal network that mediates the behavioral effects of IS.

To establish causality it would also be desirable to determine whether manipulations that reduce DRN 5-HT_{1A}-R inhibitory function, in the absence of IS treatment, also produce typical IS-induced behaviors. Indeed, acute administration of fluoxetine produces typical IS-induced behaviors, 24 hr after injection (Greenwood et al., 2008). Although not measured by Greenwood et al. (2008), acute fluoxetine is known to internalize 5-HT_{1A}-R in the DRN (Riad et al., 2004). Finally, it would be desirable to determine whether preventing 5-HT_{1A}-R desensitization during IS blocks the behavioral effects of IS. Essentially, the behavioral immunization experiment above provides such data. In addition it should be noted that pharmacological blockade of DRN 5-HT activation during IS (Maier et al., 1995a), which

would prevent 5-HT_{1A}-R desensitization since extracellular DRN 5-HT would not rise, blocks the behavioral effects of IS.

The present experiments used *ex vivo* extracellular single unit recording in the DRN to investigate the relationship between stressor controllability and 5-HT receptor sensitivity. Others have also used this approach to assess the consequences of a stress experience on receptor function (Laaris et al., 1999; Froger et al., 2004). Although it is remarkable that the effects of stressor controllability carried into the slice preparation, it should be noted that single unit recording has limitations. Although 33-66% of cells in the DRN are serotonergic, extracellular single unit recording cannot definitively identify cell type. Furthermore, despite the well-documented firing characteristics of 5-HT cells (VanderMaelen and Aghajanian, 1983), these characteristics may be insufficient for serotonergic cell identification (Kirby et al., 2003). Additionally, extracellular single unit recording can only assess functionality, although this was our goal. Using the present procedures it cannot be concluded whether IS produces a downregulation of 5-HT_{1A}-Rs or an uncoupling of the G-protein to the 5-HT_{1A}-R. Lastly, an obvious limitation of extracellular single unit recording is that cell firing is being recorded in an artificial environment. However, the addition of phenylephrine hydrochloride to aCSF mimics *in vivo* firing of 5-HT neurons (VanderMaelen and Aghajanian, 1983) and the objective of the present studies was to assess 5-HT_{1A}-R function after stress.

The present experiments were not designed to determine the cause(s) of 5-HT_{1A}-R desensitization. Although stress-induced desensitization of DRN 5-HT_{1A}-Rs can occur via glucocorticoid receptor stimulation (Laaris et al., 1995), this is an unlikely mechanism for the stress-group differences observed here, as the ES and IS treatments used presently causes similar release of glucocorticoids (Maier et al., 1986). However, IS and ES do lead to very different levels of extracellular 5-HT within the DRN (Maswood et al., 1998; Amat et al., 2005), with IS producing large and sustained elevations. Since increased 5-HT has been shown to desensitize DRN 5-HT_{1A}-Rs (Le Poul et al., 1995; Hervas et al., 2001), this would be a likely cause. Furthermore, inhibition of the mPFC with muscimol during the stressor not only leads ES to produce the behavioral outcomes normally produced by only IS, but it also leads ES to produce the same high levels of extracellular 5-HT within the DRN as does IS (Amat et al., 2005). Conversely, a prior experience with ES not only blocks the behavioral effects of later IS, but it also prevents the IS-induced increase in extracellular 5-HT within the DRN (Amat et al., 2006). Thus, whether 5-HT_{1A}-R desensitization was or was not produced by a given manipulation in the present studies is perfectly predicted by whether the manipulation does or does not lead to elevated levels of extracellular 5-HT within the DRN.

The functional desensitization of DRN 5-HT_{1A}-Rs following IS resembles clinical findings reporting a relationship between raphe 5-HT_{1A}-R alterations

and anxiety- and depression-related psychopathologies. Indeed, patients with depression show reduced binding of the 5-HT_{1A}-R in the raphe (Drevets et al., 1999; Meltzer et al., 2004; Drevets et al., 2007). Additionally, reduced binding of 5-HT_{1A}-Rs in the raphe is also observed in social anxiety disorder (Lanzenberger et al., 2007) and panic disorder (Nash et al., 2008). These clinical findings encourage the notion that IS may capitulate some of the endophenotypes associated with depression and anxiety.

Chapter 3:

General Discussion

Uncontrollable stress (inescapable stress, IS) can produce a variety of neurochemical and behavioral changes in a trans-situational fashion for a number of days. Many of the behaviors produced by IS are thought of as anxiety- or depression-like behaviors. These behavioral outcomes are not observed if the stressor is controllable (escapable stress, ES). In fact, the behavior of ES subjects resembles that of non-stressed homecage (HC) controls. Previous findings demonstrate that alterations in serotonergic (5-hydroxytryptamine, 5-HT) function in the dorsal raphe nucleus (DRN) mediates the behavioral outcomes of IS; however, the exact mechanisms are unknown. Identifying the mechanisms responsible for IS-induced behavioral effects is of great interest as the findings may offer insight into the genesis of several psychopathologies that are precipitated by stress, such as post-traumatic stress disorder (PTSD), substance use disorder, and depression. The experiments reported above were designed to determine such a mechanism by using a number of stress protocols that either produce or block IS-induced behavioral changes. The dissociations that we observed among the stress protocols that block or produce IS behavioral changes informed us of the possible mechanisms that are responsible for producing IS-induced behavioral outcomes.

The above experiments revealed that (i) IS, but not ES, desensitized serotonin-1A receptors (5-HT_{1A}-Rs) in the DRN (ii) IS desensitized DRN 5-HT_{1A}-Rs for the same duration as IS-induced behavioral changes (iii)

activation of the medial prefrontal cortex (mPFC) during ES is necessary to block stress-induced desensitization of DRN 5-HT_{1A}-Rs and (iv) when ES was administered prior to IS, IS did not desensitize DRN 5-HT_{1A}-Rs.

The Dorsal Raphe Nucleus and Stressor Controllability

These findings support the hypothesis that IS "drives" or activates 5-HT cells in the DRN. This IS-induced activation of DRN 5-HT cells leads to a functional desensitization of DRN 5-HT_{1A}-Rs. However, when behavioral control is present during stress, as during ES, the mPFC becomes activated. This activation of the mPFC inhibits DRN 5-HT cells and consequently reduces the level of extracellular DRN 5-HT. It is this reduction of extracellular DRN 5-HT that is thought to preserve the sensitivity of DRN 5-HT_{1A}-Rs. Moreover, these protective effects of ES are enduring because a subsequent experience with IS will no longer desensitize 5-HT_{1A}-Rs in the DRN.

The functional desensitization of DRN 5-HT_{1A}-Rs by IS is consistent with several studies demonstrating sensitized extracellular 5-HT 24 hr following IS. Indeed, 24 hr following IS, but not ES, a number of different stimuli produce a sensitized 5-HT response including 2 foot shocks, subcutaneous morphine injection, and exposure to a juvenile conspecific (Amat et al., 1998a; Bland et al., 2003; Christianson et al., 2010). This

sensitized 5-HT response is necessary to produce the behavioral outcomes of IS (Bland et al., 2004; Christianson et al., 2010). Therefore, it is hypothesized that the behavioral differences between ES and IS subjects are only revealed if the behavior being measured activates 5-HT cells in the DRN, and thus activates the sensitized DRN 5-HT cells of IS subjects. Indeed, stimuli that do not activate DRN 5-HT cells do not produce the typical behavioral outcomes of IS (Der-Avakian et al., 2007b; Der-Avakian et al., 2007a).

The effects of ES and IS on DRN 5-HT_{1A}-R sensitivity was measured in the mid and caudal regions of the DRN. More specifically, all extracellular single unit recordings were restricted to the dorsomedial DRN (DRD). The mid and caudal region of the DRN were chosen as single unit recording sites based on previous findings that IS, but not ES, selectively activated these regions (Grahn et al., 1999; Amat et al., 2005). Additionally, the DRD was chosen as a recording site because this region contains serotonergic efferents that terminate in several stress- and anxiety-responsive brain regions including the basolateral amygdala (BLA) and the mPFC; moreover, the DRD is also activated by several different behavioral stressors and anxiogenic drugs (Lowry et al., 2008b). One limitation of the experiments presented above is that they did not contain recordings from other subregions of the DRN. Since 100 inescapable tail shocks activated the entire DRN as measured by immunohistochemistry (IHC) (Takase et al.,

2004) it is possible that DRN 5-HT_{1A}-Rs are desensitized in other subregions besides the DRD. However, the Takase et al. (2004) study did not contain a controllable stress manipulation and therefore lacked the critical comparison of DRN activation between ES and IS groups.

Additionally, the studies reported in Chapter 2 did not assess the number of tail shocks required to desensitize DRN 5-HT_{1A}-Rs. The IS protocols that desensitized DRN 5-HT_{1A}-Rs in the experiments presented in Chapter 2 used 80 or 100 inescapable tail shocks. However, we hypothesize that as few as 50 inescapable tail shocks would desensitize DRN 5-HT_{1A}-Rs as 50 inescapable tail shocks produces failure to escape in the shuttlebox 24 hr after stress (Takase et al., 2005). It remains unknown whether the number of tail shocks is the critical component that produces desensitization of DRN 5-HT_{1A}-Rs or whether it is the prolonged high level of extracellular DRN 5-HT that is responsible for the desensitization. Presumably, the number of tail shocks is an abstraction and it is the amount of extracellular DRN 5-HT produced by tail shock that is the critical component. Indeed, others have shown that increased binding at 5-HT receptors in the DRN by pharmacological methods desensitizes DRN 5-HT_{1A}-Rs (Hervas et al., 2001; Riad et al., 2004; Zimmer et al., 2004).

A number of investigators have examined the interaction of stress exposure and DRN 5-HT_{1A}-R function. However, several of these studies used chronic, rather than acute, stress exposure. Although the chronic stress

procedures used are varied, many stress protocols led to impaired DRN 5-HT_{1A}-R function (Lanfumey et al., 1999; Froger et al., 2004; Bambico et al., 2009). However, and somewhat puzzling, early maternal separation produced a decrease in the amount of stimulated extracellular 5-HT, as would be expected following desensitization of DRN 5-HT_{1A}-Rs. However, a functional impairment of DRN 5-HT_{1A}-Rs was not observed (Gartside et al., 2003). Despite the somewhat common finding that chronic stress can produce a functional desensitization of DRN 5-HT_{1A}-Rs, whether the receptor is downregulated or un-coupled seems dependent upon the stressor used and the factors determining downregulation or un-coupling remain unknown (Flugge, 1995; Lanfumey et al., 1999; Froger et al., 2004; Leventopoulos et al., 2009).

A few studies have examined the interaction of acute stressors and DRN 5-HT_{1A}-Rs. Restraint lasting 30 min produced no functional change in DRN 5-HT_{1A}-Rs (Bambico et al., 2009). However, Lanfumey and colleagues found that a 16 hr exposure to a relatively mild uncontrollable novel environment functionally impaired DRN 5-HT_{1A}-Rs (Laaris et al., 1997; Laaris et al., 1999). The functional impairment of DRN 5-HT_{1A}-Rs observed following novel environmental stress is thought to involve an un-coupling of DRN 5-HT_{1A}-Rs to their G-proteins. Indeed, quantitative autoradiography using both agonist and antagonist radioligands, revealed no change in 5-HT_{1A}-R binding sites in the DRN. This result implies that the functional

impairment of DRN 5-HT_{1A}-Rs is not due to downregulation, but to un-coupling. Lastly, the un-coupling of DRN 5-HT_{1A}-Rs by a novel environment appears to be mediated by stress-induced increases of glucocorticoids, as adrenalectomy blocked the stress-induced functional desensitization of DRN 5-HT_{1A}-Rs.

Others have reported that 30 min of exposure to water increased binding of 5-HT_{1A}-R antagonist, but not agonist, radioligands (Raghupathi and McGonigle, 1997). The authors interpreted these results as either an increase in the number of DRN 5-HT_{1A}-Rs or an un-coupling of the receptor because antagonist radioligands tend to bind receptors despite un-coupling (Gozlan et al., 1995). Although a limited amount of evidence is available, it appears that acute stressors are more likely to produce functional desensitization of DRN 5-HT1A-Rs by perhaps un-coupling the receptor from its G_i-protein or antagonizing inwardly rectifying K+ channels.

An obvious limitation of the experiments reported in Chapter 2 is that they do not address whether IS internalizes or un-couples DRN 5-HT_{1A}-Rs. However, we are certain that the functional impairment of DRN 5-HT_{1A}-Rs following IS is not mediated by stress-induced increases in plasma corticosterone as ES and IS evoke similar corticosterone responses (Maier et al., 1986). Additional studies to determine whether IS impairs DRN 5-HT_{1A}-R signaling by un-coupling or internalizing DRN 5-HT_{1A}-Rs are warranted.

The present results demonstrate that functional desensitization of DRN 5-HT_{1A}-Rs persists for the same length of time as the behavioral consequences of IS. The finding that IS produces a desensitization of DRN 5-HT_{1A}-Rs only at 24 hr provides correlative evidence that the behavioral effects of IS are due to 5-HT_{1A}-R desensitization. Again, since it is unknown how IS leads to functional desensitization of DRN 5-HT_{1A}-Rs, it is difficult to ascertain why desensitization is not observed immediately following IS (0 hr) and the factors that lead to a recovery of DRN 5-HT_{1A}-R function 7 days after IS.

The Medial Prefrontal Cortex and Stressor Controllability

The present results are, to our knowledge, the first to demonstrate that activation of the mPFC during aversive stimuli can lead to preservation of DRN 5-HT_{1A}-R function. This is inferred because an intra-mPFC microinjection of muscimol during ES produced a functional desensitization of DRN 5-HT_{1A}-Rs as compared to saline microinjected ES subjects. This same procedure has been shown to produce an IS behavioral phenotype in ES subjects (Amat et al., 2005; Christianson et al., 2009; Rozeske et al., 2009). These findings led us to conclude that activation of the mPFC during ES prevented the expression of stress-induced behaviors by preserving the sensitivity of DRN 5-HT_{1A}-Rs.

Importantly, intra-mPFC microinjection of muscimol does not interfere with wheel-turn escape learning (Amat et al., 2005). That is, the wheel-turn response can be acquired, but without mPFC activation this learning is not protective. The dorsal striatum is likely involved in mediating learning of the wheel-turn response during mPFC inactivation. Indeed, activation of the dorsal striatum supports stimulus-response learning (Yin et al., 2006). Although activation of the dorsal striatum may support wheel-turn learning during tail shock, the absence of a direct dorsal striatum to DRN projection (Lowry et al., 2008a) reduces the likelihood of concurrent inhibition of DRN 5-HT cells. This may be one reason why wheel-turning during mPFC inactivation is not protective against the behavioral effects of tail shock.

The mPFC inactivation study also suggests that the effects of ES are the result of an "active" phenomenon. That is, it appears that when behavioral control is present during a stressor the mPFC becomes activated and inhibits shock-induced activation of DRN 5-HT cells and prevents DRN 5-HT_{1A}-R desensitization. Through this process ES subjects subsequently behave as do HC controls. The idea that the mPFC can exert "top-down" modulation or "executive" control of limbic and brainstem structures is not a new one (Robbins, 2000). Indeed, others have found that the mPFC can inhibit limbic brain structures associated with both the expression of fear (Quirk and Beer, 2006) and responding for drugs of abuse (Kalivas et al., 2006). Similarly, during ES aversive stimuli "drive" limbic and brainstem

structures, but when behavioral control is present the mPFC becomes activated and inhibits brainstem structures that are activated by tail shock. This top-down regulation by the mPFC, as demonstrated in Chapter 2, is another example of executive control by this structure.

This executive regulation of stress-responsive brain regions by the mPFC during aversive stimuli is necessary to protect against desensitization of DRN 5-HT_{1A}-Rs. As discussed above, activation of the mPFC is the critical factor that determines the behavioral consequences of tail shock. Again, simply learning the wheel-turn response is not sufficient. Since learning the wheel-turn response is no longer viewed as the critical factor, it is possible that simple pharmacological activation of the mPFC during IS could block the typical behavioral outcomes of IS. A number of studies have investigated this hypothesis. Activation of mPFC with the $GABA_A$ receptor antagonist picrotoxin, during stress, is sufficient to block the neurochemical and behavioral outcomes of IS (Amat et al., 2008; Christianson et al., 2009; Rozeske et al., 2009). Because activation of the mPFC prevents the IS-induced levels of extracellular DRN 5-HT, we hypothesize that DRN 5-HT_{1A}-Rs would not become desensitized if the mPFC were activated during IS. These mPFC activation studies demonstrate that it is not behavioral control *per se* that is critical to produce stress-resistance or resilience; rather, it is activation of the mPFC during aversive stimuli that is the critical factor.

Behavioral immunization is a behavioral paradigm also designed to determine the factors that produce stress-resistance or resilience. Again, the paradigm involves testing whether a prior experience with ES can block the effects of a subsequent experience with IS. In the experiments reported above, prior ES blocked subsequent IS-induced desensitization of DRN 5-HT_{1A}-Rs. Interestingly, ES and IS were administered in different environments; this supports the notion that ES produced stressor immunization that was not context specific. Indeed, others have shown that behavioral immunization blocked subsequent IS-induced behavioral and neurochemical consequences in a general fashion (Amat et al., 2006; Christianson et al., 2008; Amat et al., 2010). That is, behavioral immunization is protective across different environments as well as across different stressors.

Given that DRN 5-HT cells must be activated to observe the behavioral effects of IS it is hypothesized that behavioral immunization produces its stress immunizing effects by preventing stress-induced activation of DRN 5-HT cells. An obvious candidate structure responsible for the inhibition of DRN 5-HT cells during subsequent IS is the mPFC. Indeed, activation of the mPFC during aversive stimuli is both necessary and sufficient to produce behavioral immunization (Amat et al., 2006; Amat et al., 2008). However, activation of the mPFC in the absence of aversive stimuli is not sufficient to produce behavioral immunization.

Interestingly, behavioral immunization produces long-lasting effects as compared to the relatively short-lived 5-HT_{1A}-R changes that occur in the DRN following IS. For this reason, whether the mPFC is the critical site mediating long-term stress-induced plasticity was investigated. To test whether plastic changes occurred in the mPFC during ES *de novo* protein synthesis was blocked in the mPFC during ES (Amat et al., 2006). Indeed, preventing *de novo* protein synthesis in the mPFC during ES blocked behavioral immunization. ES-induced protein synthesis is thought to be required for behavioral immunization so that the architectural and biochemical changes necessary for synaptic strengthening can occur. Moreover, plasticity in the mPFC following ES is hypothesized to produce a general buffering effect to subsequent challenges, controllable or uncontrollable. For example, an initial experience with ES prevents the normal consequences of aversive stimuli that does not involve tail shock, such as social defeat (Amat et al., 2010) and fear conditioning (Baratta et al., 2008). These findings indicate that the mPFC may serve as a common structure in a pathway mediating resistance to the behavioral consequences of a variety of aversive stimuli.

Although the motivation for the experiments reported above was discovery of the mechanisms responsible for the behavioral differences following ES and IS, it has become clear that an executive role of the mPFC may be generalizable to a number of different circumstances. Indeed, given

the anatomy of the mPFC, its role in emotional regulation, temporal organization of goal-directed behaviors, and decision making tasks (Miller and Cohen, 2001), it is not inconceivable that the mPFC could be implicated in a number of psychiatric disorders for which the hallmark symptoms involve emotional dysregulation, habitual behavior, perseveration, and an inability to prepare for future events. Indeed, it has been hypothesized that treatments that produce increased activation of the mPFC may alleviate symptoms associated with psychiatric disorders, such as depression, by facilitating top-down inhibitory control over limbic brain structures (DeRubeis et al., 2008; Pittenger and Duman, 2008; Fales et al., 2009).

Although a wealth of evidence has demonstrated a role of mPFC dysfunction in psychiatric disorders (Baker et al., 1997; Davidson, 2002; Kalivas et al., 2005; Etkin and Wager, 2007; Drevets et al., 2008), previous research has not extensively investigated the relationship between the mPFC and the DRN in these disorders. A number of studies have implicated increased activity of 5-HT cells in depressed patients by observing either increased tryptophan hydroxylase 2 in the DRN or a decreased density of 5-HT_{1A}-Rs in the DRN (Drevets et al., 1999; Underwood et al., 1999; Arango et al., 2001; Boldrini et al., 2005; Bach-Mizrachi et al., 2006). However, no studies to our knowledge have investigated mPFC regulation of DRN 5-HT cells and subsequent mitigation of depression symptoms. Although the studies in Chapter 2 focused on mPFC activation as a preventative event

during stress exposure, these studies may provide a novel mechanism by which the mPFC could alleviate symptoms of depression as well as other psychiatric disorders whose genesis depends on dysfunction of DRN 5-HT cells.

Currently, activation of the frontal cortex is one of the most effective treatments for depression. Indeed, deep brain stimulation of the cingulate gyrus, a region argued to be homologous to the rat mPFC (Gabbott et al., 2003), can dramatically reduce symptoms of depression in a treatment-resistant clinical population (Mayberg et al., 2005; Lozano et al., 2008; Kennedy et al., 2011). Since these studies were performed in clinical populations and also required implantation of electrodes, the exact consequences of electrical stimulation on the activation of other brain regions could not readily be measured with magnetic resonance imaging. However, given the evidence that implicates dysfunction of the serotonergic system in depressed patients, it is plausible that electrical stimulation of the cingulate gyrus reduces symptoms of depression by regulating DRN 5-HT cell activity.

In conclusion, the experiments presented in this thesis were designed to understand the mechanisms involved in producing anxiety- and depression-like behaviors following an uncontrollable stressor. Based on the dissociation that IS sensitizes DRN 5-HT cells and that activation of the mPFC during ES prevents sensitization of DRN 5-HT cells, the mechanism

responsible for the development of IS-induced behaviors was hypothesized to be localized in the DRN. The data presented in Chapter 2 provides evidence that IS desensitizes DRN 5-HT_{1A}-Rs, which leads to the expression of IS behavioral outcomes. However, activation of the mPFC can prevent stress-induced desensitization of DRN 5-HT_{1A}-Rs in a long-term, trans-situational fashion. Additional studies investigating how the mPFC becomes activated during aversive stimuli are warranted. Indeed, a detailed understanding of how the mPFC can produce long-term stress-resistance could provide novel preventative and therapeutic applications for at risk clinical populations.

References

Abrams JK JP, Hay-Schmidt A, Mikkelsen JD, Shekhar A, Lowry CA (2005) Serotonergic systems associated with arousal and vigilance behaviors following administration of anxiogenic drugs. Neuroscience 133:983-997.

Aghajanian GK (1985) Modulation of a transient outward current in serotonergic neurones by alpha 1-adrenoceptors. Nature 315:501-503.

Amat J, Matus-Amat P, Watkins LR, Maier SF (1998a) Escapable and inescapable stress differentially alter extracellular levels of 5-HT in the basolateral amygdala of the rat. Brain Res 812:113-120.

Amat J, Matus-Amat P, Watkins LR, Maier SF (1998b) Escapable and inescapable stress differentially and selectively alter extracellular levels of 5-HT in the ventral hippocampus and dorsal periaqueductal gray of the rat. Brain Res 797:12-22.

Amat J, Paul E, Watkins LR, Maier SF (2008) Activation of the ventral medial prefrontal cortex during an uncontrollable stressor reproduces both the immediate and long-term protective effects of behavioral control. Neuroscience 154:1178-1186.

Amat J, Paul E, Zarza C, Watkins LR, Maier SF (2006) Previous experience with behavioral control over stress blocks the behavioral and dorsal raphe nucleus activating effects of later uncontrollable stress: role of the ventral medial prefrontal cortex. J Neurosci 26:13264-13272.

Amat J, Aleksejev RM, Paul E, Watkins LR, Maier SF (2010) Behavioral control over shock blocks behavioral and neurochemical effects of later social defeat. Neuroscience 165:1031-1038.

Amat J, Sparks PD, Matus-Amat P, Griggs J, Watkins LR, Maier SF (2001) The role of the habenular complex in the elevation of dorsal raphe nucleus serotonin and the changes in the behavioral responses produced by uncontrollable stress. Brain Res 917:118-126.

Amat J, Baratta MV, Paul E, Bland ST, Watkins LR, Maier SF (2005) Medial prefrontal cortex determines how stressor controllability affects behavior and dorsal raphe nucleus. Nat Neurosci 8:365-371.

Amat J, Tamblyn JP, Paul ED, Bland ST, Amat P, Foster AC, Watkins LR, Maier SF (2004) Microinjection of urocortin 2 into the dorsal raphe nucleus activates serotonergic neurons and increases extracellular serotonin in the basolateral amygdala. Neuroscience 129:509-519.

Andrade R, Aghajanian GK (1985) Opiate- and alpha 2-adrenoceptor-induced hyperpolarizations of locus ceruleus neurons in brain slices: reversal by cyclic adenosine 3':5'-monophosphate analogues. J Neurosci 5:2359-2364.

Arango V, Underwood MD, Boldrini M, Tamir H, Kassir SA, Hsiung S, Chen JJ, Mann JJ (2001) Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. Neuropsychopharmacology 25:892-903.

Bach-Mizrachi H, Underwood MD, Kassir SA, Bakalian MJ, Sibille E, Tamir H, Mann JJ, Arango V (2006) Neuronal tryptophan hydroxylase mRNA expression in the human dorsal and median raphe nuclei: major depression and suicide. Neuropsychopharmacology 31:814-824.

Baker SC, Frith CD, Dolan RJ (1997) The interaction between mood and cognitive function studied with PET. Psychol Med 27:565-578.

Bambico FR, Nguyen NT, Gobbi G (2009) Decline in serotonergic firing activity and desensitization of 5-HT1A autoreceptors after chronic unpredictable stress. Eur Neuropsychopharmacol 19:215-228.

Bandler R (1988) Brain mechanisms of aggression as revealed by electrical and chemical stimulation. Suggestion of a central role for the midbrain periaqueductal gray region. Progress in Physio Psych and Psychobio 13:67-155.

Baratta MV, Lucero TR, Amat J, Watkins LR, Maier SF (2008) Role of the ventral medial prefrontal cortex in mediating behavioral control-induced reduction of later conditioned fear. Learn Mem 15:84-87.

Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. Neuropharmacology 38:1083-1152.

Beer M, Kennett GA, Curzon G (1990) A single dose of 8-OH-DPAT reduces raphe binding of [3H]8-OH-DPAT and increases the effect of raphe stimulation on 5-HT metabolism. Eur J Pharmacol 178:179-187.

Bland ST, Schmid MJ, Watkins LR, Maier SF (2004) Prefrontal cortex serotonin, stress, and morphine-induced nucleus accumbens dopamine. Neuroreport 15:2637-2641.

Bland ST, Hargrave D, Pepin JL, Amat J, Watkins LR, Maier SF (2003) Stressor controllability modulates stress-induced dopamine and serotonin efflux and morphine-induced serotonin efflux in the medial prefrontal cortex. Neuropsychopharmacology 28:1589-1596.

Boldrini M, Underwood MD, Mann JJ, Arango V (2005) More tryptophan hydroxylase in the brainstem dorsal raphe nucleus in depressed suicides. Brain Res 1041:19-28.

Bonvento G, Scatton B, Claustre Y, Rouquier L (1992) Effect of local injection of 8-OH-DPAT into the dorsal or median raphe nuclei on extracellular levels of serotonin in serotonergic projection areas in the rat brain. Neurosci Lett 137:101-104.

Casanovas JM, Lesourd M, Artigas F (1997) The effect of the selective 5-HT1A agonists alnespirone (S-20499) and 8-OH-DPAT on extracellular 5-hydroxytryptamine in different regions of rat brain. Br J Pharmacol 122:733-741.

Chalmers DT, Lovenberg TW, De Souza EB (1995) Localization of novel corticotropin-releasing factor receptor (CRF2) mRNA expression to specific subcortical nuclei in rat brain: comparison with CRF1 receptor mRNA expression. J Neurosci 15:6340-6350.

Christianson JP, Thompson BM, Watkins LR, Maier SF (2009) Medial prefrontal cortical activation modulates the impact of controllable and uncontrollable stressor exposure on a social exploration test of anxiety in the rat. Stress 12:445-450.

Christianson JP, Paul ED, Irani M, Thompson BM, Kubala KH, Yirmiya R, Watkins LR, Maier SF (2008) The role of prior stressor controllability and the dorsal raphe nucleus in sucrose preference and social exploration. Behav Brain Res 193:87-93.

Christianson JP, Ragole T, Amat J, Greenwood BN, Strong PV, Paul ED, Fleshner M, Watkins LR, Maier SF (2010) 5-hydroxytryptamine 2C receptors in the basolateral amygdala are involved in the expression of anxiety after uncontrollable traumatic stress. Biol Psychiatry 67:339-345.

Clark RB, Knoll BJ, Barber R (1999) Partial agonists and G protein-coupled receptor desensitization. Trends Pharmacol Sci 20:279-286.

Clement HW, Gemsa D, Wesemann W (1992) The effect of adrenergic drugs on serotonin metabolism in the nucleus raphe dorsalis of the rat, studied by in vivo voltammetry. Eur J Pharmacol 217:43-48.

Davidson RJ (2002) Anxiety and affective style: role of prefrontal cortex and amygdala. Biol Psychiatry 51:68-80.

Day HE, Greenwood BN, Hammack SE, Watkins LR, Fleshner M, Maier SF, Campeau S (2004) Differential expression of 5HT-1A, alpha 1b adrenergic, CRF-R1, and CRF-R2 receptor mRNA in serotonergic, gamma-aminobutyric acidergic, and catecholaminergic cells of the rat dorsal raphe nucleus. J Comp Neurol 474:364-378.

De Oca BM, DeCola JP, Maren S, Fanselow MS (1998) Distinct regions of the periaqueductal gray are involved in the acquisition and expression of defensive responses. J Neurosci 18:3426-3432.

Der-Avakian A, Rozeske RR, Bland ST, Watkins LR, Maier SF (2007a) The effects of a single session of inescapable tailshock on the subsequent locomotor response to brief footshock and cocaine administration in rats. Psychopharmacology (Berl) 191:899-907.

Der-Avakian A, Bland ST, Rozeske RR, Tamblyn JP, Hutchinson MR, Watkins LR, Maier SF (2007b) The effects of a single exposure to uncontrollable stress on the subsequent conditioned place preference responses to oxycodone, cocaine, and ethanol in rats. Psychopharmacology (Berl) 191:909-917.

DeRubeis RJ, Siegle GJ, Hollon SD (2008) Cognitive therapy versus medication for depression: treatment outcomes and neural mechanisms. Nat Rev Neurosci 9:788-796.

Dong J, de Montigny C, Blier P (1997) Effect of acute and repeated versus sustained administration of the 5-HT1A receptor agonist ipsapirone: electrophysiological studies in the rat hippocampus and dorsal raphe. Naunyn Schmiedebergs Arch Pharmacol 356:303-311.

Drevets WC, Price JL, Furey ML (2008) Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Struct Funct 213:93-118.

Drevets WC, Thase ME, Moses-Kolko EL, Price J, Frank E, Kupfer DJ, Mathis C (2007) Serotonin-1A receptor imaging in recurrent depression: replication and literature review. Nucl Med Biol 34:865-877.

Drevets WC, Frank E, Price JC, Kupfer DJ, Holt D, Greer PJ, Huang Y, Gautier C, Mathis C (1999) PET imaging of serotonin 1A receptor binding in depression. Biol Psychiatry 46:1375-1387.

Dunn AJ, Berridge CW (1990) Is corticotropin-releasing factor a mediator of stress responses? Ann N Y Acad Sci 579:183-191.

Etkin A, Wager TD (2007) Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry 164:1476-1488.

Fairchild G, Leitch MM, Ingram CD (2003) Acute and chronic effects of corticosterone on 5-HT1A receptor-mediated autoinhibition in the rat dorsal raphe nucleus. Neuropharmacology 45:925-934.

Fales CL, Barch DM, Rundle MM, Mintun MA, Mathews J, Snyder AZ, Sheline YI (2009) Antidepressant treatment normalizes hypoactivity in dorsolateral prefrontal cortex during emotional interference processing in major depression. J Affect Disord 112:206-211.

Fanselow MS, Lester LS, Helmstetter FJ (1988) Changes in feeding and foraging patterns as an antipredator defensive strategy: a laboratory simulation using aversive stimulation in a closed economy. J Exp Anal Behav 50:361-374.

File SE, Gonzalez LE (1996) Anxiolytic effects in the plus-maze of 5-HT1A-receptor ligands in dorsal raphe and ventral hippocampus. Pharmacol Biochem Behav 54:123-128.

Fincham DS, Altes LK, Stein DJ, Seedat S (2009) Posttraumatic stress disorder symptoms in adolescents: risk factors versus resilience moderation. Compr Psychiatry 50:193-199.

Flugge G (1995) Dynamics of central nervous 5-HT1A-receptors under psychosocial stress. J Neurosci 15:7132-7140.

Froger N, Palazzo E, Boni C, Hanoun N, Saurini F, Joubert C, Dutriez-Casteloot I, Enache M, Maccari S, Barden N, Cohen-Salmon C, Hamon M, Lanfumey L (2004) Neurochemical and behavioral alterations in glucocorticoid receptor-impaired transgenic mice after chronic mild stress. J Neurosci 24:2787-2796.

Fuster JM (1985) The prefrontal cortex, mediator of cross-temporal contingencies. Hum Neurobiol 4:169-179.

Gabbott PL, Warner TA, Jays PR, Bacon SJ (2003) Areal and synaptic interconnectivity of prelimbic (area 32), infralimbic (area 25) and insular cortices in the rat. Brain Res 993:59-71.

Gao B, Fritschy JM, Benke D, Mohler H (1993) Neuron-specific expression of GABAA-receptor subtypes: differential association of the alpha 1- and alpha 3-subunits with serotonergic and GABAergic neurons. Neuroscience 54:881-892.

Gartside SE, Johnson DA, Leitch MM, Troakes C, Ingram CD (2003) Early life adversity programs changes in central 5-HT neuronal function in adulthood. Eur J Neurosci 17:2401-2408.

Glaser T, Rath M, Traber J, Zilles K, Schleicher A (1985) Autoradiographic identification and topographical analyses of high affinity serotonin receptor subtypes as a target for the novel putative anxiolytic TVX Q 7821. Brain Res 358:129-136.

Glazer HI, Weiss JM (1976) Long-Term and Transitory Interference Effects. J Exp Psychol Anim Behav Process 2:191-201.

Goldman-Rakic PS (1995) Cellular basis of working memory. Neuron 14:477-485.

Gozlan H, Thibault S, Laporte AM, Lima L, Hamon M (1995) The selective 5-HT1A antagonist radioligand [3H]WAY 100635 labels both G-protein-coupled and free 5-HT1A receptors in rat brain membranes. Eur J Pharmacol 288:173-186.

Grahn RE, Watkins LR, Maier SF (2000) Impaired escape performance and enhanced conditioned fear in rats following exposure to an uncontrollable stressor are mediated by glutamate and nitric oxide in the dorsal raphe nucleus. Behav Brain Res 112:33-41.

Grahn RE, Will MJ, Hammack SE, Maswood S, McQueen MB, Watkins LR, Maier SF (1999) Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. Brain Res 826:35-43.

Grahn RE, Hammack SE, Will MJ, O'Connor KA, Deak T, Sparks PD, Watkins LR, Maier SF (2002) Blockade of alpha1 adrenoreceptors in the dorsal raphe nucleus prevents enhanced conditioned fear and impaired escape performance following uncontrollable stressor exposure in rats. Behav Brain Res 134:387-392.

Grau JW, Hyson RL, Maier SF, Madden Jt, Barchas JD (1981) Long-term stress-induced analgesia and activation of the opiate system. Science 213:1409-1411.

Gray TS, Magnuson DJ (1992) Peptide immunoreactive neurons in the amygdala and the bed nucleus of the stria terminalis project to the midbrain central gray in the rat. Peptides 13:451-460.

Greenwood BN, Strong PV, Brooks L, Fleshner M (2008) Anxiety-like behaviors produced by acute fluoxetine administration in male Fischer

344 rats are prevented by prior exercise. Psychopharmacology (Berl) 199:209-222.

Greenwood BN, Foley TE, Day HE, Campisi J, Hammack SH, Campeau S, Maier SF, Fleshner M (2003) Freewheel running prevents learned helplessness/behavioral depression: role of dorsal raphe serotonergic neurons. J Neurosci 23:2889-2898.

Hajos M, Richards CD, Szekely AD, Sharp T (1998) An electrophysiological and neuroanatomical study of the medial prefrontal cortical projection to the midbrain raphe nuclei in the rat. Neuroscience 87:95-108.

Hammack SE, Richey KJ, Watkins LR, Maier SF (2004) Chemical lesion of the bed nucleus of the stria terminalis blocks the behavioral consequences of uncontrollable stress. Behav Neurosci 118:443-448.

Hammack SE, Schmid MJ, LoPresti ML, Der-Avakian A, Pellymounter MA, Foster AC, Watkins LR, Maier SF (2003) Corticotropin releasing hormone type 2 receptors in the dorsal raphe nucleus mediate the behavioral consequences of uncontrollable stress. J Neurosci 23:1019-1025.

Harrington MA, Shaw K, Zhong P, Ciaranello RD (1994) Agonist-induced desensitization and loss of high-affinity binding sites of stably expressed human 5-HT1A receptors. J Pharmacol Exp Ther 268:1098-1106.

Heim C, Nemeroff CB (2009) Neurobiology of posttraumatic stress disorder. CNS Spectr 14:13-24.

Heinrichs SC, Koob GF (2004) Corticotropin-releasing factor in brain: a role in activation, arousal, and affect regulation. J Pharmacol Exp Ther 311:427-440.

Hensler JG (2002) Differential regulation of 5-HT1A receptor-G protein interactions in brain following chronic antidepressant administration. Neuropsychopharmacology 26:565-573.

Hervas I, Vilaro MT, Romero L, Scorza MC, Mengod G, Artigas F (2001) Desensitization of 5-HT(1A) autoreceptors by a low chronic fluoxetine dose effect of the concurrent administration of WAY-100635. Neuropsychopharmacology 24:11-20.

Hoover WB, Vertes RP (2007) Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212:149-179.

Hornung J-P (2010) The Neuroanatomy of the Serotonergic System. In: Handbook of the Behavioral Neurobiology of Serotonin (Muller CP, Jacobs BL, eds). London: Elsevier.

Innis RB, Nestler EJ, Aghajanian GK (1988) Evidence for G protein mediation of serotonin- and GABAB induced hyperpolarization of rat dorsal raphe neurons. Brain Res 459:27-36.

Jackson RL, Maier SF, Rapaport RM (1978) Exposure to inescapable shock produces both activity and associative deficits in the rat. Learn Motiv 9:69-98.

Jacobs BL, Fornal CA (1991) Activity of brain serotonergic neurons in the behaving animal. Pharmacol Rev 43:563-578.

Jankowski MP, Sesack SR (2004) Prefrontal cortical projections to the rat dorsal raphe nucleus: ultrastructural features and associations with serotonin and gamma-aminobutyric acid neurons. J Comp Neurol 468:518-529.

Kalen P, Karlson M, Wiklund L (1985) Possible excitatory amino acid afferents to nucleus raphe dorsalis of the rat investigated with retrograde wheat germ agglutinin and D-[3H]aspartate tracing. Brain Res 360:285-297.

Kalen P, Pritzel M, Nieoullon A, Wiklund L (1986) Further evidence for excitatory amino acid transmission in the lateral habenular projection to the rostral raphe nuclei: lesion-induced decrease of high affinity glutamate uptake. Neurosci Lett 68:35-40.

Kalen P, Strecker RE, Rosengren E, Bjorklund A (1989) Regulation of striatal serotonin release by the lateral habenula-dorsal raphe pathway in the rat as demonstrated by in vivo microdialysis: role of excitatory amino acids and GABA. Brain Res 492:187-202.

Kalivas PW, Volkow N, Seamans J (2005) Unmanageable motivation in addiction: a pathology in prefrontal-accumbens glutamate transmission. Neuron 45:647-650.

Kalivas PW, Peters J, Knackstedt L (2006) Animal models and brain circuits in drug addiction. Mol Interv 6:339-344.

Kennedy SH, Giacobbe P, Rizvi SJ, Placenza FM, Nishikawa Y, Mayberg HS, Lozano AM (2011) Deep Brain Stimulation for Treatment-Resistant Depression: Follow-Up After 3 to 6 Years. Am J Psychiatry.

Kirby LG, Pernar L, Valentino RJ, Beck SG (2003) Distinguishing characteristics of serotonin and non-serotonin-containing cells in the dorsal raphe nucleus: electrophysiological and immunohistochemical studies. Neuroscience 116:669-683.

Kranz GS, Kasper S, Lanzenberger R (2010) Reward and the serotonergic system. Neuroscience 166:1023-1035.

Laaris N, Haj-Dahmane S, Hamon M, Lanfumey L (1995) Glucocorticoid receptor-mediated inhibition by corticosterone of 5-HT1A autoreceptor functioning in the rat dorsal raphe nucleus. Neuropharmacology 34:1201-1210.

Laaris N, Le Poul E, Hamon M, Lanfumey L (1997) Stress-induced alterations of somatodendritic 5-HT1A autoreceptor sensitivity in the rat dorsal raphe nucleus--in vitro electrophysiological evidence. Fundam Clin Pharmacol 11:206-214.

Laaris N, Le Poul E, Laporte AM, Hamon M, Lanfumey L (1999) Differential effects of stress on presynaptic and postsynaptic 5-hydroxytryptamine-1A receptors in the rat brain: an in vitro electrophysiological study. Neuroscience 91:947-958.

Lanfumey L, Pardon MC, Laaris N, Joubert C, Hanoun N, Hamon M, Cohen-Salmon C (1999) 5-HT1A autoreceptor desensitization by chronic ultramild stress in mice. Neuroreport 10:3369-3374.

Lanzenberger RR, Mitterhauser M, Spindelegger C, Wadsak W, Klein N, Mien LK, Holik A, Attarbaschi T, Mossaheb N, Sacher J, Geiss-Granadia T, Kletter K, Kasper S, Tauscher J (2007) Reduced serotonin-1A receptor binding in social anxiety disorder. Biol Psychiatry 61:1081-1089.

Le Poul E, Laaris N, Doucet E, Laporte AM, Hamon M, Lanfumey L (1995) Early desensitization of somato-dendritic 5-HT1A autoreceptors in rats treated with fluoxetine or paroxetine. Naunyn Schmiedebergs Arch Pharmacol 352:141-148.

Lee Y, Davis M (1997) Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. J Neurosci 17:6434-6446.

Leventopoulos M, Russig H, Feldon J, Pryce CR, Opacka-Juffry J (2009) Early deprivation leads to long-term reductions in motivation for reward and 5-HT1A binding and both effects are reversed by fluoxetine. Neuropharmacology 56:692-701.

Li Q, Muma NA, van de Kar LD (1996) Chronic fluoxetine induces a gradual desensitization of 5-HT1A receptors: reductions in hypothalamic and midbrain Gi and G(o) proteins and in neuroendocrine responses to a 5-HT1A agonist. J Pharmacol Exp Ther 279:1035-1042.

Liang KC, Melia KR, Campeau S, Falls WA, Miserendino MJ, Davis M (1992) Lesions of the central nucleus of the amygdala, but not the paraventricular nucleus of the hypothalamus, block the excitatory effects of corticotropin-releasing factor on the acoustic startle reflex. J Neurosci 12:2313-2320.

Lowry CA, Moore FL (2006) Regulation of behavioral responses by corticotropin-releasing factor. Gen Comp Endocrinol 146:19-27.

Lowry CA, Rodda JE, Lightman SL, Ingram CD (2000) Corticotropin-releasing factor increases in vitro firing rates of serotonergic neurons in the rat dorsal raphe nucleus: evidence for activation of a topographically organized mesolimbocortical serotonergic system. J Neurosci 20:7728-7736.

Lowry CA, Evans AK, Gasser PJ, Hale MW, Staub DR, Shekhar A (2008a) Topographic organization and chemoarchitecture of the dorsal raphe nucleus and the median raphe nucleus. In: Serotonin and Sleep: Molecular, Functional, and Clinical Aspects (Monti JM, Pandi-Perumal SR, Jacobs BL, Nutt DJ, eds). Verlag/Switzerland: Birkhauser.

Lowry CA, Hale MW, Evans AK, Heerkens J, Staub DR, Gasser PJ, Shekhar A (2008b) Serotonergic systems, anxiety, and affective disorder: focus on the dorsomedial part of the dorsal raphe nucleus. Ann N Y Acad Sci 1148:86-94.

Lozano AM, Mayberg HS, Giacobbe P, Hamani C, Craddock RC, Kennedy SH (2008) Subcallosal cingulate gyrus deep brain stimulation for treatment-resistant depression. Biol Psychiatry 64:461-467.

Maier SF (1990) Role of fear in mediating shuttle escape learning deficit produced by inescapable shock. J Exp Psychol Anim Behav Process 16:137-149.

Maier SF (2001) Exposure to the stressor environment prevents the temporal dissipation of behavioral depression/learned helplessness. Biol Psychiatry 49:763-773.

Maier SF, Seligman MEP (1976) Learned helplessness: theory and evidence. J Exp Psychol Gen 105:3-46.

Maier SF, Watkins LR (2005) Stressor controllability and learned helplessness: the roles of the dorsal raphe nucleus, serotonin, and corticotropin-releasing factor. Neurosci Biobehav Rev 29:829-841.

Maier SF, Kalman BA, Grahn RE (1994) Chlordiazepoxide microinjected into the region of the dorsal raphe nucleus eliminates the interference with escape responding produced by inescapable shock whether administered before inescapable shock or escape testing. Behav Neurosci 108:121-130.

Maier SF, Grahn RE, Watkins LR (1995a) 8-OH-DPAT microinjected in the region of the dorsal raphe nucleus blocks and reverses the enhancement of fear conditioning and interference with escape produced by exposure to inescapable shock. Behav Neurosci 109:404-412.

Maier SF, Ryan SM, Barksdale CM, Kalin NH (1986) Stressor controllability and the pituitary-adrenal system. Behav Neurosci 100:669-674.

Maier SF, Busch CR, Maswood S, Grahn RE, Watkins LR (1995b) The dorsal raphe nucleus is a site of action mediating the behavioral effects of the benzodiazepine receptor inverse agonist DMCM. Behav Neurosci 109:759-766.

Maswood S, Barter JE, Watkins LR, Maier SF (1998) Exposure to inescapable but not escapable shock increases extracellular levels of 5-HT in the dorsal raphe nucleus of the rat. Brain Res 783:115-120.

Mayberg HS, Lozano AM, Voon V, McNeely HE, Seminowicz D, Hamani C, Schwalb JM, Kennedy SH (2005) Deep brain stimulation for treatment-resistant depression. Neuron 45:651-660.

McDevitt RA, Szot P, Baratta MV, Bland ST, White SS, Maier SF, Neumaier JF (2009) Stress-induced activity in the locus coeruleus is not sensitive to stressor controllability. Brain Res 1285:109-118.

Meltzer CC, Price JC, Mathis CA, Butters MA, Ziolko SK, Moses-Kolko E, Mazumdar S, Mulsant BH, Houck PR, Lopresti BJ, Weissfeld LA, Reynolds CF (2004) Serotonin 1A receptor binding and treatment response in late-life depression. Neuropsychopharmacology 29:2258-2265.

Mendelson SD (1992) A review and reevaluation of the role of serotonin in the modulation of lordosis behavior in the female rat. Neurosci Biobehav Rev 16:309-350.

Michelsen KA, Schmitz C, Steinbusch HW (2007) The dorsal raphe nucleus--from silver stainings to a role in depression. Brain Res Rev 55:329-342.

Miczek KA, Fish EW, De Bold JF, De Almeida RM (2002) Social and neural determinants of aggressive behavior: pharmacotherapeutic targets at serotonin, dopamine and gamma-aminobutyric acid systems. Psychopharmacology (Berl) 163:434-458.

Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. Annu Rev Neurosci 24:167-202.

Nash JR, Sargent PA, Rabiner EA, Hood SD, Argyropoulos SV, Potokar JP, Grasby PM, Nutt DJ (2008) Serotonin 5-HT1A receptor binding in people with panic disorder: positron emission tomography study. Br J Psychiatry 193:229-234.

Nemeroff CB, Vale WW (2005) The neurobiology of depression: inroads to treatment and new drug discovery. J Clin Psychiatry 66 Suppl 7:5-13.

Ostlund SB, Balleine BW (2005) Lesions of medial prefrontal cortex disrupt the acquisition but not the expression of goal-directed learning. J Neurosci 25:7763-7770.

Paxinos G, Watson C (1998) The Rat Brain in Stereotaxic Coordinates. San Diego: Academic.

Penington NJ, Kelly JS, Fox AP (1993) Whole-cell recordings of inwardly rectifying K+ currents activated by 5-HT1A receptors on dorsal raphe neurones of the adult rat. J Physiol 469:387-405.

Peyron C, Luppi PH, Fort P, Rampon C, Jouvet M (1996) Lower brainstem catecholamine afferents to the rat dorsal raphe nucleus. J Comp Neurol 364:402-413.

Peyron C, Petit JM, Rampon C, Jouvet M, Luppi PH (1998) Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods. Neuroscience 82:443-468.

Picazo O, Lopez-Rubalcava C, Fernandez-Guasti A (1995) Anxiolytic effect of the 5-HT1A compounds 8-hydroxy-2-(di-n-propylamino) tetralin and ipsapirone in the social interaction paradigm: evidence of a presynaptic action. Brain Res Bull 37:169-175.

Pittenger C, Duman RS (2008) Stress, depression, and neuroplasticity: a convergence of mechanisms. Neuropsychopharmacology 33:88-109.

Quirk GJ, Beer JS (2006) Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. Curr Opin Neurobiol 16:723-727.

Raghupathi RK, McGonigle P (1997) Differential effects of three acute stressors on the serotonin 5-HT1A receptor system in rat brain. Neuroendocrinology 65:246-258.

Riad M, Zimmer L, Rbah L, Watkins KC, Hamon M, Descarries L (2004) Acute treatment with the antidepressant fluoxetine internalizes 5-HT1A autoreceptors and reduces the in vivo binding of the PET radioligand [18F]MPPF in the nucleus raphe dorsalis of rat. J Neurosci 24:5420-5426.

Richardson-Jones JW, Craige CP, Guiard BP, Stephen A, Metzger KL, Kung HF, Gardier AM, Dranovsky A, David DJ, Beck SG, Hen R, Leonardo ED (2010) 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. Neuron 65:40-52.

Robbins TW (2000) From arousal to cognition: the integrative position of the prefrontal cortex. Prog Brain Res 126:469-483.

Rozeske RR, Der-Avakian A, Bland ST, Beckley JT, Watkins LR, Maier SF (2009) The medial prefrontal cortex regulates the differential expression of morphine-conditioned place preference following a single exposure to controllable or uncontrollable stress. Neuropsychopharmacology 34:834-843.

Rueter LR, Fornal CA, Jacobs BL (1997) A Critical Review of 5-HT Brain Microdialysis and Behavior. Reviews in the Neurosciences 8:117-137.

Sakanaka M, Shibasaki T, Lederis K (1987) Corticotropin releasing factor-like immunoreactivity in the rat brain as revealed by a modified cobalt-glucose oxidase-diaminobenzidine method. J Comp Neurol 260:256-298.

Schutz MT, de Aguiar JC, Graeff FG (1985) Anti-aversive role of serotonin in the dorsal periaqueductal grey matter. Psychopharmacology (Berl) 85:340-345.

Seligman ME, Maier SF (1967) Failure to escape traumatic shock. J Exp Psychol 74:1-9.

Sesack SR, Deutch AY, Roth RH, Bunney BS (1989) Topographical organization of the efferent projections of the medial prefrontal cortex

in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp Neurol 290:213-242.

Simson PG, Weiss JM, Hoffman LJ, Ambrose MJ (1986) Reversal of behavioral depression by infusion of an alpha-2 adrenergic agonist into the locus coeruleus. Neuropharmacology 25:385-389.

Sinha R (2008) Chronic stress, drug use, and vulnerability to addiction. Ann N Y Acad Sci 1141:105-130.

Solomon Z, Mikulincer M (2006) Trajectories of PTSD: a 20-year longitudinal study. Am J Psychiatry 163:659-666.

Sotelo C, Cholley B, El Mestikawy S, Gozlan H, Hamon M (1990) Direct Immunohistochemical Evidence of the Existence of 5-HT1A Autoreceptors on Serotoninergic Neurons in the Midbrain Raphe Nuclei. Eur J Neurosci 2:1144-1154.

Sprouse JS, Aghajanian GK (1987) Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. Synapse 1:3-9.

Stamford JA, Davidson C, McLaughlin DP, Hopwood SE (2000) Control of dorsal raphe 5-HT function by multiple 5-HT(1) autoreceptors: parallel purposes or pointless plurality? Trends Neurosci 23:459-465.

Steinbusch HMW, Nieuwenhuys R (1983) The raphe nuclei of the rat brainstem: a cytoarchitectonic and immunohistochemical study. In, pp 131-209. New York: Raven Press.

Swanson LW, Hartman BK (1975) The central adrenergic system. An immunofluorescence study of the location of cell bodies and their efferent connections in the rat utilizing dopamine-beta-hydroxylase as a marker. J Comp Neurol 163:467-505.

Takase LF, Nogueira MI, Baratta M, Bland ST, Watkins LR, Maier SF, Fornal CA, Jacobs BL (2004) Inescapable shock activates serotonergic neurons in all raphe nuclei of rat. Behav Brain Res 153:233-239.

Takase LF, Nogueira MI, Bland ST, Baratta M, Watkins LR, Maier SF, Fornal CA, Jacobs BL (2005) Effect of number of tailshocks on learned helplessness and activation of serotonergic and noradrenergic neurons in the rat. Behav Brain Res 162:299-306.

Tang SS, Fox SH (2001) Traumatic experiences and the mental health of Senegalese refugees. J Nerv Ment Dis 189:507-512.

Tao R MZ, Auerbach SB (2000) Differential effect of local infusion of serotonin reuptake inhibitors in the raphe versus forebrain and the role of depolarization-induced release in increased extracellular serotonin. J Pharmacol Exp Ther 294:571-579.

Trulson ME, Crisp T (1984) Role of norepinephrine in regulating the activity of serotonin-containing dorsal raphe neurons. Life Sci 35:511-515.

Underwood MD, Khaibulina AA, Ellis SP, Moran A, Rice PM, Mann JJ, Arango V (1999) Morphometry of the dorsal raphe nucleus serotonergic neurons in suicide victims. Biol Psychiatry 46:473-483.

van Huizen F, Bansse MT, Stam NJ (1993) Agonist-induced down-regulation of human 5-HT1A and 5-HT2 receptors in Swiss 3T3 cells. Neuroreport 4:1327-1330.

VanderMaelen CP, Aghajanian GK (1983) Electrophysiological and pharmacological characterization of serotoninergic dorsal raphe neurons recorded extracellularly and intracellularly in rat brain slices. Brain Res 289:109-119.

Varga V, Szekely AD, Csillag A, Sharp T, Hajos M (2001) Evidence for a role of GABA interneurones in the cortical modulation of midbrain 5-hydroxytryptamine neurones. Neuroscience 106:783-792.

Vertes RP (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51:32-58.

Vertes RP, Crane AM (1997) Distribution, quantification, and morphological characteristics of serotonin-immunoreactive cells of the supralemniscal nucleus (B9) and pontomesencephalic reticular formation in the rat. J Comp Neurol 378:411-424.

Wang QP, Ochiai H, Nakai Y (1992) GABAergic innervation of serotonergic neurons in the dorsal raphe nucleus of the rat studied by electron microscopy double immunostaining. Brain Res Bull 29:943-948.

Weiss JM, Stone EA, Harrell N (1970) Coping behavior and brain norepinephrine level in rats. J Comp Physiol Psychol 72:153-160.

Weiss JM, Goodman PA, Losita BA, Corrigan S, Charry JM, Bailey WH (1981) Behavioral depression produced by an uncontrollable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. Brain Res Rev 3:36-97.

Weiss JM, Stout JC, Aaron MF, Quan N, Owens MJ, Butler PD, Nemeroff CB (1994) Depression and anxiety: role of the locus coeruleus and corticotropin-releasing factor. Brain Res Bull 35:561-572.

Weiss JM GP, Losita BA, Corrigan S, Charry JM, Bailey WH (1981) Behavioral depression produced by an uncontrol- lable stressor: relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain. Brain Res Rev 3:36-97.

Will MJ, Watkins LR, Maier SF (1998) Uncontrollable stress potentiates morphine's rewarding properties. Pharmacol Biochem Behav 60:655-664.

Will MJ, Der-Avakian A, Bland ST, Grahn RE, Hammack SE, Sparks PD, Pepin JL, Watkins LR, Maier SF (2004) Electrolytic lesions and pharmacological inhibition of the dorsal raphe nucleus prevent stressor potentiation of morphine conditioned place preference in rats. Psychopharmacology (Berl) 171:191-198.

Williams JL, Maier SF (1977) Transituational immunization and therapy of learned helplessness in the rat. . J Exp Psychol Anim Behav Process 3:240-253.

Williams JT, Colmers WF, Pan ZZ (1988) Voltage- and ligand-activated inwardly rectifying currents in dorsal raphe neurons in vitro. J Neurosci 8:3499-3506.

Wingo AP, Wrenn G, Pelletier T, Gutman AR, Bradley B, Ressler KJ (2010) Moderating effects of resilience on depression in individuals with a history of childhood abuse or trauma exposure. J Affect Disord 126:411-414.

Wirtshafter D, Sheppard AC (2001) Localization of GABA(B) receptors in midbrain monoamine containing neurons in the rat. Brain Res Bull 56:1-5.

Yin HH, Knowlton BJ, Balleine BW (2006) Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning. Behav Brain Res 166:189-196.

Young WS, 3rd, Kuhar MJ (1980) Noradrenergic alpha 1 and alpha 2 receptors: light microscopic autoradiographic localization. Proc Natl Acad Sci U S A 77:1696-1700.

Zimmer L, Riad M, Rbah L, Belkacem-Kahlouli A, Le Bars D, Renaud B, Descarries L (2004) Toward brain imaging of serotonin 5-HT1A autoreceptor internalization. Neuroimage 22:1421-1426.