The Posterior Dorsal Medial Striatum and the Effects of Controllability during Stress

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Defended March 31st, 2014

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ABSTRACT

Traumatic events often have adverse effects on an individual's behavior. However, the outcomes of such an experience vary, some individuals being more resilient than others. It has been determined that the presence of control can block the development of learned helplessness in rats when they are subjected to escapable shock (ES) rather than inescapable shock (IS). When the presence of control is recognized, the ventral medial prefrontal cortex (vmPFC) pyramidal neurons inhibits the dorsal raphe nucleus (DRN) preventing prolonged excitation and subsequent sensitization of the DRN. The objective here was to ascertain if NMDA receptors in the posterior dorsal medial striatum (pDMS) is involved in modulating the DRN response during stress by local microinjection of the NMDA receptor antagonist AP5.

Both the acute and long term impact of stress was observed and compared to the behavior of rats who received no such treatment (home cage controls, HC). Rats that received AP5 injections into the pDMS showed similar results to rats subjected to IS despite undergoing ES. They displayed higher serotonin (5-HT) concentrations in the DRN during stress and lower social exploration times 24 hours afterwards. When intra-pDMS AP5 injected rats were given IS a week after ES, they showed a deficit in shuttlebox escape learning in addition to the reduced social exploration times. The results imply that, in addition to the vmPFC, the pDMS is also involved in behavioral immunization.

ACKNOWLEDGMENTS

First, I would like to thank my primary thesis advisor, Dr. Alison Vigers, for the advice she has given me in the process of writing my thesis. Though this project initially intimidated me, her encouragement and support has motivated me to pursue it. She taught my first MCDB course and inspired me to pursue this major. This led me to an unforgettable undergraduate experience which will contribute greatly to my success in the future.

I would also like to thank Dr. Steve Maier, for the opportunity to work in his lab. Additionally, Dr. Jose Amat, and Roman Aleksejev have provided invaluable support over the years I have worked and have my heartfelt thanks. Without them, I would have never been able to undertake this thesis project. The skill and patience the have exhibited while teaching me the experimental techniques and concepts was exceptional. I would have never been able to grasp them on my own. The experience they have provided me has not only contributed greatly to my development as a scientist but also as a person.

Additionally, I want to thank Dr. Andrea Feldman for all the help she has given me in the writing process and for taking the time to read through the many versions of my thesis. Her suggestions had a large influence on the final product. Finally, I would like to thank the additional members of my thesis committee Dr. Charles Judd and Dr. Christy Fillman for the support they have given me during my undergraduate years and for teaching the courses that have increased my growth as a student.

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INTRODUCTION

It has long been known that a traumatic experience can lead to behavioral deficiencies and contribute to the development of psychopathologies such as depression and PTSD (Manchini & Bonanno, 2009). However, not all individuals exposed to such events acquire these disorders. This observation has fostered curiosity in the research community as to exactly why some individuals appear to be more or less susceptible to developing detrimental behaviors in the aftermath of these adverse experiences. Although genetic influence is undoubtedly a factor, cognition and how one evaluates a stressful situation play a role as well.

Rats that are exposed to uncontrollable tail shocks, also called inescapable shock (IS), have been observed to display a spectrum of behavioral abnormalities such as increased anxiety and learning deficits, known as learned helplessness. However, rats who undergo escapable shock (ES) do not show any of these deficits despite receiving identical sequence and shock intensity. The difference between these two conditions is that in ES, rats are exposed to tail shocks that they can terminate by turning a wheel mounted in front of their cage; however, during IS, the wheel is locked and the rat cannot turn off the shock. As such, the perceived presence of control has a strong influence over the impact of that traumatic experience (Maier & Seligman, 1976).

Past research has indicated that these effects are a consequence of a strong and prolonged activation of serotonergic (5-HT) neurons in the dorsal raphe nucleus (DRN) during IS, an effect not observed in rats subjected to ES. Such prolonged exposure to high levels of 5-HT sensitizes the DRN, so that future excitatory inputs will release exaggerated amounts of 5-HT within the DRN and its projection targets (Grahn et al., 1999). During ES, there is an initial increase of 5-HT concentrations; however, when control is perceived by the rat, 5-HT levels quickly return to

baseline due to the inhibitory actions of pyramidal neurons from the ventral medial prefrontal cortex (vmPFC) that project to the DRN (Jankowski & Sesack, 2004, Amat et al., 2005). The effect of this is two-fold; first, ES blocks the acute detrimental effects of stress and learned helplessness is not observed in these rats. Second, ES has been shown to have a longer term, protective impact called immunization. When rats are given ES and then IS a week later, the adverse effects of the latter session of IS are blunted. It has been hypothesized that the vmPFC would be activated during the second round of stress and thereby inhibit the DRN (Amat et al., 2010).

The posterior dorsal medial striatum (pDMS) is a structure believed to be involved in "goal-seeking" behavior, meaning that it is responsible for creating associations between actions and its outcomes, such as the association between turning the wheel and turning off the shock (Balleine & O'Doherty, 2010). There are two objectives of this paper: 1) to ascertain if the pDMS is also involved in modulating the DRN during ES and 2) to test if the pDMS activity during ES is necessary for immunization. To test for these hypothesis, pDMS function will be altered using an NMDA receptor antagonist (AP5). The impact of the stressor will be compared between rats that have been injected with AP5 and rats that have been injected with saline as a control. If a difference is observed, it would support the hypothesis that pDMS is involved in the outcomes of stress. Neurophysiological data will be taken through microdialysis where 5-HT concentrations in the DRN will be measured and behavioral data will be collected through social exploration and shuttlebox.

BACKGROUND

Traumatic events can have a negative impact on an individual's behavior. However, the outcome of these events are not always consistent and some people have been observed to be more or less resilient. The ability to cope has been tied to a perception of control (Diehl & Hay, 2010). Not only does this perceived control decrease the immediate negative impact of the stressor, but it also offers long-term protective effects against future stressors. (Williams & Maier, 1977).

Scientists have argued about the effects of a person's perceived ability to cope, whether that involves religious faith or sociopolitical effectiveness. These beliefs have been hypothesized to offer protection against the negative impact of stressors since they generate a sense of control. Individuals who do not perceive to have control can develop increased anxiety later in life (Chorpita & Barlow, 1998). However, detailed neurocircuitry and neurochemical workings of the brain cannot be studied in humans as modern-day neuroimaging techniques have limitations. As such, animals have been an alternative as there are many similarities between humans and other mammalian species such as rats.

Learned Helplessness

In the late 1960s, it was observed that dogs that were exposed to uncontrollable shocks later displayed a change in behavior characterized by decreased motivation, cognitive deficits, and greater emotional disruption (Overmier & Seligman, 1967). This has been observed numerous times under different conditions. An example is when IS rats fail to learn escape tasks such as shuttlebox and will freeze rather than actively run around thus implying a decreased motivation to control future stressors. Cognitive deficiencies are seen in an animal's decreased

ability to learn that a certain response is successful in controlling a stressor. Dogs that receive uncontrollable stress require more successes to be able to learn than unstressed, naïve dogs. Emotional deficits have been measured in a variety of ways including an increase in ulcer development and decreased food and water intake which indicate an increase in anxiety and stress. This led to the learned helplessness hypothesis, which is a model explaining that uncontrollable stressors contribute to the development of behavioral depression not seen if the stressor is controllable (Maier & Seligman, 1976).

Dorsal Raphe Nucleus

The DRN is one of many brainstem nuclei and provides 5-HT innervation to various areas of the brain by projecting to the hippocampus, hypothalamus, and other cortical and limbic structures. 5-HT is known to be involved in many functions such as escape behavior, sleep, feeding, anxiety, circadian rhythm, and pain sensitivity (Vertes, 1991). Two specific regions, the dorsolateral periaqueductal gray (Lovick, 1994), which is responsible for escape learning, and the basolateral amygdala (Ma et al., 1991), which is involved with fear conditioning, are innervated by these 5-HT projections from the caudal DRN. These connections suggest that the DRN is involved in producing learned helplessness behaviors seen in animals subjected to uncontrollable stressors. Higher DRN activity has been observed in animals that undergo IS, increasing the concentration of 5-HT in the area, which persists for several hours after IS (Grahn et al., 1999). This prolonged exposure to high levels of serotonin is believed to sensitize the 5-HT neurons of the DRN to future stimuli so that animals exhibit exaggerated responses in behavioral testing that occurs 24 hours later (Amat et al., 1998). In addition to projecting to other areas of the brain, the axon collaterals from the DRN also project back onto itself. 5-HT1_A

receptors located on the soma and dendrites of DRN neurons inhibit 5-HT synthesis and release when activated by 5-HT binding thus creating a negative feedback loop. 5-HT1_A receptors are prone to desensitization when exposed to high levels of 5-HT. It is hypothesized that IS desensitizes these receptors and therefore sensitizing the neuron (Maier & Watkins, 2005). On the other hand, 5-HT levels do not remain elevated for such an extended period of time during ES. Despite an initial peak in 5-HT concentrations, levels return to baseline soon after (Matos et al., 1996). Prolonged activation and sensitization of the DRN is required and sufficient to produce learned helplessness behaviors (Maier et al., 1995). As a small brain stem structure, it receives no direct input from either primary sensory or motor regions, which provide the information needed to recognize the presence of control. Therefore, it is unable to distinguish between ES and IS.

Ventral Medial Prefrontal Cortex

The prefrontal cortex (PFC) is associated with executive function and is involved in registering the presence of control during ES. The DRN receives much of its cortical input from the vmPFC (Vertes, 2004). These glutamatergic projections from the vmPFC, when activated, act on GABAergic neurons in the DRN (Jankowski & Sesack, 2004) which in turn inhibits 5-HT neurons. Though muscimol injections into the vmPFC in IS rats did not further worsen subsequent behavioral deficits, it did affect rats with control. Muscimol is a GABA agonist and thus works to inhibit vmPFC function. ES rats are not prevented from learning wheel-turn escape, but the protective effects of ES are still blocked and ultimately, these rats displayed learned helplessness behaviors much like their IS counterparts. So although the vmPFC is not involved in learning wheel turn escape, it likely participates in processing information about

controllability and regulates other circuits responsible for the development of learned helplessness. Additionally, 5-HT inhibits the vmPFC which may further increase DRN activation during IS (Amat et al., 2005). It has been observed that during ES, DRN-projecting vmPFC pyramidal neurons are activated (Barratta et al., 2009) thus inhibiting DRN activation during ES. Additionally, activating the vmPFC in IS rats is sufficient to create the protective effects of ES (Amat et al., 2008). However, the vmPFC has many projections in addition to the DRN, such as the pDMS, which may also influence the development of detrimental behaviors after uncontrollable stress.

Posterior Dorsal Medial Striatum

Traditionally, the striatum was viewed as a regulator of motor movement through output to the pallidum, thalamus, and motor cortices (Mink, 1996). However, more recently, the pDMS has been linked to executive functions. Of interest is its role in decision-making based on reward. The pDMS is believed to be involved in act/outcome, goal-orientated behavior which is dependent on contingencies and the value of the reward (Shiflett & Balleine, 2011). Contingency has been defined to be the difference between the probability of undergoing a specific action and receiving a reward and the probability of gaining that same reward without that action (Liljeholm et al., 2011). Though the ideas of goal-orientated reward and control may seem different, the concepts behind both are similar. Previously, Maier and Seligman (1976) stated that control or the lack thereof over a stressor was defined by two parts: the first being the probability that a specific action will terminate the stressor and the second being the probability of that same action having no effect. If the two probabilities equal each other, than the subject is considered to lack control.

On the other hand, another part of the striatum, the posterior dorsal lateral striatum (pDLS) is thought to be involved in habit-forming behaviors which are governed by learned stimulus-responses. This does not involve an associative link between the action and the outcome (Balleine & O'Doherty, 2010). Additionally, the vmPFC, involved in the perception of control, has known connections to the pDMS but not the pDLS. These two systems of reward learning have been shown to involve distinct neurological circuits under different conditions (Shiflett & Balleine, 2011).

Immunization

Though the acute impact of stress lasts for about 72 hours, the effects of immunization can last up to a month after the initial exposure to a controllable stressor. Immunization is seen when rats that receive ES are protected against IS performed a week later. Normally during IS, rats display elevated levels of 5-HT in the DRN for the duration of stress. However, in rats that have received previous experience of control, 5-HT levels during subsequent IS look similar to that of rats undergoing ES (Amat et al., 2006). It has been observed that these protective effects are general and nonspecific, meaning that immunization can protect an individual from stressors that are quite different from the initial controllable event (Amat et al., 2010). It is believed that plasticity in the vmPFC may contribute to the formation of immunization (Amat et al., 2006). Interestingly, ES is still considered a "stressful" event as rats show increased levels in adrenocorticotrophin releasing hormone and corticosterone which is equal to that released by IS rats (Helmreich et al., 2012). Despite this, rats that undergo ES and then subsequent IS do not

show the same learning deficits as rats that receive IS and then another round of IS in tasks such as the shuttlebox escape task (Williams & Maier 1977).

METHODS

Rats: The experiments used male Sprague-Dawley rats weighing 275-350g. Rats were housed in pairs under a 12 hour light/dark cycle. Lights were on starting at 7:00 and turned off at 19:00. All tests were conducted sometime between 9:00 and 16:00 and followed international guidelines on ethical animal experimentation in addition to being approved by the Institutional Animal Care and Use Committee of the University of Colorado at Boulder.

Surgery and cannulation: Rats were anesthetized with halothane (1.5-3% in O_2) during surgery. Microinjection and microdialysis guide cannulae were embedded into the rat's head using steel screws and acrylic cement. Measurements for cannulae placement were made from the intersection of bregma and the latitudinal fissure. Rats for microinjections only had cannulae guides inserted bilaterally either in the pDMS (0.2mm in the caudal direction, ±2.2mm laterally, and 3.5mm from the skull surface) or pDLS (0.2mm in the caudal direction, ±4.3mm laterally, and 3.6mm from the skull surface). Rats for both microinjection and microdialysis had a microdialysis cannula guide implanted just above the caudal DRN (8.3mm in the caudal direction, at the midline, 5.5mm from the skull surface) along with the microinjection guides. To protect the skull assembly during microdialysis, a screw cap from a 15mL conical centrifuge tube with the center removed was affixed with acrylic cement to the skull. Post-surgery, each rat was given a subcutaneous injection of 0.25mL/kg penicillin and of a 0.5mg/kg nonsteroidal

analgesic (Loxicam). Before experimentation, rats were given 1-2 weeks to recover from the surgery.

Wheel-turn escape/yoked inescapable stress procedure:

In this experiment, rats were given either ES or IS. Shock was administered on the tail through electrodes. The ES rat could terminate the shock by turning a wheel mounted in front of its cage; however, the wheel in front of the rat given IS was locked. Subjects were connected in series as yoked pairs within the same circuit to ensure that both rats were given identical amounts of shock.

A Plexiglas box (14x11x17cm) with a wheel mounted in front and a Plexiglas rod extending in the back contained the rats during stress. Rats were placed with their tail taped to the rod, facing the wheel. In addition to the tape, the rat's tail was affixed with copper electrodes. Each treatment had 100 shocks with an average intertrial interval of 60 sec. Shocks began and ended at the same time for both rats. The shock was terminated when the ES rat met the response criterion or after 30 sec if the requirement was not reached. The criterion began as a quarter turn of the wheel. If the requirement was reached within five sec for three consecutive trials, it was increased by 50% after each successful termination until a maximum of four full turns was reached. If the rat failed to reach the criterion within 30s, the shock ended and the requirement returned to the initial single quarter turn. Shock intensity began at 1.0mA for the first 30 trials, 1.3 mA for the next 30 trials, and then increased to 1.6 mA for the rest of the trials to maintain sufficient escape response from the rats. Home cage control (HC) rats were not shocked and remained in the colony room.

To test for immunization, rats were given a round of ES or no stress if they were HC rats. Then a week later, the same rats underwent a round of IS. Subsequent IS was conducted in narrow Plexiglas tubes lacking a wheel. The tail was fixed to an extending rod. Copper electrodes were than fastened to the tail so that shocks could be administered. Each treatment had approximately 100 shocks with an average intertrial interval of 60 sec. Each shock lasted 30 sec.

AP5 microinjections: 0.5µL of a 30mM solution of AP5 was injected into the pDMS or pDLS of rats about five min before ES or IS. Vehicle rats were given artificial cerebrospinal fluid (aCSF, pH 7.2). For injection, dual 33-guage microinjectors which protrude from the guides by 1mm were inserted through the cannulae. The gauge microinjectors were attached to PE-50 tubing which was in turn connected to a 25µL syringe. The syringes were mounted onto a Kopf microinjection unit (Model 5000). The solution was injected over 30s and then given two min to diffuse while the injector was in place.

In vivo microdialysis: A microdialysis probe (0.5mm in diameter) was inserted through the cannula guide so that the tip was within the caudal DRN. This was done in the afternoon the day before the stress. Dialysis tubing was threaded through a portion of a 15mL Eppendorf tube, which was then attached to the probe that was inserted into the cannula. The tubes were protected within a metal spring and the portion of the Eppendorf tube that was screwed onto the skull-mounted screw cap. The rats were individually placed in a Plexiglas bowl overnight where they were infused with aCSF at a rate of 0.2 µL/min. At about 9:00 the day of experimentation, the flow rate was increased to 1.5 µL/min which would be the rate used throughout the rest of the experiment. After a 90 min stabilization period, three baseline samples were collected every 20

min. AP5 or vehicle solution was injected into the pDMS or pDLS approximately five minutes before rats were placed into the Plexiglas wheel-turn boxes specially designed for microdialysis. They were given 100 ES or yoked IS tail shocks during which five samples were collected. After the session of stress, the rats were returned to the Plexiglas bowls where three more samples were taken. Since a rat's head movements could cause a possible 5-HT increase during dialysis, brisk movements of the skull-mounted screw cap were performed while the last sample was collected. If there was a 5-HT increase, then the data from that rat were discarded.

Juvenile social exploration test: Twenty-four hours after the ES/IS yoked procedure previously described, social exploration testing was conducted. Each rat was placed in its own plastic cage for about 45 min before a 28 (±2) day old juvenile was planted into the experimental subject's cage. An observer, blind to treatment to prevent bias, timed exploratory behaviors (sniffing, pinning, and allogrooming) initiated by the subject for three minutes. Though juveniles were used for multiple tests, the individuals were never used twice for the same adult.

Shuttlebox escape learning: Escape learning was conducted in shuttle boxes (46.0 cm x 27.7 cm x20 cm) with shock intensity of 0.6 mA. Rats were given five min to adapt to the boxes before five fixed ratio 1 (FR-1) escape trials were administered at one min intervals. The rat was required to cross to the other side of the box to end the shock. Afterwards, 25 FR-2 trials were given where the rat was required to cross to the opposite side and then return to end the shock. These trials were analyzed in blocks of five. If the rat did not escape, the shock was terminated after 30 sec.

5-HT analysis: Electrochemical detection from high pressure liquid chromatography (HPLC) was used to measure 5-HT concentrations in dialysates. The system was made of an ESA 5600A Coularray detector with an ESA 5014V analytical cell and an ESA 5020 guard cell. The column was an ESA HR-80X3.2 at 38°C and the mobile phase was ESA buffer MD-TM. The analytical cell potentials were kept at -100mV and +200mV and the guard cell at +220mV. Dialysates were kept at 6°C and 25µL were injected with an ESA 542 autosampler. External standards were run to quantify 5-HT concentrations.

Cannulae Placement Verification: Rats were overdosed with pentobarbital and brains were extracted and frozen. A cryostat was used to take 40µm slices that were later stained with cresyl violet to verify cannulae placement. If the injector tip or probe tract reached the target structure, then the data from that rat was included.

RESULTS

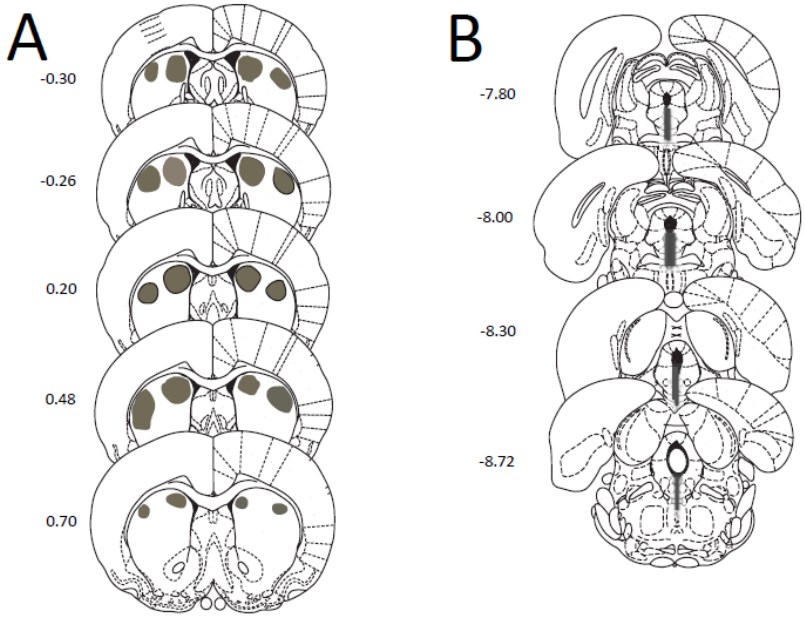

Figure 1: Location of injection cannulae tips within the pDMS and pDLS (A) marked in gray and microdialysis probes (2mm lines) in the DRN (B) marked by gray lines. The pDMS probe locations are

indicated by two more medial marks while the pDLS probe locations are represented by the two more lateral shaded areas. Not all cannulae are individually shown due to overlapping placements. Numerals represent millimeters from bregma on the anteroposterior axis.

Wheel Turn Escape Latencies

No significant difference was found between the different conditions (Fig. 2). AP5 injections in either the pDMS or pDLS did not affect the rats' ability to learn the wheel turn escape task and no effect of drug or injection site was found with repeated-measures ANOVA ($F_{2, 23} = .120$).

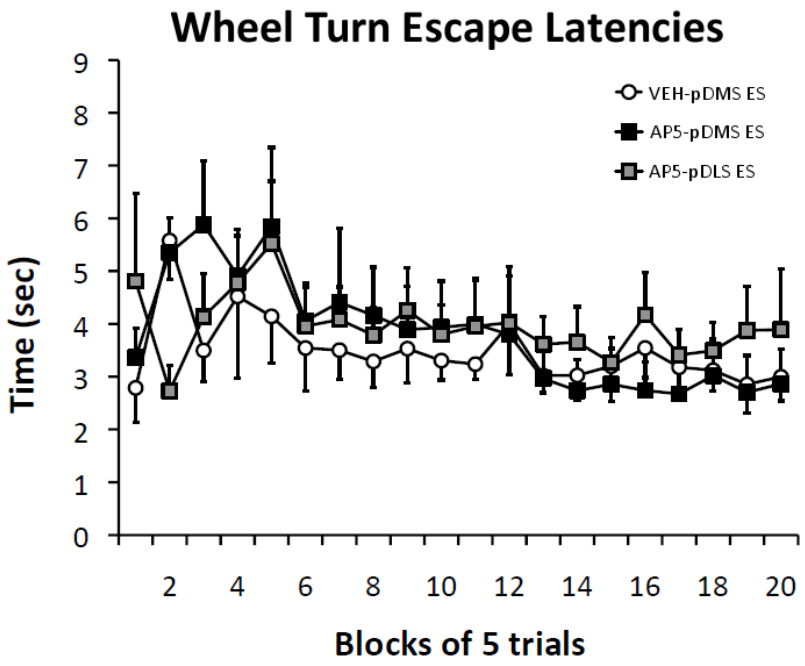

Figure 2: Wheel-turn escape latencies show no significant difference between different treatment types. Rats in each condition learned wheel-turn escape during ES. Repeated-measures ANOVA ($F_{2, 23} = .120$) did not find effects of drug or injection site.

5-HT Levels during Stress

Data from Amat et al. (2005) shows that rats given IS display prolonged, high levels of 5-HT in the DRN that persist through stress to the end of microdialysis sampling. ES rats show an initial increase of 5-HT concentration that soon return back to baseline levels. Though rats

injected with AP5 in the pDMS were given ES, they showed similar patterns to those of IS rats. On the other hand, subjects given AP5 injections in the pDLS were comparable to those of ES rats (Fig. 3). Baseline levels between groups prior to stress did not differ. However, during and after stress the differences were significant. Post hoc analysis ($p < 0.05$) shows that 5-HT levels in the pDMS were significantly higher than that of pDLS injections after the initial peak in 5-HT about 40 minutes after stress was initiated until the end of sampling. ES and IS 5-HT concentration data taken from Amat et al. (2005) for comparison.

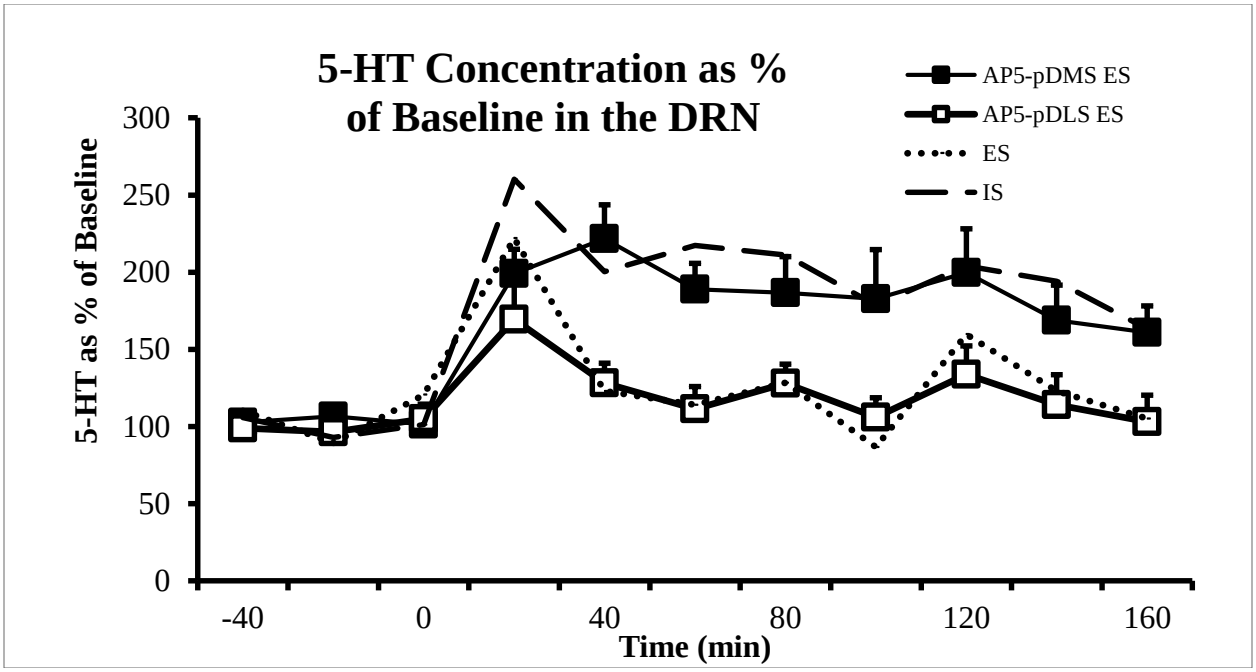

Figure 3: Serotonin concentrations in the DRN during stress as a percentage of baseline levels. Baseline levels before stress did not differ between groups. A two-way ANOVA was run with injection region as a between subjects factor and sample as a repeated measure identified a main effect of region ($F_{1, 15} = 8.633$, $p = 0.010$), a main effect of sample, ($F_{10, 150} = 7.580$, $p < 0.0001$), and their interaction ($F_{10, 150} = 2.307$, $p = 0.015$). Post hoc, Fischer PLSD comparisons identified significant differences at between pDMS and pDLS injections 40 min after the start of stress until the end of sampling ($p < 0.05$). AP5 injected into the pDMS leads to prolonged high levels of 5-HT in the DRN whereas AP5 injected into the pDLS does not prevent the rapid inhibition of 5HT release during ES. Data representing 5-HT levels in ES and IS taken from Amat et al, 2005.

Acute Effects of AP5 Injection

Injections were given just prior to stress. To observe the acute effects of stress, social exploration was run 24 hours later (Fig. 4). As vehicle-injected HC rats did not differ in social exploration times to AP5-injected HC rats (t-test, $p < 0.05$), the two groups were pooled together. A significant main effect of treatment was determined by a one-way ANOVA ($F_{4, 51} = 7.461$, $p < 0.001$). Post hoc analysis ($p < 0.05$) showed that VEH-injected rats that received ES showed no significant difference from HC rats and ES rats given intra-pDLS AP5 injections. However, ES rats that received intra-pDMS AP5 injections and VEH-injected IS rats had significantly reduced social exploration times that were similar to each other, but not the other groups. Shuttlebox escape task (data not shown) showed inconsistent data within each treatment condition with no significant difference between groups.

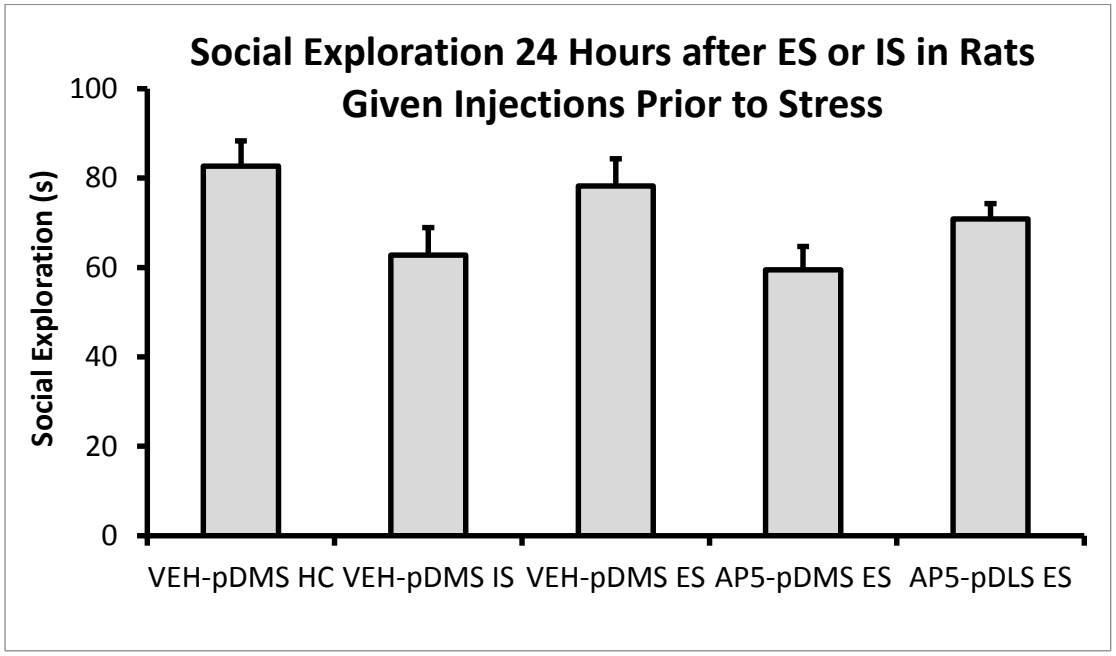

Figure 4: Mean times interacting with a 4 week-old juvenile rat in a 3 min time period during social exploration 24 hours after ES. Vehicle-injected HC rats did not differ in social exploration times compared to AP5-injected HC rats (t-test, $p < 0.05$) and were pooled together. A one-way ANOVA yielded a significant main effect of treatment ($F_{4, 51} = 7.461$, $p < 0.001$). Post hoc analysis showed that rats which received ES did not show a significant reduction in social exploration times in comparison to HC

rats whereas rats which received IS explored significantly less (pDMS HC vs. VEH-pDMS ES and pDMS HC vs. VEH-pDMS IS, $p < 0.05$). Rats with AP5 injected into the pDMS displayed significantly reduced times (AP5-pDMS ES vs. VEH-pDMS ES and AP5-pDMS ES vs. pDMS HC, $p < 0.05$), but not rats injected with intra-pDLS AP5 injections (pDMS HC vs. AP5-pDLS ES, $p < 0.05$).

AP5 Injection Effect on Immunization

To observe the effects on immunization, rats were given injections just prior to the first round of stress. A week after the initial exposure, rats were given IS and then 24 hours later, rats were given either the shuttlebox escape task (Fig. 5) or social exploration (Fig. 6). For the shuttlebox escape task, a repeated measures ANOVA showed a significant difference between groups ($F_{3, 33} = 14.72$, $p < 0.00001$). Post-hoc analysis with Fischer PLSD ($p < 0.05$) showed that subjects given IS displayed the highest FR-2 latencies in comparison to all other groups. Although rats that had AP5 injected into the pDMS showed decreased escape times compared to HC/IS rats, they had increased times in comparison to vehicle-injected rats and rats that were given AP5 injection into the pDLS. Rats given intra-pDMS vehicle injections and intra-pDLS AP5 injections showed similar escape times that were significantly less than the other two groups. The average escape time of these groups is similar to that of rats that did not receive any previous stress, as found in experiments done by previous studies. HC/HC data was taken from Amat et al. (2010) for comparison. No between-group differences were found in FR-1 latencies (data not shown).

Social exploration in rats 24 hours after IS showed significant difference between treatment types. A one-way ANOVA yielded a significant main effect of treatment ($F_{3, 32} = 5.07$, $p = 0.003$). Post-hoc analysis ($p < 0.05$) showed that HC/IS rats had significantly lower social exploration times than vehicle injected rats that had received previous ES. Rats that were given intra-pDMS AP5 injections showed similar reduced times to that of HC/IS, whereas rats with intra-pDLS AP5 injections did not display a social deficit.

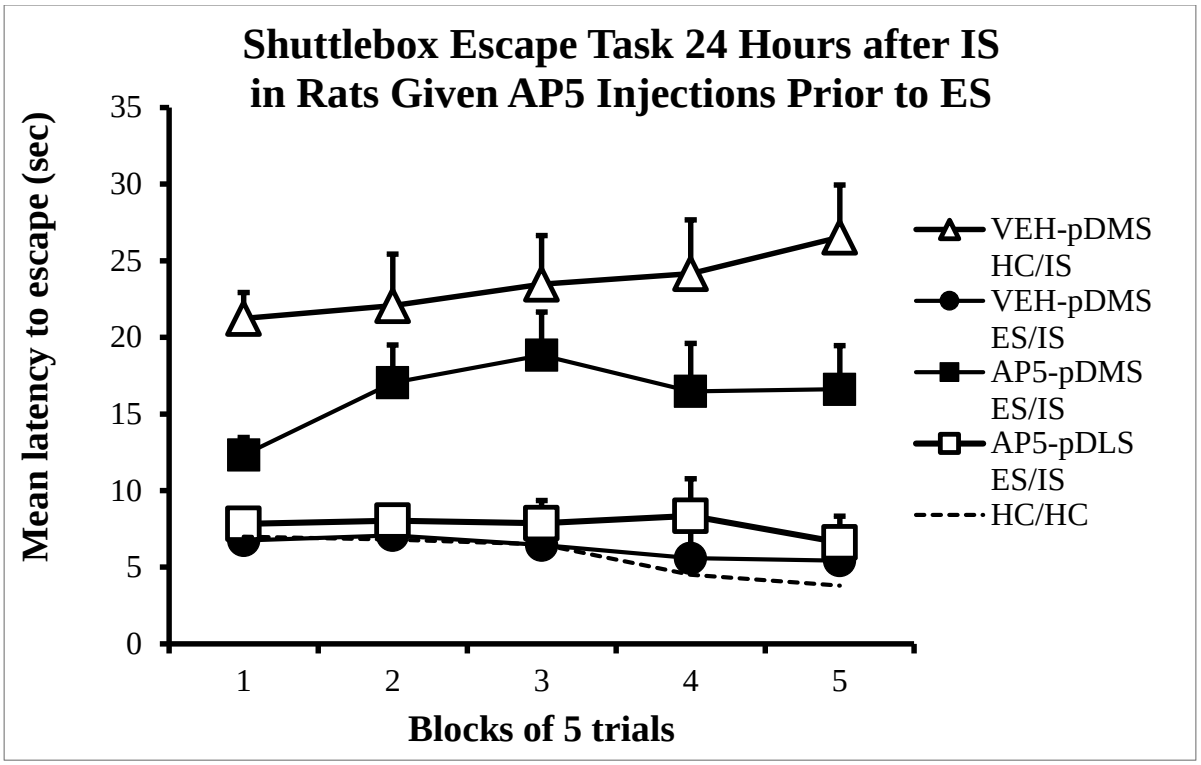

Figure 5: FR-2 mean latency to escape in shuttlebox escape task 24 hours after IS. A repeated measures ANOVA was run. Treatment condition ($F_{3, 33} = 14.72$, $p < 0.00001$) showed a significant effect between subjects, but within subjects trial blocks ($F_{4, 148} = 1.85$), and interaction between groups and trial blocks ($F_{12, 148} = 1.88$) showed no significant difference. Post hoc analysis ($p < 0.05$) indicated that HC rats that received IS without previous ES showed an increased latency in shuttlebox escape times compared to vehicle-injected rats that did receive ES a week before IS (VEH-pDMS HC/IS vs. VEH-pDMS ES/IS). Rats that received AP5 injection in the pDLS showed similar times to vehicle-injected rats that received ES prior to IS and displayed no learning deficit (VEH-pDMS ES/IS vs. AP5-pDLS ES/IS). However, rats that were given intra-pDMS AP5 injections showed increased escape times in comparison to vehicle injected rats given prior ES (AP5-pDMS ES/IS vs. VEH-pDMS ES/IS), but decreased times compared to HC/IS rats (AP5-pDMS ES/IS vs. VEH-pDMS HC/IS). The dotted line shows escape latencies for HC/HC rats from Amat et al, 2010 for comparison.

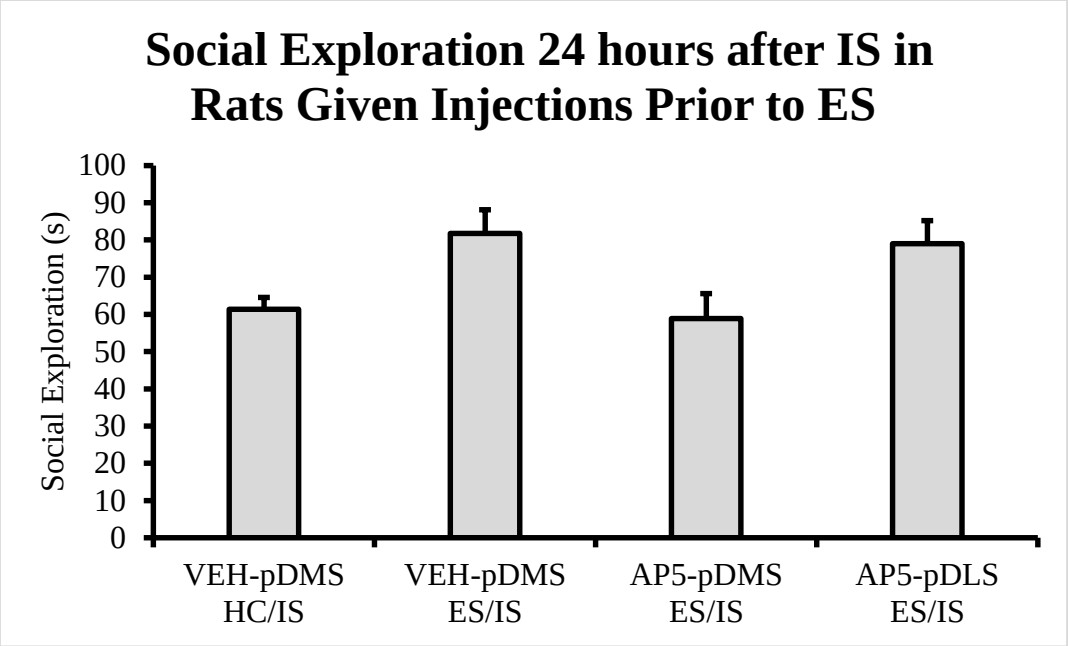

Figure 6: Mean social exploration times given 24 hours after IS. A one-way ANOVA yielded a significant main effect of treatment ($F_{3, 32} = 5.07$, $p = 0.003$). Fischer PLSD tests indicated that vehicle-injected HC rats that did not receive previous ES showed reduced social exploration times after IS that were not seen in rats that received ES a week previous to IS (VEH-pDMS HC/IS vs. VEH-pDMS ES/IS, $p < 0.05$). Rats that were given intra-pDMS AP5 injections before ES had similarly reduced times (VEH-pDMS HC/IS vs. AP5-pDMS ES/IS, $p < 0.05$). However, rats injected with AP5 in the pDLS did not show a significant reduction in social exploration times and were comparable to that of vehicle-injected rats that received previous ES (AP5-pDLS ES/IS vs. VEH-pDMS ES/IS, $p < 0.05$).

DISCUSSION

The results suggest that activation of the pDMS is essential for the both the short term and long term protective effects of ES, as AP-5 in the pDMS but not the pDLS blocks these effects. NMDA receptor blockade with intra-pDMS AP5 injection prior to ES resulted in prolonged DRN activation similar to that of IS rats. As a consequence, the post-stress, acute behavioral impact observed from this condition is also comparable to that of IS rats. Rats showed increased anxiety as reflected in decreased social exploration times not seen in vehicle-injected rats. Additionally, immunization was not seen in rats that were injected with AP5 in the pDMS

before ES. These rats showed deficits in both social exploration and shuttlebox escape task run 24 hours after IS was administered.

Shuttlebox escape tasks did not result in reliable data for acute conditions 24 hours after ES. The results were inconsistent and while some subjects within each condition successfully escaped, others did not. It is unclear why this did not work, as previous experiments done by Amat et al. (2005) acquired reliable shuttlebox data to observe the acute effects of stress. Of note is that a slightly different shuttlebox was used for the experiment. In the previous study, the rat crossing to the other side of the box was detected by a slight tilt in the floor. The shuttlebox used for this experiment emits a small beam light that is interrupted when the rat crosses. This is detected by a device installed on the opposite side. Another possibility is that paw lesions sometimes acquired through stress are unable to heal before acute testing, discouraging the rat from the movement required to escape. Since the second round of stress is performed in tubes rather than the wheel-mounted boxes, some rats may receive less injury and after a week, the lesions may have healed more, leading to more consistent results.

Wheel turn escape learning was neither affected by injection type nor the region of injection. Rats that were given AP5 injections in either the pDMS or pDLS were able to terminate tailshocks efficiently. This result implies that just the action of control during stress is not sufficient to create protection. However, rats given the intra-pDMS AP5 injection still showed behavioral deficits similar to rats given IS when tests were conducted 24 hours later. This implies that the vmPFC-pDMS circuit needs to be activated during stress to develop the protective effects of ES. The observation that merely turning the wheel did not protect intra-pDMS AP5 injected rats, suggests that the vmPFC and pDMS is involved in perceiving control. This implicates the vmPFC in two potentially distinct functions: 1) detecting the presence of

control and 2) subsequently inhibiting reactions to the stressor. In addition to the glutamatergic projections from the vmPFC activating GABA neurons in the DRN during ES, vmPFC glutamatergic projections may activate the pDMS which may take further actions to inhibit the DRN, a pathway that can be elucidated with further research. It is possible that the pDMS which projects to the globus pallidus, could, from there, then influence the outputs from the lateral habenula to the DRN. The globus pallidus has been associated with the lateral habenula in reward systems (Wickens, 2008) and lateral habenula has been shown to be involved in activating the DRN during stress. Habenular lesions have eliminated the development of learned helplessness in IS rats (Amat et al., 2001). It is possible that the activation of the pDMS inhibits the activation of the habenula, thus removing excitatory input into the DRN from this region.

In addition to blocking the acute impact of stress, which was observed by injecting the rat before stress and then conducting behavioral testing 24 hours later, ES also blunts the impact of future stressors regardless of their controllability (Amat et al., 2010). In this experiment, rats were given injections and then immediately afterwards, ES. A week later, they were given IS, 24 hours after which behavioral testing was carried out. Though only the acquisition was studied with intra-pDMS AP5 injections before ES, it logically follows that the expression of these protective effects should be tested as well. Though as of yet unpublished, Amat et al., have found that AP5 injections prior to the second round of stress did not result in a deficit in learning in the shuttlebox escape task. These rats showed protection from the prior session of ES. This suggests that NMDA neurons in the pDMS are not involved in expression of immunization. However, intra-pDMS muscimol injections before IS prevented learning in the shuttlebox escape task implying that perhaps a different type of receptor is responsible for expression of immunization.

We have observed that blocking NMDA receptors in the pDMS leads to higher levels of 5-HT in the DRN during stress despite the presence of control, which leads to acute behavioral deficits demonstrated by reduced social exploration times. Additionally, intra-pDMS AP5 injections before ES blunts immunization. After a second round of stress, rats that have received this treatment show decreased cognitive abilities as well as increased anxiety. Immunization has been connected to plastic changes in the vmPFC (Varela et al., 2012). As our results indicate that NMDA neurons in the pDMS are involved in forming the immunizing effects of ES, it is possible that plasticity also occurs in this region of the brain or the circuits connecting the two areas. Plasticity in these areas may also be involved in developing the protective effects seen in cognitive behavioral therapies. As behavioral therapies often address control, it is possible that the vmPFC and pDMS plasticity is involved in this as well.

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