Previous Inescapable Stress Interferes with the Immunization, but not the Acute, Effect of Later Escapable Stress

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Defended

April 11, 2017

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Abstract

For many years it has been known that perceived behavioral control over an adverse event, referred to here as stress, can prevent the immediate, or acute, behavioral and neurochemical impacts typically associated with an adverse event. Additionally, behavioral control has been shown to immunize, or protect, against the behavioral and neurochemical effects of future stress, even in the absence of control. However, there are no previous studies which investigate the proactive impact that an initial experience of stress without behavioral control might have on the acute protection and immunization provided by future controllability. Here, stress over which the subject had no behavioral control was administered prior to stress over which the subject perceived control in order to determine the effect on the acute and immunization effects typically associated with controllability. Behavioral results suggest that prior stress without the experience of control has no impact on the acute protection provided by future controllability, but blunts the ability of later control to immunize against future adverse events. Clinically, these results imply that prior trauma may impact the ability to reestablish behavioral control over future stress, which may prevent trauma survivors from developing resistance or resilience to cope with stress via behavioral control.

Acknowledgements

I would first like to thank my thesis advisor, Dr. Steven F. Maier, for the opportunity to work in his lab over the last three and a half years. The projects that I have been a part of have allowed me to gain insight into the process of research in an academic environment, be an author on a publication, and present research at a national conference. These experiences, and many more throughout my time in the Maier Watkins lab, have been invaluable to my undergraduate experience at CU Boulder, to my development as a student and as a researcher, and have influenced my long term career and life goals.

The patient guidance and unwavering support of Dr. Jose Amat and former PRA Scott Tilden in my training and day-to-day mentorship has been instrumental to my development as a researcher and to the accomplishment of this project. Without their countless hours of work, this thesis would never have been feasible. I am extremely grateful for all of the time that Jose has dedicated to mentor me not only in the techniques necessary to accomplish the experiments, but also the foundations behind them, and in critical thinking.

In addition to my direct mentors, the support, guidance, and friendship of the entire Maier Watkins lab family has made my lab experience not only educational, but a true community. PRAs Tim Fabisiak and Suzanne Fulgham and post-doctoral fellow Mike Baratta, especially, have offered me support both emotionally and intellectually, and without them, as well as many others in the lab family, my research experience would have been a dull comparison.

I would like to give special thanks to Dr. Nancy Guild and Dr. Christy Fillman for the opportunity to participate in the Phage Genomics Lab my freshman year. The mentorship and guidance of these two inspiring, kind, and incredibly successful women is truly what inspired me to continue my education in biological sciences and pursue research.

I am grateful for the departments of both Psychology and Neuroscience and Molecular Cellular Developmental Biology for the challenging and engaging courses and the professors who have inspired and educated me. Specifically, I would like to thank Dr. Jerry Rudy, my honor's council representative, for making me feel valued and always fueling my progress with guidance and coffee, as well as Dr. Jenifer Martin for providing an excellent role model as a woman in science to aspire to, and always having an open door for puppy therapy on especially hard days.

Finally, I cannot express enough gratitude to the family and friends who have supported and encouraged me over the last four years. Without the support of my community, I could not have become who I am personally or academically. The balance and joy they have brought to my life has kept me sane and motivated in times of stress and chaos.

This work was supported by funding from the Biological Sciences Initiative Biological Undergraduate Research Skills and Training (BSI-BURST), Undergraduate Research Opportunities Program (UROP), and Biological Sciences Initiative Grants. Without the financial and interpersonal support provided by these programs, I would have been unable to undertake this project.

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Introduction

Traumatic experience has long been known to be associated with subsequent development of anxiety-based disorders such as depression, social anxiety disorder, and PTSD (Brewer et al., 2000).Response to traumatic events can vary widely between any two individuals, and can be attributed to a large number of different genetic, developmental, and environmental factors (Mancini and Bonnano, 2009). While each person responds to trauma with unique coping mechanisms, the majority of people cope relatively effectively and appear to be resilient after potential traumatic events, and the mechanism of resilience manifests itself in a diverse variety of ways between individuals (Bonano 2005).

One factor which has been shown to influence resilience, or coping, with traumatic stressors in human models, as well as animal models, is known as learned helplessness. Learned helplessness is a phenomenon in which the detrimental effect of a stressful or traumatic event can be attributed to the perceived lack of control over a stress or anxiety inducing stimulus, rather than to the effects of the stimulus itself (Maier 1976).

The learned helplessness phenomenon has formed the foundation for an animal model of stress which differentiates between the physical effects of stress itself and the psychological effects associated with behavioral controllability or lack thereof. In this model, one animal has behavioral control over a stressful stimulus, while a second animal receives exactly equal stressful stimuli, but lacks the ability to exert behavioral control (Maier 1984). The animal which has is able to exert behavioral control over the event is protected from many of the immediate adverse behavioral and neurochemical outcomes typically associated with stress (Maier and

Watkins, 1998). Additionally, prior experience with controllability appears to provide an immunization effect to the effects of later stress, even in the absence of behavioral control (Williams and Maier, 1977).

The ability of prior control to prevent the negative effects of uncontrollable stress has been exploited in various behavioral therapies which utilize controllability in a clinical setting as a means to attenuate the later effects of a stressor in its endogenous environment (Hoffman, 2008). While it is known that previous experience with control prevents the adverse effects of later stress regardless of controllability, no previous studies have addressed whether a previous experience with inescapable stress could interfere with the protection that is offered by controllability. Clinical data suggest that prior trauma may increase the likelihood of developing anxiety or stress related disorders later in life (Heim and Nemeroff, 2001), which could implicate interference of prior experience with inescapability in the prevention of immunization.

The purpose of the current research is two-fold: 1) Determine whether prior experience with stress over which the subject has no behavioral control blunts the acute, short term protection of subsequent stress over which the subject can exert behavioral control and 2) Ascertain whether previous experience with uncontrollable stress disrupts the ability of future controllability to provide immunization against the effects of future stress.

Background

The response of people to adverse events, referred to here as stressors, is highly variable. Some are less likely to develop long lasting behavioral or mental disorders and have a shorter period of recovery after a traumatic stress experience, i.e. stress resistance/resilience. The variability in resistance and resilience between any two individuals can be attributed to numerous genetic, psychological, environmental, and physiological factors which can vary widely between people (Mancini and Bonanno, 2009).

In the paradigm utilized here, an animal model is used to differentiate between the physical and psychological effects of stress, in order to better isolate the psychological factors which may contribute to stress resistance and resilience. In this paradigm, one animal receives uncontrollable stress, also known as inescapable stress (IS), while another receives controllable, or escapable, stress (ES). Both animals receive the exact same physical stress, in this case electric tail shocks, however the ES subject is able to turn a wheel which terminates the shock for both itself and its yoked IS partner. Since each animal receives exactly equal physical stress, the different behavioral and neurochemical effects between ES and IS can be attributed uniquely to the perception of control, and not to any difference in the physical stress experienced.

Acute Effect of Stress

Behavioral control over an adverse event has long been known to attenuate the neurochemical and behavioral effects of stress (Seligman and Maier, 1967). Inescapable stress, but not escapable, has been correlated with aversive behavioral effects such as learned helplessness,

failure to escape aversive situations, and increased anxiety that are not seen in escapable stress or unstressed controls (Maier and Watkins 1998). These acute behavioral effects have been shown to persist for up to 72 hours after stress is administered.

Previous research has implicated the role of serotonin (5HT) in the adaptive response to aversive events (Deakin and Graeff, 1991). When extracellular 5HT levels were measured in vivo in the basolateral amygdala (BLA) throughout the duration of stress, ES and IS animals both showed an initial spike in extracellular 5HT levels. However, levels in animals which received ES quickly return to baseline, while 5HT levels of IS animals remain elevated even after 24 hours (Amat et al., 1998).

The elevated levels of 5HT in the BLA observed during and immediately after IS are thought to be due to increased input from the Dorsal Raphae Nucleus (DRN) (Maier and Watkins, 2005). IS, but not ES, has been shown to selectively desensitize inhibitory 5HT1A auto-receptors, resulting in reduced inhibitory feedback input on DRN firing, and thus DRN enhanced activation (Rozeske, et al., 2011). DRN activation results in increased serotonin release in projections to various brain regions associated with the aversive stress response behaviors commonly seen after IS, including the periaqueductal gray, the dorsal PAG, and the basolateral amygdala, which are responsible for escape learning, fight/flight, and fear conditioning, respectively (Zanoveli, et al. 2003; Brandao et al., 1994; Fanselow, et al., 2003).

The experience of behavioral control (ES) is not associated with enhanced DRN activation seen in IS. In ES, the experience of behavioral control over the shocks results in increased excitability of pyramidal cell neurons in the prelimbic (PL) region of the medial prefrontal cortex (mPFC)

(Varela et al., 2012). These glutamatergic pyramidal cells in the mPFC do synaptic contact with GABA-ergic cells in the DRN resulting in inhibition of 5HT neurons (i.e. decreasing DRN serotonin output), and therefore a decrease in the behavioral disorders which result from the DRN hyper activity associated with IS (Amat et al., 2008).

Immunization

In addition to the acute effects which modulate behavioral and neurochemical outcomes of an adverse event, ES has been shown to provide a behavioral immunization effect, which blunts the aversive effects of future stress, even in the absence of control during the future traumatic experience (Williams and Maier, 1977). This immunization effect has been shown to endure for up to several weeks after the original ES, as compared to the relatively short duration of the acute behavioral effects associated with IS. The phenomenon of immunization has been attributed to the perception of control over the stress during ES, not to the experience of stress itself (Varela et al., 2012).

Behavioral control alone is necessary but not sufficient to provide immunization from future IS. Activation of both the mPFC and dorsomedial striatum (DMS) during the experience of controllability is necessary for the perception of control and the subsequent immunization from future stress (Amat et al., 2005, 2014). The combination of perceived behavioral control and mPFC/DMS activation results in plastic changes in the PL of the mPFC, a pathway which is potentiated by ES and later activated by future stress events, resulting in protection, even in the absence of control (Baratta, et al., 2009; Maier 2015). Recently, pharmacological agents which target the circuitry involved in stressor controllability, such as ketamine, have been

shown both to prevent the acute effects of IS and to provide immunization against future IS events, even in the absence of controllability (Amat et al., 2016).

Clinical Implications of Controllability

There is also literature on the effect of stressor controllability in humans resulting in outcomes comparable to those of this model. Self-efficacy, or control, over a stressful stimulus has been shown to reduce anxiety and subjective distress in human studies (Bandura et al., 1998). Human subjects are relatively unaffected by stressors about which they feel a high level of self-efficacy to manage; but exhibit increased levels of disstress and anxiety when a stimulus surpasses their perceived ability to control (Benight and Bandura, 2004). Increased perception of self-efficacy to cope with trauma has been implicated in improved resilience and recovery after traumatic stressors including military trauma and domestic violence (Solomon et al., 1991; Lerner and Kennedy, 2000).

The neural mechanisms implicated in our ES/IS model are also implicated in human anxiety disorders which are thought to be related to stress controllability. Disorders such as PTSD and depression have been linked to abnormalities in mPFC functioning (Drevets, 2000; Bremmer et al., 1999). These abnormalities are hypothesized to be correlated with stressor controllability, or perceived self-efficacy, with worse cases of PTSD exhibiting decreased mPFC response to stressful stimuli, and therefore exaggerated symptoms due to decreased mPFC inhibition of the amygdala (Maier et al., 2006). The ability of a similar stressor to result in PTSD in some subjects, but not others, may be a result of previous experience with perceived stressor controllability,

which provides immunization from the detrimental behavioral and neurochemical changes of a later uncontrollable stressor.

Behavioral therapies for a wide range of anxiety disorders including post-traumatic stress disorder, phobias, panic disorder, and obsessive compulsive disorder exploit the immunizing effect of behavioral control on future traumatic experiences by exposing patients to the source of their fear or anxiety in a setting in which they perceive having control (e.g. Foa et al., 1999; Ost et al., 2001; Clum and Surls 1993; Abramowitz, 1997). In these therapies, the experience of control in a clinical setting may protect against the aversive behavioral effects of the anxiety-inducing stimulus in its natural context by changing harm expectancy and therefore perceived self-efficacy over the stress (Hoffman, 2008).

Effects of a previous IS experience on the protective effects of ES

While much is known about the effect of controllability and the ability of ES to provide protection from future IS experiences, little is known about the effects of IS administered prior to ES. There are no previous studies on whether the resilience circuitry of ES could be affected by a previous IS experience. If this were the case, it could have important implications for the field of stress and stress disorders. If a previous trauma is sufficient to prevent the immunization effect of ES, a subject who had undergone a previous traumatic stressor would not be protected by experiencing control, at least for some time, due to the proactive interference of the previous stressor on the neural mechanisms of controllability and immunization. The goal of the current study is to ascertain the neurochemical and behavioral effects of an IS experience on both the acute and immunization effects of later ES.

Materials and Methods

Subjects

All experiments were performed on male Sprague Dawley rats (Harlan Laboratories, Indianapolis, Indiana) weighing between 275 and 350 grams. All animals were housed two per cage and kept on a 12 hour light dark cycle (7 am-7 pm) and were allowed free access to standard rat food and water. Experiments were performed between the hours of 8am and 5pm. Subjects were allowed at least one week between arrival to the lab and initiating any experimentation to acclimate. All experiments were approved by the *Institutional Animal Care and Use Committee* (IACUC) of CU Boulder and performed in accordance with the recommendations set forth by the National Institutes of Health (NIH) in the *Guide for the Care and Use of Laboratory Animals.*

Study Design

The experiment was designed to determine the behavioral and neurochemical effects of prior IS on the acute and immunization effects of ES on a later IS. The design included three rounds of treatment of varying combinations of inescapable stress, escapable stress, or home cage control (no treatment). The second treatment was administered five days after the first (day 5), and the third treatment was administered seven days after the second (day 12). Juvenile social exploration was performed 24 hours after the second and third treatment (days 6 and 13) and additional behavioral tests including shuttle box and fear conditioning were conducted 24 hours after the final treatment (day 13). A subset of animals was implanted with a cannula in the BLA

and in vivo microdialysis was performed during the final treatment to measure BLA extracellular 5HT levels induced by IS.

During the first and second treatments, IS was delivered as a series of variable duration tail shocks which were yoked to the ES subjects in which the ES animals had control over the duration of the administered shock, and the IS animals received the exact number and duration of shock as did the ES. This allowed the behavioral control over the stress to be isolated from the physical experience of stress itself as both experimental groups received identical shock but differential behavioral control. As has been widely supported, the behavioral control over a stressor can prevent the typical aversive effects seen after stress (Maier Watkins, 2005). However, during the third treatment, as no subjects received ES, IS consisted of a series of fixed-duration of random tail shocks, as the behavioral control of the third stressor was not a contributing variable to the effect studied. However, it should be noted that previous studies suggest that the neurochemical and behavioral effects of IS are identical whether administered as a series of variable duration tail shocks yoked to the control of an ES subject or as a series of fixed duration tail shocks (Amat et al., 1998).

Tail Shock Procedure

Escapable and inescapable tail shock were performed as previously outlined (Amat et al., 2005), (Christianson 2010). Each animal was placed in a Plexiglas box (14cmx11cmx17cm) with a wheel affixed to the front. A Plexiglas rod extended from the back, to which the tail was taped and affixed with two copper electrodes. These electrodes were then yoked so that the animals received shock in yoked pairs. Each session consisted of 100 shocks with an average trial

interval of 60 seconds. The shock was delivered at the exact same time for both animals, and the ES animal was able to terminate the shock by turning the wheel in the front of the box. Initially, a quarter turn of the wheel terminated the shock, but if the ES animal turned the wheel to terminate the shock in under 5 seconds for three consecutive trials, the requirement to terminate the shock was increased by a quarter turn. Subsequent latencies under 5s increased the requirement to terminate by 50%, up to a maximum of requiring 4 full turns to terminate the shock. Any trial in which the ES animal failed to escape after the maximum duration of the shock of 30 seconds reduced the requirement to terminate the shock back to a quarter of a turn. This increasing requirement to terminate the shock ensured that ES animals learned the appropriate response while still maintaining a minimum level of shock required to induce the typical IS effect (Amat et al., 1998).

Three rounds of treatment were administered, with the second being administered 5 days after the first, and the final treatment being administered 7 days after the second. Each animal received a combination of inescapable stress (IS), escapable stress (ES) and home cage control (HC) in varying orders. Subjects were evenly assigned to each experimental group based on baseline JSI score. During all shock treatments, shock intensity was 1.0 mA for the first 30 min, 1.3 mA for the second 30 min, and 1.6 mA for the final 40 min. All home cage control (HC) animals were handled identically and brought out of the colony along with the animals receiving treatment and placed undisturbed in a neighboring room.

Behavioral Outcome Measures

Juvenile Social Exploration

Juvenile social exploration (JSI) was performed as a measure of anxiety as outlined previously (Christianson 2008). Each animal was removed from their home cage and singly placed into a standard plastic cage with bedding and a wire top. The animals were then allowed to acclimate to the new cage for approximately 60 minutes. After the acclimation period, a juvenile rat aged 28±5 days was placed into the cage with the adult animal. An observer, blind to experimental condition, then timed exploratory advances initiated by the adult rat for 3 minutes. These included sniffing, pinning, grooming, and pursuing.

JSI was performed three times with each subject. 24 hours before the initial treatment to establish a baseline level of social interaction was measured, and animals were then evenly distributed to experimental conditions based on baseline JSI. JSI was performed again 24 hours after each the second and third treatments.

Animals that showed signs of pain or injury after the stress session were administered .2mg of loxiocom and monitored carefully. Animals which continued to show signs of pain and injury were excluded from behavioral testing. These most often included inflamed or irritated hind paws or toenails. Though every effort was made to prevent injury during the stress procedure, several animals were excluded from the remainder of the study due to injury.

Fear Conditioning

24 hours following the third treatment exposure, fear conditioning behavioral test was performed as previously outlined in Amat et al., 2008. Each animal received three 0.6mA shocks

in a shuttle box, that could be terminated by crossing to the other side of the shuttle box (FR-1). Fear conditioning to the environmental context was assessed by recording freezing in the environment for 20 minutes. Freezing or not freezing behavior was recorded every 10 seconds for the 20 minute duration. Freezing was defined as the absence of all movement not required for respiration.

Neurochemical Outcome

Cannula Placement

Surgical procedure was followed as described in Amat et al., 2006. After being anaesthetized with inhaled isolfourane anaesthesia, animals were placed in a stereotaxic surgical frame. The skull was adjusted to be level, and a cannula guide was implanted, terminating directly above the BLA for microdialysis . The cannula was placed as described previously (Amat et al., 2016) [from bregma in mm: AP, 3.0; mediolateral (ML), 4.8; dorsoventral (DV), 6.2] according to coordinates in the atlas Paxinos and Watson (1998). The cannula was held in place with dental cement affixed to four screws placed into the skull and a 15 mL conical screw top was affixed to the top in order to protect the cannula and provide a method to affix the microdialysis equipment. Upon termination of the surgery, animals were administered .25mL/kg penicillin and .5mg/kg Loxicom analgesic subcutaneously. All animals were given 1-2 weeks recovery time before any experimentation.

In vivo microdialysis

Approximately 18 hours prior to the experiment, animals were removed from their home cage and a Scripo microdialysis probe (MAB 6.14.2: 0.6 mm in diameter, 2 mm membrane) was

inserted through the cannula guide so that the tip rested just past the end of the cannula guide in the BLA. A portion of a 15 mL conical tube was affixed to the screw top on the skull, through which the microdialysis tubing was passed, and attached to the probe. Each animal was placed individually into a plexiglass bowl, and artificial CSF was infused through the microdialysis tubing at a rate of .2µl/min overnight. 90 minutes before the experiment the following morning, the rate was increased to 1.5µl/min, and remained constant for the duration of the experiment. The animals were placed in wheel turn boxes as previously described that were specialized to accommodate the microdialysis equipment, where they received 100 either escapable or inescapable tail shocks. After the shock session was terminated, all animals were returned to their individual Plexiglass bowl. Samples were taken every 20 minutes for 3-4 baseline samples before stress, throughout the approximately 100 minute ES/IS shock section, and for 3-4 post stress samples. This protocol was repeated during the final shock section seven days later.

5HT quantification

High Performance Liquid Chromatography (HPLC) with electrochemical detection was performed to quantify 5HT concentrations from the samples taken for microdialysis as previously described (Amat. et al., 2015).

Tissue Analysis

Upon termination of the experiment, animals were given a lethal dose of sodium pentobarbital (60mg/kg) and the brains were extracted and frozen in cold isopentane. The brains were then sliced into 40nm sections and stained with cresyl violet and cannula placement in the BLA was confirmed via microscopy.

Statistics

Data were analyzed by one way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. P values were set at 0.05. Data are presented as mean ± standard error of the mean.

Results

Effect of Previous IS on Acute Behavioral Outcomes

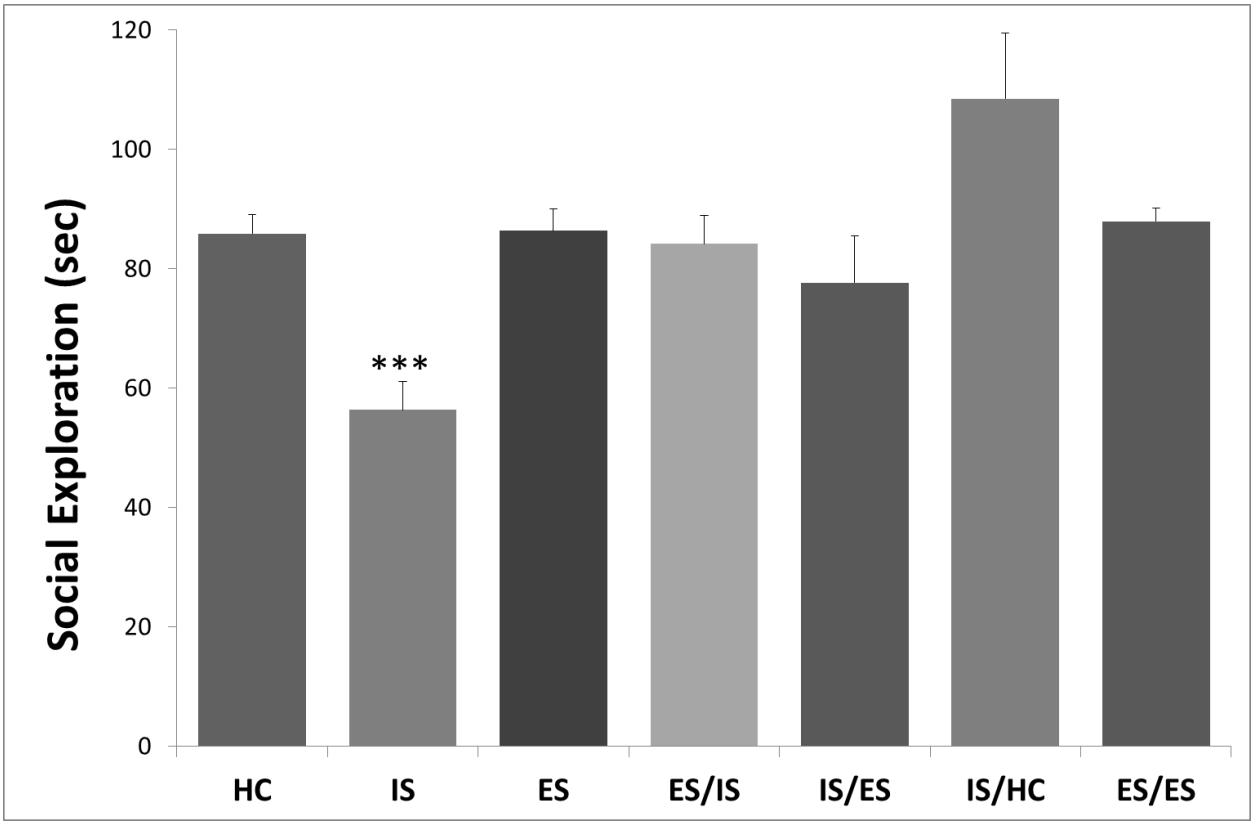

Figure 1: Effect on juvenile social exploration of a primary stress event followed by a secondary stress event 5 days later. Experimental groups include ES/IS (n=8); IS/ES (n=8); IS/HC (n=12); and ES/ES (n=4). Single treatment groups of HC (n=8), ES (n=8), and IS (n=8) are included for reference. (*** p< .01).

All rats received a primary stress of 100 tail shocks of either IS or ES, followed by a second round of treatment consisting of either IS, ES, or HC. Social anxiety was assessed by juvenile social exploration 24 hours after the secondary stress event. Figure 1 shows the time spent in social exploration for the four experimental groups, as well as 3 single treatment groups of IS, ES, or HC for comparison. ANOVA yielded significant effects of treatment ($F_{(6,84)}$=4.80, p<.01). Bonferroni multiple comparisons indicated significant reduction in time spent in social

exploration as compared to baseline for a single administration of IS (p<.001), and no significant deviation from control for any other experimental group. Prior experience with escapability offers protection from the future reduction JSI associated with IS, as has been supported previously. Previous experience with IS does not appear to cause a reduction in time spent in juvenile exploration 24 hours after either HC or ES, and exploration times for these groups do not differ significantly from either HC or ES alone.

Effect of Previous IS on Behavioral Outcomes of ES Immunization

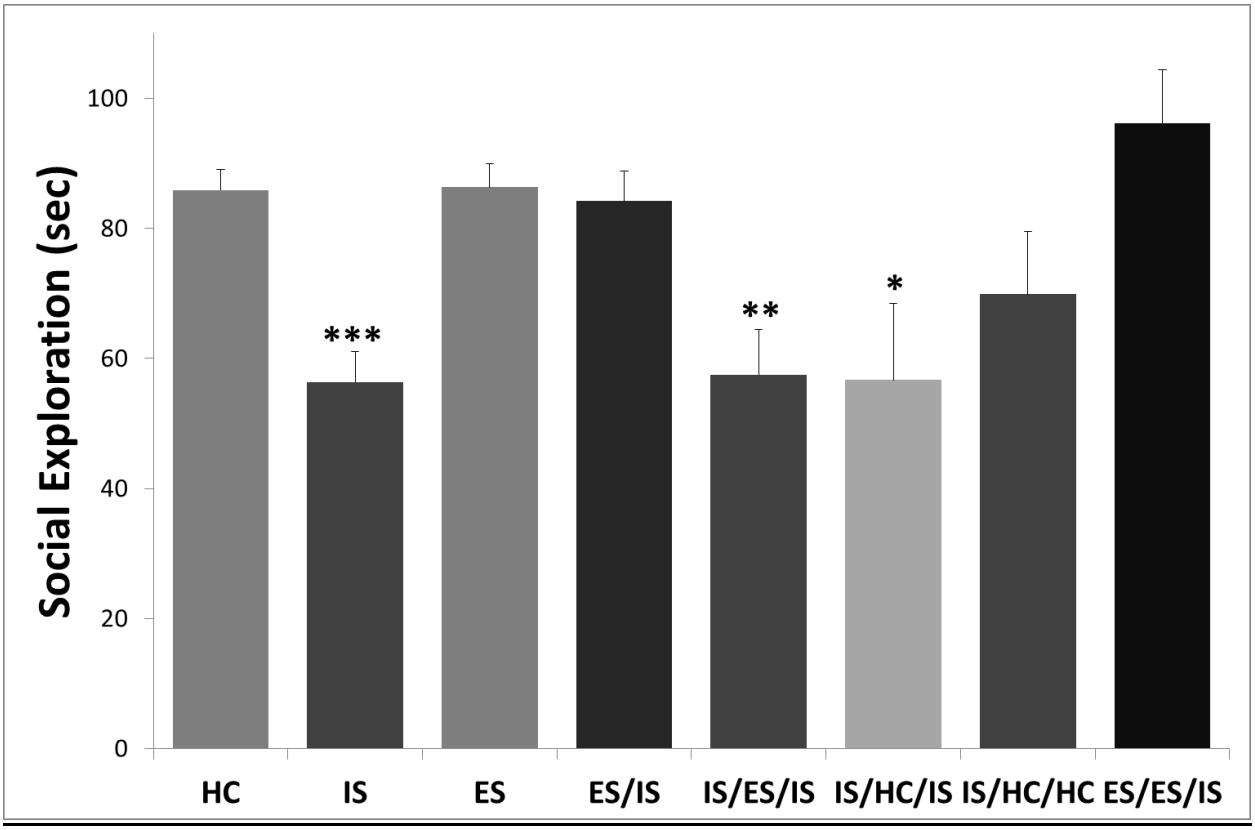

Figure 2: Effect on juvenile social exploration of a primary stress event followed by secondary and tertiary stress events 5 and 12 days later. Experimental groups include IS/ES/IS (n=10); IS/HC/IS (n=6); IS/HC/HC (n=4); and ES/ES/IS (n=4). Single treatment groups of HC (n=8), IS (n=8), ES (n=8) and a two treatment group of ES/IS (n=8) are included for reference. (*** p<0.001; **p <.01; * p< 0.05).

Rats received a primary stress exposure consisting of 100 IS or ES tail shocks. 5 days later, each rat received a second treatment of IS, ES, or HC. 7 days later, each rat received a third treatment of IS, ES, or HC. Social anxiety was assessed by juvenile social exploration 24 hours after the third treatment. Figure 2 shows the time spent in social exploration for each of the 4 experimental groups as well as 4 groups of one or two treatments for comparison. ANOVA yielded a significant effect of treatment ($F_{(7,48)}$=6.19, p<.01). Bonferroni multiple comparisons indicated significant reduction in social interaction time as compared to HC for IS (p<.001), IS/ES/IS (p<.01), and IS/HC/IS (p<.05) while all other groups exhibited no significant deviation from unstressed controls. Animals which received IS previously exhibit decreased levels of social interaction when given ES then IS 5 days later and 12 days later, respectively.

Freezing was recorded every 10 seconds over a 20 minute period 24 hours after the final stress exposure session. Figure 3 shows average number of 10 second intervals spent frozen over each 2 minute block for each experimental condition. Statistical analysis via ANOVA and Bonferroni multiple comparisons yielded no significant difference between treatment groups, however, this is likely due to the few animals in each treatment group and may yield statistically significant different time spent freezing upon an increase in group size.

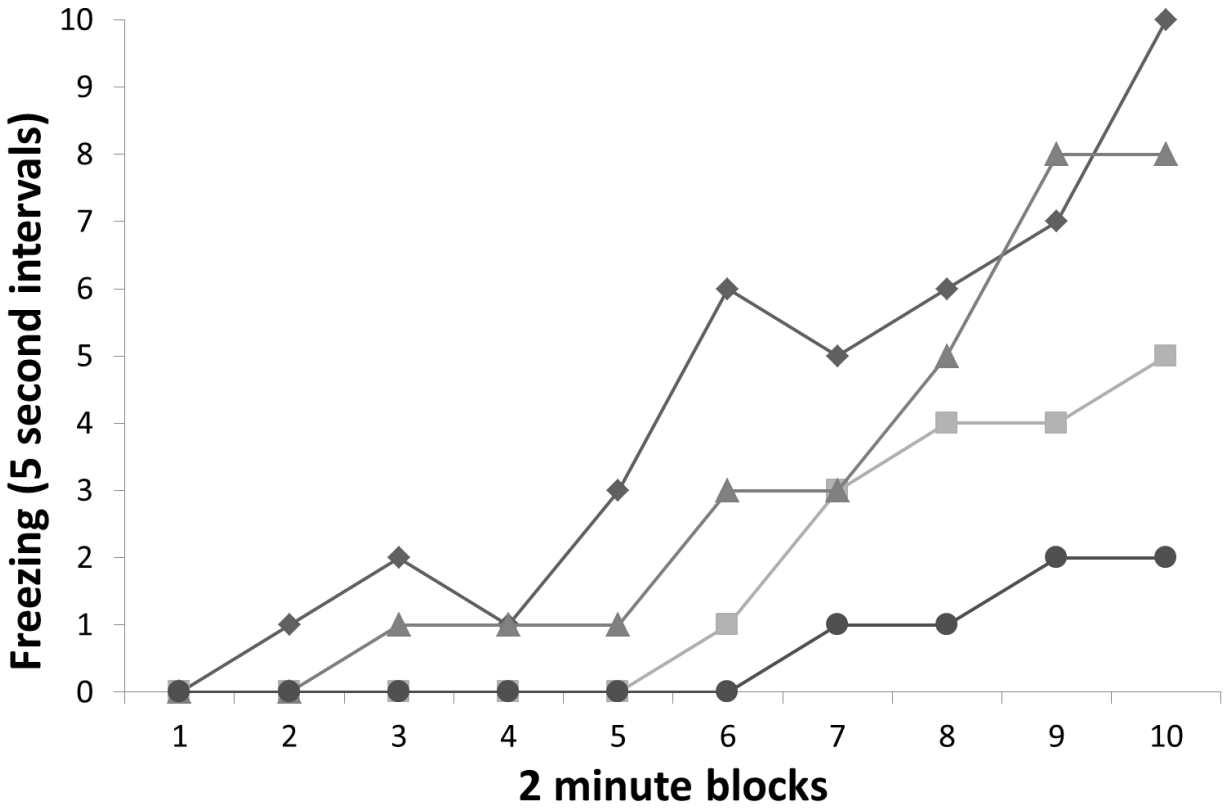

Figure 3: Mean number of 10 second trials spent freezing over each two minute period during fear conditioning after a primary stress event followed by secondary and tertiary stress events 5 and 12 days later, respectively. Experimental groups include ES/ES/IS (n=4, diamonds), IS/HC/HC (n=4, triangles), IS/ES/IS (n=4, squares), and IS/HC/IS (n=3, circles).

Discussion

Prior IS has no effect on the acute behavioral impact of ES administered 5 days later, indicating that the acute protection offered by control persists regardless of prior exposure to IS. Juvenile social exploration time of animals which receive either ES or HC 5 days after IS shows no significant deviation from that of animals which receive HC or ES alone (Figure 1). This implies that the protection from acute (short term) effects of stress that is provided by controllability is still present, even if the animal received IS previously, indicating that the circuitry involved in the acute protection offered by ES remains intact. Activation of the mPFC, and subsequent inhibition of DRN enhanced activation, which has been shown to attenuate the acute effects of stress in previous experiments (Amat et al., 2008) appears to be unaffected by previous experience with inescapable stress.

While the behavioral results of the present study suggest that the same circuitry involved in ES protection from the acute effects associated with stress remains intact when the animal is exposed to previous IS, this conclusion must be confirmed by repetition of previous experiments which confirmed the involvement of the PL in the acute effects of ES. Grahn et al. (1999) demonstrated that Fos expression in DRN 5HT neurons is selectively activated by IS, but not ES, and Baratta, et al. (2009) demonstrated that the PL-DRN pathway is preferentially activated by controllable stress. Taken together, these two studies suggest the role of the PL in attenuating DRN 5HT activation when exposed to controllable, as opposed to uncontrollable stress. These experiments should be repeated after initial IS exposure in order to confirm the neural circuitry of ES remains intact, and acute protection is not provided by some other mechanism, when exposed to prior IS.

Although the acute effects associated with behavioral control appear to be intact, the immunization that previous experience with controllability has on future IS appears to be blunted by an experience of IS 5 days before the ES experience. Previous experience with ES has been shown to blunt the reduction in JSI exploration time typically produced by IS (Williams and Maier, 1977). However, the protective effect of ES from the behavioral consequence of a future IS is blocked when IS is administered 5 days before ES. Animals which received IS/ES/IS showed significant reduction in JSI time ($p<.01$) 24 h after the second IS experience, similar to that of IS alone, while animals which received ES/IS with no prior IS show no reduction in JSI time (Figure 2). Importantly, the fact that the behavioral effect of IS/ES/IS and IS/HC/IS are the same, indicate that this ES impact is not different from no treatment at all (HC) between the two IS experiences. The results of fear conditioning as a behavioral comparison of each treatment group are inconclusive due to relatively small sample size, but additional subjects may provide insight into the behavioral outcomes of IS interference (Figure 3).

The neural mechanisms behind the immunization provided by escapability are thought to be due to plastic changes in the PL of the mPFC which occur in response to behavioral control, a circuitry which is then reactivated in response to subsequent stressors (Baratta, et al, 2009). While the current data suggest that IS may interfere with the ability of the PL of the mPFC to undergo the necessary plastic changes to provide immunization, no previous studies have investigated this phenomenon or the possible mechanisms by which it could prevent the experience of control from protecting against future adverse events. The mechanism by which IS interferes with later plastic changes necessary to induce immunization in the presence of

behavioral control must be better understood in order to provide possible targets for reestablishing controllability and immunization even after a prior experience with IS.

IS interference with later immunization provided by ES is likely a result of either the inability to detect/recall the presence of behavioral control, or of the ability of the circuitry potentiated by control to be reactivated by future stress. The detection of control has been shown to occur in the dorsal medial striatum (DMS), and if the DMS is inhibited, the animal still learns to turn the wheel, but the protection of control from the acute effects of stress are not seen (Amat et al., 2014). Since behavioral control in this case still provides protection from acute effects associated with IS, it is unlikely that detection of behavioral control is prevented by a prior IS experience. Conversely, the interference prior IS has on the immunization effect of ES could be due to failure to potentiate the PL of the mPFC and then reactivate this pathway in the absence of control. Neural plasticity in the PL and resulting activation of the mPFC during subsequent stressors, even in the absence of control has been attributed to an increase in pyramidal cell neuron excitability in the PL of the mPFC during the experience of control (Varela et al., 2012) and to activation of NMDAR and ERK signaling pathways in the mPFC (Christianson et al., 2014). These mechanisms are thought to be crucial in the induction of plasticity in the PL and corresponding immunization effect of ES. The inhibition of these pathways may provide potential explanation for the observed interference IS has on immunization while maintaining the acute protective effects of ES, and are therefore likely mechanisms for future understanding of IS interference, as well as potential targets for behavioral or pharmacological manipulations to restore or mimic the immunization offered by behavioral control.

The behavioral effects discussed here provide strong evidence that the acute protective effects of ES are maintained while its ability to immunize from future IS is blunted by a previous experience with IS. However, behavioral data alone without supporting neurochemical data are insufficient to determine interference an initial experience with IS might have on later ES. It is known that the decreased social interaction associated with IS is correlated with increased 5HT release in the BLA (Christianson et al., 2010). Throughout the duration of this experiment, in vivo microdialysis was performed in the BLA during both the second and third experimental treatments. However, due to equipment and resource limitations, these samples have not yet been analyzed via HPLC to determine 5HT levels in the BLA. In order to draw reliable conclusions about the effect an initial IS experience might have on subsequent ES, the 5HT levels in these samples must be analyzed in order to determine whether the neurochemical effect a previous IS experience has on the acute and immunization effects of ES corresponds with the effects seen behaviorally.

The ability of ES to immunize against the effects of future IS has been utilized extensively as an experimental method of protecting against later uncontrollable stressors (Williams and Maier, 1977), has been shown to persist throughout the lifetime of the animal (Kubala 2012), and appears to be trans-situational and applicable to contexts outside that of the original stressor (Amat et al., 2010). These findings have long been observed in human subjects, where perceived control plays an important role in coping and resilience (Folkman 1984). Those who feel they have control over life changes have been shown to be less likely to develop depression or anxiety disorders (Johnson and Sarason, 1978). Results from the present study implicate an interfering effect of prior trauma on the ability to benefit from later protection provided by

controllability, and therefore challenge the ability of control or self-efficacy to be protective or beneficial, especially in people with previous exposure to traumatic experiences.

Similar findings to these have already been observed in humans, and it has been suggested that previous trauma may decrease ability to cope with future stress, and severe childhood trauma predicts an increased likelihood of the development of anxiety disorders throughout the lifespan (Pynoos et al., 1999). Exposure to traumatic experiences during childhood has been associated with neurobiological changes which are correlated with increased likelihood of the development of depression and anxiety disorders (Heim and Nemeroff, 2001). For example, instances of abusive or neglectful childhood have been found to have lifelong implications on mental health (McEwen, 2003). In a study on predictors of developing posttraumatic symptoms after a terrorist attack, those who had previously survived a traumatic event, such as the holocaust, were found to be significantly more likely to develop aversive, posttraumatic effects of the event than those who had no previous recorded experience with trauma (Lamet et al., 2008). These data are consistent with the current study, and indicate that prior experience with a traumatic event may prevent the coping and resilience mechanisms that controllability can provide. This could explain why some disorders, such as social anxiety disorder, are often cyclical and lifelong, despite significant clinical intervention in which control is established (Keller 2003).

The potential for prior stress or trauma to interfere with the ability to cope and recover from future stressors carries important implications for clinical treatment of anxiety disorders. Many behavioral therapies for treating various anxiety disorders are based on establishing a sense of self-efficacy or control over the environment in which control is created in a clinical

environment, which then attenuates the anxiety and stress caused by the source of anxiety in its endogenous environment (Hoffman, 2008). However, if a previous trauma renders these therapies ineffective or only partially effective, it would have important implications on the efficacy of behavioral therapy via controllability on a large portion of the affected population. Pharmacological agents such as ketamine have been found to mimic the acute effects of controllability even when administered IS (Amat et al., 2016), and may provide a potential avenue to "rescue" from IS interference on later immunization by ES. The immunization effect of ketamine against future IS has not yet been studied, but may provide a potential clinical solution in the absence of protection via control.

It is interesting to note that ES and IS each interfere proactively with later response to stress, which raises the question of which one will become more prominent over a lifetime. People experience various phases of perceived controllability or lack of control over the course of their lives. Whether ES immunization from future IS or IS interference with immunization dominates perception of control and resistance/resilience to future stressors may be a result of simply order of occurrence, frequency of occurrence, occurrence at some crucial point in development, or determined by predisposition due to environmental and/or genetic factors. This development can only serve to convolute the already complex combination of factors which determine individual resistance/resilience to stress.

The behavioral data discussed here, taken together, indicate an interference effect of prior IS on the immunization effect, but not the acute effect, of later ES. These two effects are under control of different neural circuitry, and operate independently of one another. Further studies

must be performed to confirm that prior IS affects the neurochemical effect in the same way as the behavioral effects, and to understand the circuitry involved in IS interference on immunization by ES. IS interference on later immunization provided by escapability could have enormous implications on the understanding of stress and resilience, and on the ability of control in clinical behavioral intervention to reduce the symptoms of stress related disorders such as PTSD, panic disorder, phobias, OCD and depression in patients who have experienced previous trauma. This may necessitate the development of pharmacological agents which imitate the effect of controllability in order to increase resistance/resilience to stress or anxiety in patients with a history of trauma.

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