Published in final edited form as:

Physiol Behav. 2013 October 2; 0: . doi:10.1016/j.physbeh.2013.04.008.

Manganese-enhanced magnetic resonance imaging (MEMRI) reveals brain circuitry involved in responding to an acute novel stress in rats with a history of repeated social stress

Debra A. Bangasser^{1,2}, Catherine S. Lee¹, Philip A. Cook³, James C. Gee³, Seema Bhatnagar¹, and Rita J. Valentino¹

¹Department of Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, Philadelphia, PA 19104

²Department of Psychology and Neuroscience Program, Temple University, Philadelphia, PA 19122

³Department of Radiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

Abstract

Responses to acute stressors are determined in part by stress history. For example, a history of chronic stress results in facilitated responses to a novel stressor and this facilitation is considered to be adaptive. We previously demonstrated that repeated exposure of rats to the resident-intruder model of social stress results in the emergence of two subpopulations that are characterized by different coping responses to stress. The submissive subpopulation failed to show facilitation to a novel stressor and developed a passive strategy in the Porsolt forced swim test. Because a passive stress coping response has been implicated in the propensity to develop certain psychiatric disorders, understanding the unique circuitry engaged by exposure to a novel stressor in these subpopulations would advance our understanding of the etiology of stress-related pathology. An *ex vivo* functional imaging technique, manganese-enhanced magnetic resonance imaging (MEMRI), was used to identify and distinguish brain regions that are differentially activated by an acute swim stress (15 min) in rats with a history of social stress compared to controls. Specifically, Mn²⁺ was administered intracerebroventricularly prior to swim stress and brains were later imaged *ex vivo* to reveal activated structures. When compared to controls, all rats with a history of social stress showed greater activation in specific striatal, hippocampal, hypothalamic, and midbrain regions. The submissive subpopulation of rats was further distinguished by significantly greater activation in amygdala, bed nucleus of the stria terminalis, and septum, suggesting that these regions may form a circuit mediating responses to novel stress in individuals that adopt passive coping strategies. The finding that different circuits are engaged by a novel stressor in the two subpopulations of rats exposed to social stress implicates a role for these circuits in determining individual strategies for responding to stressors. Finally, these data underscore the utility of *ex vivo* MEMRI to identify and distinguish circuits engaged in behavioral responses.

© 2013 Elsevier Inc. All rights reserved

Corresponding author information: Debra Bangasser, PhD Temple University 1701 North 13th Street 873 Weiss Hall Philadelphia, PA 19122 Phone: 215-204-1015 debra.bangasser@temple.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

Stressors occur repeatedly throughout the lifespan. Fortunately, biological mechanisms are in place to help animals adapt to repeated or chronic stressor exposure. For example, endocrine and behavioral responses to the same stressor (homotypic stress) habituate with repeated exposures, a process that conserves energy resources and prevents chronic exposure to stress hormones [1–4]. In contrast, the presentation of a novel stressor (heterotypic stress) following repeated exposure to homotypic stress, results in facilitation of the hypothalamic pituitary adrenal (HPA) response to the stress [5–7]. This facilitation overcomes the negative feedback induced by repeated exposure to homotypic stressors, allowing the animal to readily respond to a new, potentially threatening, stimulus [7, 8]. Failure to appropriately respond to chronic stress can precipitate or exacerbate pathology, including depression (e.g., [9, 10]). Therefore, understanding the mechanisms involved in adaptive and maladaptive stress responses would further the development of interventions aimed at mitigating the deleterious consequences of repeated stressor exposure.

One determinant of the long term effects of chronic or repeated stress is the coping strategy in response to the stressor. This can be seen with the resident-intruder social stress model. This model involves repeatedly placing a male rodent (intruder) into the cage of an aggressive male rodent (resident) to initiate antagonistic encounters towards that intruder that induce stress responses [11–14]. Both mouse and rat versions of the task use individual differences in interactions between either the intruder with the resident or the intruder with a novel conspecific to categorize intruders as vulnerable versus resilient, categories which are supported by behavioral, pharmacological, and endocrine differences between the groups [12, 14, 15]. For example, our laboratory demonstrated that exposure of rats to the resident-intruder stress causes the emergence of two distinct subpopulations characterized by their propensity to exhibit subordinate (defeat) behavior [12]. Rats that readily adopted subordinate postures exhibiting a passive coping strategy had a phenotype that resembled melancholic depression including suppressed adrenocorticotropin (ACTH) levels in response to a novel stressor, increased immobility on the second day of the Porsolt forced swim test, and alterations in the CRF system [12]. In contrast, rats that were more resistant to defeat had a more typical facilitation of the ACTH response to a novel stressor and were less immobile on the second day of the forced swim test [12]. The engagement of distinct brain regions underlying the different coping strategies may play a role in the different endocrine and behavioral consequences in these subpopulations. Identifying these brain regions will advance our understanding of the neurobiological basis of individual differences in stress vulnerability.

One tool for identifying activated neuronal networks is manganese-enhanced magnetic resonance imaging (MEMRI) [16–18]. Unlike imaging based on blood oxygenation or blood flow, MEMRI is based on the ability of paramagnetic manganese ions (Mn^{2+}) to enter activated neurons through voltage-gated calcium channels [16, 19–21]. Once inside neurons, Mn^{2+} can bind to calcium binding proteins and it is also axonally transported, secreted at synapses and taken up by the next neurons [22–25]. Thus, Mn^{2+} can enter entire activated networks. MEMRI has been used for three types of applications: enhancing whole brain contrast, tract tracing, and demarcating brain circuits activated in response to stimuli (e.g., [17, 26–28]). MEMRI typically requires anesthesia and/or restraint during the imaging procedure, thereby precluding the use of this technique for *in vivo* imaging during active behaviors. However, because Mn^{2+} remains in circuits for a relatively long duration (several days), brain imaging can occur *ex vivo* after a behavioral challenge. Thus, brain circuits that are activated while animals are unanesthetized and behaving can be visualized by imaging the perfused brain after sacrifice. Here we use this technique to identify brain regions activated by an acute novel stress in rats with a history of repeated social stress versus

controls, and we further delineate regions activated uniquely in rats that display the more submissive phenotype.

2. Methods

2.1 Subjects

Male Sprague-Dawley rats (Charles River, Wilmington MA) weighing 250 to 275 g were used. Rats were individually housed in standard cages with *ad libitum* access to food and water and were maintained on a 12 h light/dark cycle (time on 7:00am). Care and use of animals was approved by the Children's Hospital of Philadelphia Institutional Animal Care and Use Committee and was in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2 Cannula Implantation Surgeries

Rats were anesthetized with a 2% isoflurane-in-air mixture administered through a nose cone and positioned in a stereotaxic instrument. Holes were drilled for the insertion of anchoring skull screws and a cannula guide aimed at the lateral ventricle (anteroposterior, -1.0 mm relative to bregma; mediolateral, +1.5 mm from the midline; dorsoventral, -5.0 mm from the surface of the skull) as previously described [29]. The guide cannula was secured to the skull and anchoring screws with dental cement, a stylet was placed into the guide cannula and the suture was closed. Rats were allowed at least a 72 h recovery period.

2.3 Social stress (resident-intruder) model

Following the recovery period, rats were randomly assigned to an experimental or control group. The social defeat paradigm used was modified from Miczek's original resident-intruder model (1979) and has been used previously in the laboratory [11, 12]. For 7 consecutive days, each intruder rat was placed in the cage of an unfamiliar Long-Evans resident, previously screened for high aggression, for a 30 min session. During a typical encounter, the resident's aggressive behavior caused the intruder to assume a subordinate supine posture signaling defeat. Once the defeat posture was displayed, a wire partition was placed in-between the resident and intruder to prevent further physical contact, while allowing for visual, auditory, and olfactory contact to be maintained for the remainder of the 30 min session. If an intruder resisted defeat for 15 min, the rats were separated with the wire partition for the remaining 15 min of the session. Following the session rats were returned to their home cages. The control group remained in the colony room but was placed in a cage different from their home cage without access to food and water for 30 min a day for 7 days to mimic the handling and food/water restriction associated with the social defeat task. The same cage was used throughout the 7 day control procedure.

Latency to assume the submissive defeat posture was recorded and averaged over the 7 defeat exposures. Previous studies demonstrated that intruder rats can be subdivided into two subpopulations based on their latency to display the defeat posture. Short latency (SL) animals displayed the defeat posture in less than < 500 s on average, while long latency (LL) animals display the defeat posture in greater than > 500 s on average. The same criteria were used in this study to link behavioral responses during the defeat to the activation of specific brain circuits.

2.4 Mn²⁺ administration and swim stress exposure

Twenty-four hours after the last social stress exposure or novel cage exposure for controls, all rats were infused intracerebroventricularly (i.c.v.) with Mn²⁺ solution (MnCl₂ 500 mM in artificial cerebrospinal fluid) prior to exposure to swim stress. During the microinfusion process, the stylet was replaced with an infusion cannula extending 1 mm beyond the tip of

the guide cannula (28 gauge; Plastics One). The infusion cannula attached to polyethylene tubing was connected to a 10 μ l Hamilton syringe, and a microinfuser pump infused the Mn^{2+} solution at a rate of 1 μ l/min for a total infusion volume of 3 μ l. The entire system was backloaded with distilled water, with a small air bubble separating the distilled water from the Mn^{2+} solution. After infusion, cannulas were left in place for 1 min to allow for proper diffusion. One hour after the Mn^{2+} infusion, all rats were exposed to the swim stress procedure where they were put into a cylindrical tank (46 cm high $\times$ 20 cm diameter) filled with room temperature water ($25^{\circ}C \pm 1^{\circ}C$) to a depth of 30 cm for a 15 min session. A subset of these control and socially stressed rats were videotaped to confirm behavior was not altered by the dose of manganese. The videotapes were later scored for immobility, swimming, and climbing behaviors by a rater blind to the experimental condition.

Pilot studies determined that Mn^{2+} enhancement was optimal in specific brain regions 24 h after administration. Therefore, 24 h after swim stress exposure, rats were transcardially perfused with saline 0.9%, followed by 4% paraformaldehyde in 0.1 M phosphate buffer (PB). After perfusion, brains were removed, post-fixed for at least 1 h, and then stored in 0.1 M phosphate buffer (PB) until scanning.

2.5 Image acquisition

All MRI studies were performed within 48 h of specimen harvesting on a 9.4 T vertical bore (Agilent, Palo Alto, CA) imaging system equipped with a 55 mm ID 100 gauss/cm gradient tube. Specimens were prepared for study by insertion into a custom made Delran holder containing perfluorinated tri-dodecylamine (an inert oil that is MRI invisible) in order to minimize magnetic susceptibility effects. After removal of all air bubbles, the specimen holder was sealed and mounted in the RF probe. The RF probe was a 20 mm ID resonator constructed in house specifically for the study of rodent brain specimens. Following RF calibration and acquisition of scout images, a 2D spin echo scan was performed with contiguous slices oriented in the axial dimension and spanning the entire length of the specimen (Field of View = 20×20 mm, slice thickness = 0.2 mm, slices = 90–110, matrix = 256×256 , TE = 20 msec, TR = 4 sec). Raw data was processed into images by two dimensional fourier transformation with no filtering. Voxel size in the coronal plane was $0.08\text{mm} \times 0.08\text{mm} \times 0.2\text{mm}$.

2.6 Image analysis

The images were aligned to a common space using Advanced Normalization Tools (ANTs) [30]. An unbiased average template image was constructed iteratively by aligning and averaging 10 control subject images. All images were then aligned independently to the template using the symmetric diffeomorphic deformable registration algorithm in ANTs. After spatial normalization, using ITK-SNAP, regions-of-interest (ROI) were segmented manually on the template image based on the atlas of Paxinos and Watson [31, 32]. Regions were chosen based on previous studies suggesting their involvement in stress, aggression, motor function, or other relevant behaviors. Image intensities were normalized by subtracting the average intensity of the spinal trigeminal track, a white matter region with relatively little Mn^{2+} uptake, from the average intensity within each ROI. To avoid any artifact caused by the cannula track, three structures, the septum, dorsal BNST, and dorsal hippocampus, were analyzed only on the side contralateral to the track.

2.7 Statistics

Mixed factors Analysis of Variance (ANOVA) compared behavior between the control group and social stressed group during the 15 min forced swim stress session (broken down into 5 min bins). An independent samples t-test (two-tailed) was used to compare activated regions in control rats to those that had been through the social stress procedure.

Additionally, stressed rats were subdivided based on latency to defeat into SL and LL groups. A one-way ANOVA was conducted to compare activated regions between control, LL, and SL groups. Values that exceeded 2 SDs above or below the group mean were considered outliers and dropped from these analyses. Due to multiple comparisons, a false discovery rate (FDR) correction [33] was applied and comparisons were considered significant if the t-test or ANOVA reached $p < 0.05$ with the FDR correction. Tukey's post-hoc tests were applied to significant (FDR corrected) ANOVAs.

3. Results

3.1 Behavior during social stress and acute swim stress

Fourteen rats with accurate cannula placements were exposed to social stress. Of these, four were classified as having a short latency (SL) to assume the defeat posture ($M=190$ s, $SD=88$ s). The remaining animals were classified as having a long latency (LL) to defeat ($M=555$ s, $SD=173$ s). The LL rats also showed more active coping behaviors, such as upright postures, during social stressor exposure.

Following exposure to social stress or the control condition all rats were exposed to a 15 min single session of forced swimming. Behavior during this session was analyzed for 6 control animals and 10 socially stressed animals (Fig. 1). The 15 min session was broken down into 5 min bins and over the session immobility increased [$F(2,28)=60.8$, $p < 0.05$], while swimming and climbing decreased ([$F(2,28)=4.1$, $p < 0.05$], [$F(2,28)=40.0$, $p < 0.05$], respectively). There were no differences between the control and socially stressed groups on any of the behavioral measures ([$F(1,14)=0.12$, $p > 0.05$], [$F(1,14)=0.43$, $p > 0.05$], [$F(1,14)=0.03$, $p > 0.05$] immobility, swim, and climb, respectively), as previously reported [12]. Additionally, there were no group by time interactions ([$F(2,28)=0.70$, $p > 0.05$], [$F(2,28)=0.05$, $p > 0.05$] [$F(2,28)=0.56$, $p > 0.05$], immobility, swim, and climb, respectively).

3.2 Enhanced Mn^{2+} signal in rats with a history of social stress compared to controls

Figure 2 illustrates typical signal enhancement in selected coronal sections (200 μ m anterior to posterior) following swim stress exposure from a control rat compared to a rat with a history of social stress. Signal intensities were analyzed after removing subjects that met the criteria for outliers. In the locus coeruleus (LC) region 2 control animals met the outlier criteria and were removed, however in all other regions no more than 1 control and 1 socially stressed animal were designated outliers and removed. Differences in signal intensity between control and stressed rats were found in a number of brain regions (Fig. 3). Specifically, for the socially stressed group the signal intensity was greater than controls in the prefrontal cortex [$t(18)=3.0$, $p < 0.05$], but not other cortical areas, such as the motor [$t(18)=1.74$, $p > 0.05$] or piriform cortex [$t(19)=0.99$, $p > 0.05$]. Swim stress induced a greater Mn^{2+} signal in the nucleus accumbens [$t(18)=3.7$, $p < 0.05$] of rats with a history of social stress versus controls. Several limbic regions were activated to a greater degree in socially stressed rats compared to controls, including the septum [$t(17)=2.5$, $p < 0.05$], amygdala [$t(17)=2.6$, $p < 0.05$], ventral hippocampus [$t(17)=3.8$, $p < 0.05$], fimbria [$t(18)=2.7$, $p < 0.05$], and ventral bed nucleus of the stria terminalis (vBNST) [$t(18)=3.2$, $p < 0.05$]. Additionally, there was a trend for increased Mn^{2+} signal in the dorsal BNST (dBNST) [$t(17)=2.3$, $p = 0.056$]. However, the signal was comparable in many other limbic structures, such as the diagonal band [$t(19)=1.14$, $p > 0.05$], dorsal hippocampus [$t(18)=2.02$, $p > 0.05$] and dentate gyrus within the dorsal hippocampus [$t(18)=1.76$, $p > 0.05$]. Several structures in the diencephalon were also differentially activated in the social stress group vs. the control group. The habenula [$t(19)=3.5$, $p < 0.05$], lateral hypothalamus [$t(17)=4.2$, $p < 0.05$] and medial hypothalamus [$t(18)=4.7$, $p < 0.05$] exhibited a greater Mn^{2+} signal in the rats with a history of social stress. However, the paraventricular nuclei of the hypothalamus

[$t(19)=1.18$, $p > 0.05$], and the paraventricular nucleus of the of thalamus [$t(19)=2.08$, $p > 0.05$], had comparable activation in both groups. In the midbrain, there was no difference in signal intensity in the mammillary bodies [$t(19)=0.03$, $p > 0.05$]. However, Mn^{2+} signal in the dorsal raphe and in all three subdivision of the periaqueductal gray was greater in stressed rats compared to controls (dorsal raphe: $t(18)=2.7$, $p < 0.05$; dorsal PAG: $t(19)=2.8$, $p < 0.05$, lateral PAG: $t(18)=3.4$, $p < 0.05$, ventral PAG: $t(17)=3.5$, $p < 0.05$). Finally, there was no difference in signal intensity between groups in the LC [$t(17)=0.08$, $p > 0.05$] or nucleus of the solitary tract [$t(19)=0.96$, $p > 0.05$].

3.2 Enhanced Mn^{2+} signal in SL rats versus LL and controls

Figure 4 illustrates structures that distinguished brain responses to swim stress in the SL group when compared to the LL and control groups. The SL group had a greater Mn^{2+} signal compared to the control group in the amygdala ($[F(2,17)=4.3$, $p < 0.05$], post-hoc $p < 0.05$) and dBNST ($[F(2,17)=6.2$, $p < 0.05$], post-hoc $p < 0.05$), whereas there were no significant signal differences between LL and controls for these regions (post-hoc tests $p > 0.05$) (Fig. 5). For the vBNST ($[F(2,17)=10.9$, $p < 0.05$] both SL and LL groups had greater activation than controls ($p < 0.05$), but the vBNST of SL group further activated relative to the LL group ($p < 0.05$). Additionally, the Mn^{2+} signal in the septum was greater in SL animals compared to all other groups ($[F(2,16)=7.9$, $p < 0.05$], post-hoc tests $p < 0.05$) (Fig. 5).

4. Discussion

The present study demonstrates that acute exposure to a heterotypic stressor engages different brain circuits in animals with a history of social stress compared to unstressed rats. Furthermore, the circuitry engaged by swim stress is in part related to the subject's coping strategy in response during the social stress. In this regard, the BNST, amygdala and septum are three interconnected and functionally-related regions that were selectively activated by swim stress exposure in rats that take a more subordinate strategy during the social stress procedure. Previous studies have demonstrated that the rats that adopt a passive coping strategy during the defeat have a neuroendocrine and behavioral characteristics that resembled melancholic depression, thus plasticity within an amygdala-BNST-septal circuit may underlie the development of depressive-like phenotype [12].

4.1 Using MEMRI for functional brain mapping

Unlike traditional MRI that is based on cerebral hemodynamics, MEMRI is based on the opening of calcium channels and axonal transport of Mn^{2+} , a more direct indicator of neuronal activity, which allows for better spatial resolution [34]. Additionally, because Mn^{2+} is taken up through voltage-gated calcium channels that regulate the release of neurotransmitters and influence synaptic strength, MEMRI maps regions undergoing calcium-related neuronal plasticity [19–21]. Other calcium imaging techniques have been developed that combine calcium indicators with optical imaging (e.g., two photon microscopy) (for review see [35, 36]). Although these tools have better spatial (micrometer) and temporal resolution (millisecond) than MEMRI, they are limited to visualizing calcium in the most superficial structures of the brain (within a millimeter below the surface) [35, 36]. Thus, an advantage of MEMRI is that it can gauge calcium-related plasticity in circuits activated throughout the brain. Another common technique involves performing immunohistochemistry on brain slices to detect the expression of immediate early genes (e.g., cFOS) that become activated following behavioral manipulations [37]. However, drawbacks to this approach are that it does not delineate projections, analysis is limited to two dimensions, and it only reveals neuronal plasticity that requires gene transcription.

Taken together, there are clear advantages to using MEMRI to map brain regions functionally activated by behavioral manipulations.

One challenge of MEMRI that may limit its widespread use as a functional brain mapping tool is that Mn^{2+} does not readily cross the blood brain barrier (BBB). Thus, it is difficult to find a dose of Mn^{2+} that is high enough to cross the BBB yet not so high that it damages the liver, heart, or the brain, inducing Parkinsonian-like symptoms [38, 39]. One approach to avoid toxicity is to give several low doses peripherally [40]. Although this technique works well for studies using Mn^{2+} simply as a contrast agent, studies using Mn^{2+} for functional imaging require a temporally restricted dosing regimen. Another approach has been to deliver Mn^{2+} intravenously in conjunction with BBB disruptor mannitol [41]. Here we took a different approach and directly administered Mn^{2+} into the lateral ventricle. Similarly, a recent paper successfully infused Mn^{2+} directly into the cisterna magna to image the brain [42]. The intracerebroventricular infusion ensured the Mn^{2+} entered the brain at a specific time and was available to be taken up via any voltage-gated calcium channels activated during swim stress. Mn^{2+} infused rats showed the typical behavioral pattern during the 15 min swim session where active responses (swimming and climbing) decrease, while immobility increases over the session [43]. Thus, the Mn^{2+} dose used here was sufficient to distinguish circuitry, but not so high as to impair behavior. One disadvantage to this approach was that the cannula track can create an artifact during the scanning. Thus, regions near the cannula track, including the septum, dorsal BNST, and dorsal hippocampus, were analyzed only on the side contralateral to the cannula track to avoid the artifact. Despite this caveat, this study demonstrated that central administration was an effective route for administering Mn^{2+} in a time specific manner to image circuits in a behaving animal, while avoiding peripheral toxicity.

4.2 Activated circuitry in rats with a history of social stress versus controls

Despite the fact that all of the animals in this study were exposed to swim stress, the stressor engaged a unique set of brain regions in rats with a history of social stress compared to control animals. For example, rats with a history of social stress had greater activation than controls of certain cortical, striatal, and limbic structures, including the prefrontal cortex, nucleus accumbens, ventral hippocampus, vBNST, septum, and amygdala, although the latter three regions were further differentiated by behavioral differences in the response to social stress (see below). Previous studies demonstrated that chronic stress induces structural changes (e.g., alteration in dendritic morphology, spine density, etc.) in the prefrontal cortex, nucleus accumbens, and limbic regions [44–48]. Thus, the increased MEMRI signal observed in socially stressed rats may reflect calcium-induced structural plasticity in these areas. Moreover, all of these areas are thought to comprise the circuitry critical for depression, which is exacerbated by chronic stress [49–53]. We propose that these structures act in concert to mediate responses to novel stress exposure in rats with a history of social stress. This idea is based on the fact that these regions are interconnected. For example, the prefrontal cortex projects to the amygdala and nucleus accumbens [54–56]. The nucleus accumbens also receives inputs from the hippocampus and amygdala [54, 57, 58]. Additionally, the ventral hippocampus and vBNST are highly connected with the amygdala, and together these regions modulate anxiety-related behaviors [59–62]. Notably, the dorsal hippocampus, which preferentially modulates certain types of learning (e.g., spatial), was not differentially activated in the socially stressed group, supporting recent reports of a regional distinction in hippocampal function [59, 60, 63]. The idea that these regions form a circuit that critically mediates behavior in rats exposed to repeated stress is further supported by the fact the fimbria, which connects the hippocampus and amygdala, showed an enhanced Mn^{2+} signal only in the socially stressed group. In Syrian hamsters the acquisition of fear during conditioned defeat requires the prefrontal cortex, amygdala,

BNST, and ventral hippocampus, while the expression of fear also engages the nucleus accumbens [64–67]. This circuit may be similarly engaged in repeatedly stressed rats to facilitate the acquisition and recall of critical aspects of the social interaction so that this information can be used to direct coping behavior during the novel stressor exposure.

Socially stressed rats had greater activation of the lateral hypothalamus than their counterparts. The lateral hypothalamus contains orexin neurons that have been implicated in arousal, stress, anxiety, and depression [68–72]. Evidence suggests a role for orexin projections to the paraventricular nucleus of the thalamus in the facilitation of HPA responses to novel stressors [72]. Surprisingly, however, this nucleus and the paraventricular nucleus of the hypothalamus, which have increased cFOS expression following HPA facilitation to a novel stressor, had Mn^{2+} signals that were comparable between socially stress rats and controls [7]. This may suggest that exposure to a novel stressor induces calcium-related plasticity in these regions regardless of the animal's stress history, but enduring effects such as changes in gene transcription occur only in rats with a history of chronic social stress.

The medial hypothalamus and PAG form part of a circuit that mediates defensive responses [73–75]. Previously, it has been demonstrated that the resident-intruder stress increases cFOS expression in the PAG [76, 77]. The present results extend these findings by revealing greater activation the medial hypothalamus in addition to the PAG in response to swim stress, in rats with a history of social stress compared to controls. This suggests that rats with a history of antagonistic social interactions continue to engage circuits that mediate defensive behaviors during exposure to novel stressors.

It is unlikely that regional differences in Mn^{2+} signal intensity between stress and control groups result from differences in baseline neuronal activity rather than activity elicited by the acute swim stress. Previous studies have shown that by 7 days after social stress or control manipulation baseline corticosterone levels are comparable between groups yet the response to an acute novel stressor can be distinguished [12, 78].

4.3 Brain areas differentiated by coping strategy in response to social stress

An advantage of the resident-intruder model is that coping strategy in response to the social stressor predicts the consequences of future stressful experiences. For example, previously we demonstrated that in LL rats the ACTH response to the novel stress of restraint was facilitated, whereas in SL rats this response was suppressed [12]. Because HPA facilitation is considered an adaptive response to stress, SL animals that lack HPA facilitation may be more vulnerable to stressful events. This idea is supported by data from the Porsolt forced swim test. Although behavior on the first day (15 min swim session) was similar between all groups, SL animals selectively displayed pro-depressive behaviors on the second day [12]. In the present study, we were interested identifying brain regions that were differentially activated in the SL versus LL groups to reveal circuitry that may contribute to pro-depressive behavior. The amygdala, BNST, and septum were distinguished as being uniquely activated in SL rats. One caveat of these results, however, relates to the relatively low number of rats that exhibited a SL phenotype. Usually the exposure of intruder male rats to the aggressive resident gives rise to a relatively equal distribution of the two subpopulations (SL vs. LL)[12]. However, in our experience with rats that have intracranial implantations there is a strong bias towards the LL phenotype. Although statistically significant differences were seen between the subpopulations in the Mn^{2+} signal in the amygdala, BNST, and septum, suggesting these regions form a circuit that mediates depressive-like behavior, we interpret these results with caution.

The amygdala, BNST, and septum are all implicated in mediating submissive or subordinate social behavior. For example, subordinate rats in a visible burrow system have greater expression of the stress neuropeptide corticotropin releasing factor in the BNST than dominant rats [79]. In the amygdala, dendritic remodeling occurs in rats sensitized by social defeat but not in their resilient counterparts [80]. Additionally, submissive behavior during conditioned social defeat in Syrian hamsters requires NMDA receptor activation in the amygdala [81]. Submissive behaviors in this task also require the septum, and social stress increases cFOS in the septum of subordinate but not dominant hamsters [82, 83].

The aforementioned data suggest these regions can act independently to mediate submissive behavior. However, structurally there is evidence to support an amygdala-BNST-septal circuit. The central and medial nuclei of the amygdala and the BNST are highly interconnected and share a similar cytoarchitecture, which has led to the proposal that these structures should be classified together as the extended amygdala [84]. The extended amygdala sends projections to the lateral septum [85, 86]. These regions also are major components of the vasopressin circuit that mediates male aggressive and territorial behaviors [87–89]. High vasopressin levels are often associated with low aggression characterized by submissive or passive behaviors [88, 90, 91]. Therefore, high Mn^{2+} enhancement in the amygdala-BNST-septal circuit in the SL group could reflect an increase in vasopressin that mediates passive behaviors during stressor exposure.

5. Conclusions

The present study used MEMRI to distinguish brain regions engaged by acute swim stress in rats with a history of social stress compared to controls. This approach identified cortical-striatal-limbic, habenula-raphé, and hypothalamic-PAG circuits that were uniquely activated in rats with a history of chronic social stress compared to unstressed controls. Rats that adopted a more subordinate strategy to the social stress had further enhancement of the amygdala, BNST, and septum, implicating this specific circuit in mediating passive coping response to stress. Importantly, these data highlight the utility of using central administration of Mn^{2+} combined with *ex vivo* imaging to identify circuits engaged during behavioral tasks. We believe this approach can be utilized in future studies that employ animal models to investigate a range of behaviors associated with psychiatric disorders.

Acknowledgments

We would like to thank Steve Pickup and Weixia Liu for their technical support with the scanning and Sandra Luz and Elizabeth Ver Hoeve for their assistance with the behavior. This work was supported by DARPA 58077LSDRP, MH092438, and MH093981.

References

- [1]. Jaferi A, Nowak N, Bhatnagar S. Negative feedback functions in chronically stressed rats: role of the posterior paraventricular thalamus. *Physiol Behav.* 2003; 78:365–73. [PubMed: 12676271]
- [2]. Bhatnagar S, Huber R, Nowak N, Trotter P. Lesions of the posterior paraventricular thalamus block habituation of hypothalamic-pituitary-adrenal responses to repeated restraint. *Journal of neuroendocrinology.* 2002; 14:403–10. [PubMed: 12000546]
- [3]. McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med.* 1998; 338:171–9. [PubMed: 9428819]
- [4]. Grissom N, Bhatnagar, S. Habituation to repeated stress: get used to it. *Neurobiol Learn Mem.* 2009; 92:215–24. [PubMed: 18667167]
- [5]. Bhatnagar S, Vining C. Facilitation of hypothalamic-pituitary-adrenal responses to novel stress following repeated social stress using the resident/intruder paradigm. *Horm Behav.* 2003; 43:158–65. [PubMed: 12614646]

- [6]. Dallman MF, Akana SF, Scribner KA, Bradbury MJ, Walker CD, Strack AM, et al. Stress, feedback and facilitation in the hypothalamo-pituitary-adrenal axis. *Journal of neuroendocrinology*. 1992; 4:517–26. [PubMed: 21554636]
- [7]. Bhatnagar S, Dallman M. Neuroanatomical basis for facilitation of hypothalamic-pituitary-adrenal responses to a novel stressor after chronic stress. *Neuroscience*. 1998; 84:1025–39. [PubMed: 9578393]
- [8]. Grissom N, Iyer V, Vining C, Bhatnagar S. The physical context of previous stress exposure modifies hypothalamic-pituitary-adrenal responses to a subsequent homotypic stress. *Horm Behav*. 2007; 51:95–103. [PubMed: 17054953]
- [9]. Tamashiro KL, Sakai RR, Shively CA, Karatsoreos IN, Reagan LP. Chronic stress, metabolism, and metabolic syndrome. *Stress*. 2011; 14:468–74. [PubMed: 21848434]
- [10]. Nemeroff CB, Vale WW. The neurobiology of depression: inroads to treatment and new drug discovery. *J Clin Psychiatry*. 2005; 66(Suppl 7):5–13. [PubMed: 16124836]
- [11]. Bhatnagar S, Vining C, Iyer V, Kinni V. Changes in hypothalamic-pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats. *Journal of neuroendocrinology*. 2006; 18:13–24. [PubMed: 16451216]
- [12]. Wood SK, Walker HE, Valentino RJ, Bhatnagar S. Individual differences in reactivity to social stress predict susceptibility and resilience to a depressive phenotype: role of corticotropin-releasing factor. *Endocrinology*. 2010; 151:1795–805. [PubMed: 20160137]
- [13]. Miczek KA. A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. *Psychopharmacology*. 1979; 60:253–9. [PubMed: 108702]
- [14]. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*. 2007; 131:391–404. [PubMed: 17956738]
- [15]. Cao JL, Covington HE 3rd, Friedman AK, Wilkinson MB, Walsh JJ, Cooper DC, et al. Mesolimbic dopamine neurons in the brain reward circuit mediate susceptibility to social defeat and antidepressant action. *J Neurosci*. 2010; 30:16453–8. [PubMed: 21147984]
- [16]. Watanabe T, Frahm J, Michaelis T. Functional mapping of neural pathways in rodent brain in vivo using manganese-enhanced three-dimensional magnetic resonance imaging. *NMR Biomed*. 2004; 17:554–68. [PubMed: 15617054]
- [17]. Pautler RG. In vivo, trans-synaptic tract-tracing utilizing manganese-enhanced magnetic resonance imaging (MEMRI). *NMR Biomed*. 2004; 17:595–601. [PubMed: 15761948]
- [18]. Silva AC, Bock NA. Manganese-enhanced MRI: an exceptional tool in translational neuroimaging. *Schizophr Bull*. 2008; 34:595–604. [PubMed: 18550591]
- [19]. Catterall WA, Few AP. Calcium channel regulation and presynaptic plasticity. *Neuron*. 2008; 59:882–901. [PubMed: 18817729]
- [20]. Narita K, Kawasaki F, Kita H. Mn and Mg influxes through Ca channels of motor nerve terminals are prevented by verapamil in frogs. *Brain research*. 1990; 510:289–95. [PubMed: 2158851]
- [21]. Hu TC, Pautler RG, MacGowan GA, Koretsky AP. Manganese-enhanced MRI of mouse heart during changes in inotropy. *Magn Reson Med*. 2001; 46:884–90. [PubMed: 11675639]
- [22]. Slood WN, Gramsbergen JB. Axonal transport of manganese and its relevance to selective neurotoxicity in the rat basal ganglia. *Brain research*. 1994; 657:124–32. [PubMed: 7820609]
- [23]. Takeda A, Kodama Y, Ishiwatari S, Okada S. Manganese transport in the neural circuit of rat CNS. *Brain Res Bull*. 1998; 45:149–52. [PubMed: 9443831]
- [24]. Sophianopoulos AJ, Sophianopoulos JA. Effect of the conformation of concanavalin A on its affinity for manganous ion. *Arch Biochem Biophys*. 1986; 246:572–80. [PubMed: 3754709]
- [25]. Senguen FT, Grabarek Z. X-ray structures of magnesium and manganese complexes with the N-terminal domain of calmodulin: insights into the mechanism and specificity of metal ion binding to an EF-hand. *Biochemistry*. 2012; 51:6182–94. [PubMed: 22803592]
- [26]. Watanabe T, Natt O, Boretius S, Frahm J, Michaelis T. In vivo 3D MRI staining of mouse brain after subcutaneous application of MnCl₂. *Magn Reson Med*. 2002; 48:852–9. [PubMed: 12418000]

- [27]. Silva AC, Lee JH, Wu CW, Tucciarone J, Pelled G, Aoki I, et al. Detection of cortical laminar architecture using manganese-enhanced MRI. *Journal of neuroscience methods*. 2008; 167:246–57. [PubMed: 17936913]
- [28]. Yu X, Wadghiri YZ, Sanes DH, Turnbull DH. In vivo auditory brain mapping in mice with Mn-enhanced MRI. *Nat Neurosci*. 2005; 8:961–8. [PubMed: 15924136]
- [29]. Snyder K, Wang WW, Han R, McFadden K, Valentino RJ. Corticotropin-Releasing Factor in the Norepinephrine Nucleus, Locus Coeruleus, Facilitates Behavioral Flexibility. *Neuropsychopharmacology*. 2011
- [30]. Avants BB, Epstein CL, Grossman M, Gee JC. Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. *Med Image Anal*. 2008; 12:26–41. [PubMed: 17659998]
- [31]. Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, et al. User-guided 3D active contour segmentation of anatomical structures: significantly improved efficiency and reliability. *Neuroimage*. 2006; 31:1116–28. [PubMed: 16545965]
- [32]. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. 2nd ed.. Academic Press; North Ryde: 1998.
- [33]. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society*. 1995; 57:289–300. Series B (Methodological)
- [34]. Duong TQ, Silva AC, Lee SP, Kim SG. Functional MRI of calcium-dependent synaptic activity: cross correlation with CBF and BOLD measurements. *Magn Reson Med*. 2000; 43:383–92. [PubMed: 10725881]
- [35]. Garaschuk O, Griesbeck O, Konnerth A. Troponin C-based biosensors: a new family of genetically encoded indicators for in vivo calcium imaging in the nervous system. *Cell Calcium*. 2007; 42:351–61. [PubMed: 17451806]
- [36]. Russell JT. Imaging calcium signals in vivo: a powerful tool in physiology and pharmacology. *Br J Pharmacol*. 2011; 163:1605–25. [PubMed: 20718728]
- [37]. Hoffman GE, Smith MS, Verbalis JG. c-Fos and related immediate early gene products as markers of activity in neuroendocrine systems. *Front Neuroendocrinol*. 1993; 14:173–213. [PubMed: 8349003]
- [38]. Dobson AW, Erikson KM, Aschner M. Manganese neurotoxicity. *Ann N Y Acad Sci*. 2004; 1012:115–28. [PubMed: 15105259]
- [39]. Wolf GL, Baum L. Cardiovascular toxicity and tissue proton T1 response to manganese injection in the dog and rabbit. *AJR Am J Roentgenol*. 1983; 141:193–7. [PubMed: 6305179]
- [40]. Bock NA, Paiva FF, Silva AC. Fractionated manganese-enhanced MRI. *NMR Biomed*. 2008; 21:473–8. [PubMed: 17944008]
- [41]. Lu H, Xi ZX, Gitajn L, Rea W, Yang Y, Stein EA. Cocaine-induced brain activation detected by dynamic manganese-enhanced magnetic resonance imaging (MEMRI). *Proc Natl Acad Sci U S A*. 2007; 104:2489–94. [PubMed: 17287361]
- [42]. Jeong KY, Lee C, Cho JH, Kang JH, Na HS. New method of manganese-enhanced Magnetic Resonance Imaging (MEMRI) for rat brain research. *Exp Anim*. 2012; 61:157–64. [PubMed: 22531731]
- [43]. Xu GP, Van Bockstaele E, Reyes B, Bethea T, Valentino RJ. Chronic morphine sensitizes the brain norepinephrine system to corticotropin-releasing factor and stress. *J Neurosci*. 2004; 24:8193–7. [PubMed: 15385601]
- [44]. Magarinos AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. *Neuroscience*. 1995; 69:83–8. [PubMed: 8637635]
- [45]. Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neurosci*. 2002; 22:6810–8. [PubMed: 12151561]
- [46]. Wang YC, Ho UC, Ko MC, Liao CC, Lee LJ. Differential neuronal changes in medial prefrontal cortex, basolateral amygdala and nucleus accumbens after postweaning social isolation. *Brain Struct Funct*. 2012; 217:337–51. [PubMed: 22002740]

- [47]. Christoffel DJ, Golden SA, Dumitriu D, Robison AJ, Janssen WG, Ahn HF, et al. IkappaB kinase regulates social defeat stress-induced synaptic and behavioral plasticity. *J Neurosci.* 2011; 31:314–21. [PubMed: 21209217]
- [48]. Radley JJ, Rocher AB, Rodriguez A, Ehlenberger DB, Dammann M, McEwen BS, et al. Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex. *J Comp Neurol.* 2008; 507:1141–50. [PubMed: 18157834]
- [49]. Bewernick BH, Hurlemann R, Matusch A, Kayser S, Grubert C, Hadrysiewicz B, et al. Nucleus accumbens deep brain stimulation decreases ratings of depression and anxiety in treatment-resistant depression. *Biological psychiatry.* 2010; 67:110–6. [PubMed: 19914605]
- [50]. Koolschijn PC, van Haren NE, Lensvelt-Mulders GJ, Hulshoff Pol HE, Kahn RS. Brain volume abnormalities in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. *Hum Brain Mapp.* 2009; 30:3719–35. [PubMed: 19441021]
- [51]. Bellani M, Baiano M, Brambilla P. Brain anatomy of major depression II. Focus on amygdala. *Epidemiol Psychiatr Sci.* 2011; 20:33–6. [PubMed: 21657113]
- [52]. Bellani M, Baiano M, Brambilla P. Brain anatomy of major depression I. Focus on hippocampus. *Epidemiol Psychiatr Soc.* 2010; 19:298–301. [PubMed: 21322503]
- [53]. Price JL, Drevets WC. Neural circuits underlying the pathophysiology of mood disorders. *Trends Cogn Sci.* 2012; 16:61–71. [PubMed: 22197477]
- [54]. McDonald AJ. Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. *Neuroscience.* 1991; 44:1–14. [PubMed: 1722886]
- [55]. Ding DC, Gabbott PL, Totterdell S. Differences in the laminar origin of projections from the medial prefrontal cortex to the nucleus accumbens shell and core regions in the rat. *Brain research.* 2001; 917:81–9. [PubMed: 11602231]
- [56]. Cassell MD, Wright DJ. Topography of projections from the medial prefrontal cortex to the amygdala in the rat. *Brain Res Bull.* 1986; 17:321–33. [PubMed: 2429740]
- [57]. Mulder AB, Hodenpjl MG, Lopes da Silva FH. Electrophysiology of the hippocampal and amygdaloid projections to the nucleus accumbens of the rat: convergence, segregation, and interaction of inputs. *J Neurosci.* 1998; 18:5095–102. [PubMed: 9634575]
- [58]. Wright CI, Beijer AV, Groenewegen HJ. Basal amygdaloid complex afferents to the rat nucleus accumbens are compartmentally organized. *J Neurosci.* 1996; 16:1877–93. [PubMed: 8774456]
- [59]. Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, et al. Regional dissociations within the hippocampus—memory and anxiety. *Neuroscience and biobehavioral reviews.* 2004; 28:273–83. [PubMed: 15225971]
- [60]. Trivedi MA, Coover GD. Lesions of the ventral hippocampus, but not the dorsal hippocampus, impair conditioned fear expression and inhibitory avoidance on the elevated T-maze. *Neurobiol Learn Mem.* 2004; 81:172–84. [PubMed: 15082019]
- [61]. Dong HW, Petrovich GD, Swanson LW. Topography of projections from amygdala to bed nuclei of the stria terminalis. *Brain Res Brain Res Rev.* 2001; 38:192–246. [PubMed: 11750933]
- [62]. Walker DL, Toufexis DJ, Davis M. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *Eur J Pharmacol.* 2003; 463:199–216. [PubMed: 12600711]
- [63]. Bannerman DM, Grubb M, Deacon RM, Yee BK, Feldon J, Rawlins JN. Ventral hippocampal lesions affect anxiety but not spatial learning. *Behavioural brain research.* 2003; 139:197–213. [PubMed: 12642189]
- [64]. Luckett C, Norvelle A, Huhman K. The role of the nucleus accumbens in the acquisition and expression of conditioned defeat. *Behavioural brain research.* 2012; 227:208–14. [PubMed: 22024431]
- [65]. Markham CM, Taylor SL, Huhman KL. Role of amygdala and hippocampus in the neural circuit subserving conditioned defeat in Syrian hamsters. *Learn Mem.* 2010; 17:109–16. [PubMed: 20154357]
- [66]. Markham CM, Norvelle A, Huhman KL. Role of the bed nucleus of the stria terminalis in the acquisition and expression of conditioned defeat in Syrian hamsters. *Behavioural brain research.* 2009; 198:69–73. [PubMed: 19000716]

- [67]. Markham CM, Luckett CA, Huhman KL. The medial prefrontal cortex is both necessary and sufficient for the acquisition of conditioned defeat. *Neuropharmacology*. 2012; 62:933–9. [PubMed: 22001285]
- [68]. Shahid IZ, Rahman AA, Pilowsky PM. Orexin A in rat rostral ventrolateral medulla is pressor, sympatho-excitatory, increases barosensitivity and attenuates the somato-sympathetic reflex. *Br J Pharmacol*. 2012; 165:2292–303. [PubMed: 21951179]
- [69]. Nattie E, Li A. Respiration and autonomic regulation and orexin. *Prog Brain Res*. 2012; 198:25–46. [PubMed: 22813968]
- [70]. Johnson PL, Molosh A, Fitz SD, Truitt WA, Shekhar A. Orexin, stress, and anxiety/panic states. *Prog Brain Res*. 2012; 198:133–61. [PubMed: 22813973]
- [71]. Suzuki M, Beuckmann CT, Shikata K, Ogura H, Sawai T. Orexin-A (hypocretin-1) is possibly involved in generation of anxiety-like behavior. *Brain research*. 2005; 1044:116–21. [PubMed: 15862796]
- [72]. Heydendael W, Sharma K, Iyer V, Luz S, Piel D, Beck S, et al. Orexins/hypocretins act in the posterior paraventricular thalamic nucleus during repeated stress to regulate facilitation to novel stress. *Endocrinology*. 2011; 152:4738–52. [PubMed: 21971160]
- [73]. Cezario AF, Ribeiro-Barbosa ER, Baldo MV, Canteras NS. Hypothalamic sites responding to predator threats--the role of the dorsal premammillary nucleus in unconditioned and conditioned antipredatory defensive behavior. *Eur J Neurosci*. 2008; 28:1003–15. [PubMed: 18691328]
- [74]. Canteras NS. The medial hypothalamic defensive system: hodological organization and functional implications. *Pharmacol Biochem Behav*. 2002; 71:481–91. [PubMed: 11830182]
- [75]. Zalcman SS, Siegel A. The neurobiology of aggression and rage: role of cytokines. *Brain Behav Immun*. 2006; 20:507–14. [PubMed: 16938427]
- [76]. Miczek KA, Nikulina E, Kream RM, Carter G, Espejo EF. Behavioral sensitization to cocaine after a brief social defeat stress: c-fos expression in the PAG. *Psychopharmacology*. 1999; 141:225–34. [PubMed: 10027503]
- [77]. Nikulina EM, Marchand JE, Kream RM, Miczek KA. Behavioral sensitization to cocaine after a brief social stress is accompanied by changes in fos expression in the murine brainstem. *Brain research*. 1998; 810:200–10. [PubMed: 9813326]
- [78]. Bingham B, McFadden K, Zhang X, Bhatnagar S, Beck S, Valentino R. Early adolescence as a critical window during which social stress distinctly alters behavior and brain norepinephrine activity. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*. 2011; 36:896–909. [PubMed: 21178981]
- [79]. Choi DC, Nguyen MM, Tamashiro KL, Ma LY, Sakai RR, Herman JP. Chronic social stress in the visible burrow system modulates stress-related gene expression in the bed nucleus of the stria terminalis. *Physiol Behav*. 2006; 89:301–10. [PubMed: 16949112]
- [80]. Blugeot A, Rivat C, Bouvier E, Molet J, Mouchard A, Zeau B, et al. Vulnerability to depression: from brain neuroplasticity to identification of biomarkers. *J Neurosci*. 2011; 31:12889–99. [PubMed: 21900567]
- [81]. Jasnow AM, Cooper MA, Huhman KL. N-methyl-D-aspartate receptors in the amygdala are necessary for the acquisition and expression of conditioned defeat. *Neuroscience*. 2004; 123:625–34. [PubMed: 14706775]
- [82]. Morrison KE, Curry DW, Cooper MA. Social status alters defeat-induced neural activation in Syrian hamsters. *Neuroscience*. 2012; 210:168–78. [PubMed: 22433296]
- [83]. McDonald MM, Markham CM, Norvelle A, Albers HE, Huhman KL. GABAA receptor activation in the lateral septum reduces the expression of conditioned defeat and increases aggression in Syrian hamsters. *Brain research*. 2012; 1439:27–33. [PubMed: 22265703]
- [84]. Alheid GF. Extended amygdala and basal forebrain. *Ann N Y Acad Sci*. 2003; 985:185–205. [PubMed: 12724159]
- [85]. Caffé AR, van Leeuwen FW, Luiten PG. Vasopressin cells in the medial amygdala of the rat project to the lateral septum and ventral hippocampus. *J Comp Neurol*. 1987; 261:237–52. [PubMed: 3305600]
- [86]. De Vries GJ, Buijs RM, Van Leeuwen FW. Sex differences in vasopressin and other neurotransmitter systems in the brain. *Prog Brain Res*. 1984; 61:185–203. [PubMed: 6152059]

- [87]. DeVries GJ, Buijs RM, Van Leeuwen FW, Caffé AR, Swaab DF. The vasopressinergic innervation of the brain in normal and castrated rats. *J Comp Neurol.* 1985; 233:236–54. [PubMed: 3882778]
- [88]. Koolhaas JM, Everts H, de Ruiter AJ, de Boer SF, Bohus B. Coping with stress in rats and mice: differential peptidergic modulation of the amygdala-lateral septum complex. *Prog Brain Res.* 1998; 119:437–48. [PubMed: 10074805]
- [89]. De Vries GJ, Buijs RM. The origin of the vasopressinergic and oxytocinergic innervation of the rat brain with special reference to the lateral septum. *Brain research.* 1983; 273:307–17. [PubMed: 6311351]
- [90]. Compaa JC, Koolhaas JM, Buijs RM, Pool CW, de Ruiter AJ, van Oortmerssen GA. Vasopressin and the individual differentiation in aggression in male house mice. *Ann N Y Acad Sci.* 1992; 652:458–9. [PubMed: 1626848]
- [91]. Veenema AH, Neumann ID. Neurobiological mechanisms of aggression and stress coping: a comparative study in mouse and rat selection lines. *Brain Behav Evol.* 2007; 70:274–85. [PubMed: 17914259]

Highlights

- Manganese-enhanced MRI revealed brain regions activated by an acute novel stressor in rats
- A novel stressor engaged unique brain regions in rats with a history of social stress vs. controls
- A novel stressor engaged unique brain regions in rats that express different coping strategies to social stress

Figure 1.

Behavior during the single exposure to 15 min of forced swimming was comparable for control rats and rats with a history of social stress. Points on the graph indicate the mean ($\pm$ s.e.m.) of different behaviors assessed in 5 min bins over the 15 min session. Consistent with previous reports, immobility increased, while swimming and climbing decreased across the session [43].

Figure 2.

Swim stress produces a different pattern of Mn^{2+} enhancement in rats with a history of social stress compared to controls. Coronal sections (200 μm) from anterior to posterior are shown in the following order from left to right: Paxinos and Watson atlas section, the region(s) of interest analyzed, control rat, and socially stressed rat [32]. The images have been pseudocolored, such that red indicates the regions of highest activity and dark blue indicates regions with the lowest activity.

Figure 3.

Quantification of the Mn^{2+} signal and comparison between groups. A unique set of brain regions were activated by stressor exposure in rats with a history of social stress compared to controls. The graph represents normalized intensity (x-axis) of each brain region (y-axis). Socially stressed rats had greater Mn^{2+} enhancement than controls in a number of brain regions. Asterisks indicate a significant difference from control after FDR correction ($p < 0.05$). Number sign indicates a trend after FDR correction ($p < 0.07$). Bars represent the mean ($\pm$ s.e.m.)

Figure 4.

Swim stress produces relatively different magnitudes of Mn²⁺ enhancement in certain brain regions in rats with different coping strategies. The panels illustrate differences in Mn²⁺ enhancement in the septum, BNST, and amygdala in SL compared to LL rats. Coronal sections (200 μ m) from anterior to posterior are shown in the following order from left to right: Paxinos and Watson atlas section, the region(s) of interest analyzed, control rat, LL rat, and SL rat [32]. The images have been pseudocolored, such that red indicates the regions of highest activity and dark blue indicates regions with the lowest activity.

Figure 5.

Quantification of the Mn^{2+} signal and comparison between control, SL and LL groups. Three brain regions were uniquely activated in SL group versus controls. These include the amygdala, BNST, and septum. The graph represents normalized intensity (x-axis) of each brain region (y-axis). Data are represented as the mean ($\pm$ s.e.m.). Asterisks indicate a significant difference from control after FDR correction ($p < 0.05$). Double asterisks indicate a significant difference between the SL and LL group. Number sign indicates a trend compared to controls after FDR correction ($p < 0.07$). Bars represent the mean ($\pm$ s.e.m.)