Psychopharmacology (2010) 211:69–77
DOI 10.1007/s00213-010-1869-9

ORIGINAL INVESTIGATION

The effects of repeated social defeat on long-term depressive-like behavior and short-term histone modifications in the hippocampus in male Sprague–Dawley rats

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Received: 21 May 2009 / Accepted: 16 April 2010 / Published online: 8 May 2010

Abstract
Rationale Social stress has been linked to several neuropsychiatric diseases, including depression, which is a debilitating disease that has genetic, environmental, and epigenetic underpinnings.
Objectives This study examined the effects of repeated social defeat on both depressive-like behaviors and histone acetylation in the hippocampus, amygdala, and dorsal prefrontal cortex of male Sprague-Dawley rats.
Materials and methods Subjects were exposed to four consecutive social defeats. Depressive-like behaviors were assayed in the sucrose preference, forced swim, contextual fear, and social approach and avoidance tests. Histone H3 and H4 acetylation in the hippocampus, amygdala, and prefrontal cortex were examined by Western blots under basal conditions and at several time points. We also investigated the potential involvement of *N*-methyl-D-aspartic acid (NMDA) receptors and glucocorticoid receptors (GR) by injecting respective antagonists prior to each social defeat and examining their effect on histone acetylation in the hippocampus.
Results Social defeat resulted in behavioral changes in the forced swim, social avoidance, and contextual fear tests nearly 6 weeks after defeat, with no change in sucrose preference. Additionally, histone H3 acetylation was increased in the hippocampus 30 min following the last defeat and was not blocked by antagonism of either NMDA or GR receptors. There were no changes in histone H4 acetylation.
Conclusions These results indicate that social defeat induces several long-lasting depressive-like behaviors in rats and induces a significant, short-lived increase in H3 acetylation in the hippocampus, although the underlying mechanism behind this change warrants further investigation.

Keywords Social defeat · Histone modification · Epigenetics · Depression · Stress · Behavior

Electronic supplementary material The online version of this article (doi:10.1007/s00213-010-1869-9) contains supplementary material, which is available to authorized users.

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Introduction

Depression is a serious global illness that can have devastating consequences for affected individuals and their families. The two cardinal symptoms of this disease, depressed mood and loss of interest in pleasurable activities (anhedonia), can be exhibited with seven other symptoms in patients in varying combinations and severities (DSM IV 2000). While animal models of depression are limited in their ability to encompass all aspects of human depression, it is possible to investigate several prototypical changes at both the behavioral and molecular level. We consider an animal model of social stress because stress has long been implicated as one of the many precipitators of depression (Barden 2004) and because the most common stressors for

humans are psychosocial in nature (Almeida et al. 2002; Bjorkqvist 2001; Kessler 1997). The particular model of social stress that we use is social defeat, a paradigm that relies on social conflict between members of the same species to generate emotional stress. This model has several advantages, including ecological and ethological validity, as well as a lack of habituation to stress upon repeated presentation (Tidey and Miczek 1997; Miczek et al. 2008). Repeated social defeat may result in many physiological and behavioral changes in the subordinate rat. Some of these changes in behavior can be long lasting and persist when the stressor is no longer present (Koolhaas et al. 1997; Meerlo et al. 1996a,b; Ruis et al. 1999). Social defeat has clinical relevance as rodent studies have significantly contributed to validating antidepressant efficacy, with antidepressants either reversing or attenuating defeat-induced behaviors upon administration (Willner 1995; Fuchs et al. 1996; Berton et al. 1999; Tsankova et al. 2006; Rygula et al. 2006; reviewed in Miczek et al. 2008).

While much has been investigated in terms of long-term behavioral responses following social defeat, relatively little attention has been focused on potential underlying neurobiological alterations. Such long-term changes may be the products of chromatin modification in response to the experience of social defeat. Chromatin modification is a dynamic process that regulates gene expression without alteration of DNA sequences (Crosio et al. 2003; Guan et al. 2002). This modification is primarily accomplished through modifications of histone N-terminal tails at the promoter regions of specific genes (Cheung et al. 2000). Indeed, recent studies have found changes in modifications at specific gene promoter regions in association with social defeat (Tsankova et al. 2004, 2006). One such modification is histone acetylation. Acetylation has been widely studied, and it is generally accepted that hyperacetylation leads to an increase in gene expression, while hypoacetylation has the opposing effect (Forsberg and Bresnick 2001; Ito and Adcock 2002). Specifically, acetylation of lysine 14 on histone H3 (H3K14ac) has been implicated in the activation of immediate-early genes in the brain (Tsankova et al. 2004). Furthermore, increased acetylation at specific promoter regions of genes such as brain-derived neurotrophic factor has been associated with a reversal of depressive-like behavior following electroconvulsive shock therapy, while overall increased acetylation in the nucleus accumbens has been associated with depressive-like symptoms in mice (Covington et al. 2009; Tsankova et al. 2004).

With these previous studies in mind, we first investigated the long-term behavioral effects of our social defeat paradigm to ensure that it produced depression-like behaviors. Then, we investigated changes in histone H3 and H4 acetylation in the hippocampus, the amygdala, and the dorsal prefrontal cortex at several time points following repeated social defeat in male rats. These three brain areas have been widely implicated in the symptomatology of depression both in rodent models and human imaging studies (reviewed in Drevets 2001; Nestler et al. 2002) and therefore warrant further exploration as to the exact nature of their roles in the pathology of this disease. Finally, we looked at two potential pathways that could be meditating changes in histone acetylation, the *N*-methyl-D-aspartic acid receptor (NMDA-R) and glucocorticoid receptor (GR) pathways.

Methods

Rats One hundred six male Sprague–Dawley rats weighing 250–275 g, pair-housed in plexiglass cages (48.2×26.5×20.3 cm), were used in this experiment. Additionally, 22 vasectomized male Long–Evans rats weighing 325–350 g were pair-housed with 22 female Long–Evans rats weighing 200–225 g. These Long–Evans males served as the resident attackers and were chosen for consistent aggressive behavior. All animals were ordered from Charles River Laboratories (Wilmington, MA, USA). Rats were maintained on a 12-h light/dark cycle (lights on at 7:00 A.M.) with food and water available ad libitum except during testing. All experiments were conducted during the light phase of the light/dark cycle and in accordance with the guidelines of the Animal Care and Use Committee of Florida State University and National Institutes of Health guidelines.

Experimental procedure

This study was completed in three parts using first 40, then 34, and finally 32 animals, respectively. For all studies, rats were allowed to habituate to housing conditions for 1 week before testing. During this time, they were handled, weighed, and numbered on their tails on two occasions.

Experiment I

Rats were separated into two experimental groups: non-defeated (n=20) and defeated (n=20). Rats were then subjected to the sucrose preference test, the forced swim test, the social approach and avoidance test, and the contextual fear test. All behavioral tests, except for the sucrose preference test, were videotaped and later quantified in such a manner so that the experimenter was blind to the treatments. For the time course, see Supplementary Table S1.

Experiment II

Rats were separated into two experimental groups: nondefeated (n=4) and repeated social defeat (n=18). Within the defeat group, rats were further subdivided into three groups based on the time they were sacrificed following the last exposure to defeat: 30 min (n=6), 2 h and 30 min (n=6), and 24 h (n=6). The defeat group was exposed to four consecutive defeats and then sacrificed at the specified time points. As a control, nondefeated rats were weighed and handled accordingly, and then left undisturbed in their home cages for the duration of the experiment until they were sacrificed in nonstress conditions. Their brains were rapidly dissected out and frozen. Brains were sectioned at 200 μm on a cryostat, and each brain area was tissue-punched at a diameter of 2.0 mm with a Harris Uni-Core tissue-punch tool. Proteins were isolated, and Western blots were then probed with antibodies for hyperacetylated histone H3 (detects acetylation on lysines 9 and 14), total H3 (detects H3 irrespective of modifications), hyperacetylated H4 (detects acetylation on lysine residues 5, 8, 12, and 16), and total H4 (detects H4 irrespective of modifications; Millipore, Cat. No. 06-599; Millipore, Cat. No. 05-928; Millipore, Cat. No. 06-866; Millipore; Cat. No. 05-858, respectively).

Experiment III

We explored whether defeat-induced histone acetylation would involve NMDA-R or GR activation. Therefore, rats were injected i.p. with either the NMDA receptor antagonist MK-801 (100 μg/kg; Chandramohan et al. 2007), the GR antagonist mifepristone (20 mg/kg; Koenig and Olive 2004), or a corresponding vehicle to each of the aforementioned drugs. As MK-801 is easily dissolved in saline, vehicle treatments received an equivalent volume of saline (1 ml/kg body weight). As mifepristone is hydrophobic and not easily dissolved in saline, this drug was dissolved in 20% dimethylsulfoxide (DMSO)/80% polyethylene glycol (PEG)-300. Corresponding vehicle treatments received an equivalent volume of DMSO/PEG (1 ml/kg). Animals were divided into the following experimental groups (n=4 per group): nondefeated saline, nondefeated mifepristone, nondefeated MK-801, nondefeated DMSO–PEG, defeated saline, defeated mifepristone, defeated MK-801, and defeated DMSO–PEG. The defeat groups were injected with their respective drugs 15 min prior to each social defeat exposure. Rats were then sacrificed 2 h and 30 min following the fourth defeat. Nondefeated animals were handled and injected accordingly, and then returned to their home cages following injection. On the fourth day of injection, nondefeated animals were sacrificed 2 h and 30 min following the last injection. Brains were rapidly dissected out and frozen at −80°C. Brains were sectioned at 200 μm on a cryostat, and the hippocampus was punched at a diameter of 2.0 mm with a Harris Uni-Core tissue-punch tool. Proteins were isolated, and Western blots were then probed with antibodies for hyperacetylated histone H3 and total H3.

Social defeat paradigm For these experiments, we used the protocol for social defeat as previously described by our group (Dietz et al. 2008; Kabbaj and Akil 2001; Kabbaj 2004). Briefly, the repeated social defeat paradigm consists of four consecutive encounters (for 15 min each) in the home cage of an aggressive Long–Evans male rat. Rats were allowed 5 min to physically interact. During this period, if the fighting became too intense or once the intruder adopted a submissive posture and was pinned for more than 3.0 s, the intruder rat was transferred to a wire mesh protective cage, which was then placed back in the resident's cage for the remainder of the 15-min period. This protective cage allowed for full visual, olfactory, and auditory exposure to the resident without unnecessary harm to the intruder. The cage was large enough for the intruders to move freely (10×10×15 cm). All intruders were placed in this protective cage only upon being successfully defeated.

Sucrose preference test The sucrose preference test is a two-bottle choice paradigm performed according to the procedure outlined by Bolanos et al. (2008). This test has been used extensively in evaluating stress-induced anhedonia (Willner et al. 1987). Rats were habituated to drink water from two bottles for 5 days and then exposed to ascending concentrations of sucrose (0.25–1%) for 2 days per concentration. The amount of water and sucrose solution was measured at 8:00 A.M. and 5:00 P.M. daily by experimenters blind to the treatments. The bottles were balanced between the groups once daily at 5:00 P.M. The preference for sucrose over water was used as a measure of the rats' response to a naturally rewarding stimulus.

Forced swim test The forced swim test (FST) is a 2-day procedure where rats are forced to swim in conditions where escape is impossible (Porsolt et al. 1977). The swimming apparatus is a cylindrical tube (70–80 cm) filled with 25°C water so that paws and tails do not touch the bottom. The procedure was performed as described in Detke et al. (1995), where rats were forced to swim in the cylindrical tube for 15 min on the pretest day and then again for 5 min on the test day (24 h after the pretest). The entire procedure was video-recorded, and behavior on the test day was later quantified by an experimenter blind to the treatments, for latency to become immobile and time spent immobile. Latency to immobility was defined as the time at

which the rat first adopted a stationary pose not associated with an attempt to escape. Immobility was scored when the rat remained in this stationary posture for more than 2.0 s, making only the movements necessary to maintain its head above water. Increases in behavioral immobility have been associated with exposure to chronic stress in the context of vulnerability to depression (reviewed in Cryan et al. 2005).

Contextual fear test The contextual fear test is a 5-min behavioral paradigm that measures memory of a traumatic event (Takahashi et al. 2006). In this experiment, rats were placed in the empty home cage of an unfamiliar Long–Evans aggressor, and their subsequent behavior, the time spent freezing, was measured. Freezing was defined as the absence of any movement except that necessary for respiration. Thus, the rat was considered to be freezing once all locomotor and exploratory behavior ceased.

Social approach and avoidance The social approach and avoidance test consists of three sessions, adapted from Berton et al. (2006). Each session lasted a total of 5 min. For the first session, rats were first placed in an open field to both acclimate the animals to the apparatus and measure locomotor activity. In the second session ("no target"), each rat was placed into an open field (90×90 cm) that contained an empty wire mesh cage (10×10×15 cm) on one side of the open field. The time spent by each rat investigating the cage was measured. Investigation, in this case, included sniffing and climbing on the cage, as described by File and Hyde (1978) and Kudryavtseva (2003). In the third session ("target"), each rat was placed in an open field containing a caged, unfamiliar Long–Evans male. The Long–Evans male was placed inside the wire mesh cage from the no target test in the same location as in the no target test. Each rat was then placed in the open field, and the time spent investigating the Long–Evans male was measured. In between each session, rats were placed back in their home cage for 2 min. The open field and wire mesh cage were cleaned with 75% ethanol prior to each animal testing. The sessions were sequenced so as to provide the least stressful experience for each animal. We used a combination of low light and familiar conditions to create the optimum setting for detecting a reduction in social interaction, as described by File and Seth (2003).

Western blots Total proteins extracted from 2.0-mm tissue punches of the total hippocampus (−1.88 mm−(−4.52 mm) Bregma), total amygdala (−1.60 mm−(−3.80 mm) Bregma), and dorsal prefrontal cortex (+3.70 mm−(+2.20 mm) Bregma, cingulate cortex only) were prepared using the Tri Reagent protocol (Molecular Research Center, Cincinnati, OH, USA). Proteins were run on SDS–15% polyacrylamide gels. Proteins were transferred to a nitrocellulose membrane and incubated with an antihistone H3-hyperacetylated antibody (1:1,500; Millipore, Cat. No. 06-599) or an antihistone H4-hyperacetylated antibody (1:1,500; Millipore, Cat. No. 06-866) overnight at room temperature. Membranes were then incubated with peroxidase-labeled AffiniPure goat antirabbit secondary antibodies (1:10,000; Jackson ImmunoResearch Laboratories, Inc, Westgrove, PA, USA). Labeled proteins were visualized with enhanced chemiluminescence (ECL SuperSignal West Dura substrate; Pierce Biotechnologies, Rockford, IL, USA) and exposed on Fujifilm XAR film (Fuji Film Co., Ltd; Tokyo, Japan). As an individual control, membranes were then stripped and incubated with antitotal histone H3 (1:1,500; Millipore, Cat. No. 05-928) or antitotal histone H4 antibody (1:5,000; Millipore; Cat. No. 05-858), and processed as before for visualization. Quantification was performed using AIS 6.0 Image software (Imaging Research Inc.; St. Catharines, Ontario, Canada) to look at pixel density × total target area of each band for both acetylated histone and total histone. Data were analyzed using StatView software (version 5.0.1; SAS Institute, Inc.) and are shown as the ratio of quantified amount of acetylated histone to quantified amount of total histone in each of the four groups: control nondefeated animals, animals sacrificed 30 min after the last defeat, animals sacrificed 2 h and 30 min after the last defeat, and animals sacrificed 24 h after the last defeat.

Statistical analysis For the sucrose preference test and contextual fear test, data were analyzed using one-way repeated measures analyses of variance (ANOVAs). All other behavioral studies measured data using one-way ANOVAs. Optical density quantified from Western blots was tested for significance with one-way ANOVAs. For all ANOVAs, significant effects were further analyzed using post hoc tests where appropriate. Statistical significance was defined as $p<0.05$.

Results

Behavioral tests

Repeated social defeat did not affect sucrose preference for solutions of 0.25%, 0.5%, or 1.0% sucrose content (data not shown). Both defeated and control rats similarly preferred increasing sucrose concentrations over water, suggesting that the current type of social defeat did not induce a change in sucrose intake for any of the concentrations used in this experiment. Repeatedly defeated animals had a significantly shorter latency to immobility in the FST than nondefeated rats [$F(1, 38)=16.5$; $p=0.0002$] (Fig. 1a). This repeated defeat group also spent a significantly longer time

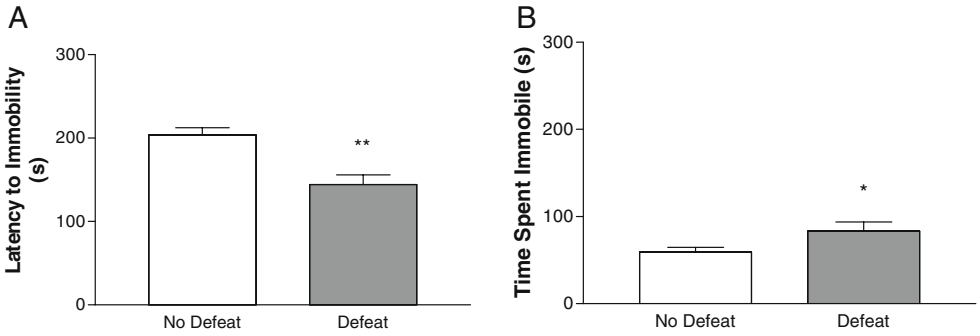

Fig. 1 Effect of repeated defeat on forced swimming behavior. **a** Defeated rats had a shorter latency to become immobile on test day (**$p<0.01$). **b** Defeated rats spent a longer time immobile compared to nondefeated rats (*$p<0.05$)

immobile than the control group [$F(1, 38)=4.3$; $p=0.046$] (Fig. 1b).

One month after social defeat, both defeated and control rats spent the same amount of time investigating an empty cage (Fig. 2a—no target), while defeated rats spent significantly less time investigating an unfamiliar Long–Evans male, when compared to controls [$F(1, 38)=4.97$ $p=0.03$] (Fig. 2b—target). Both groups exhibited similar levels of locomotor activity, as measured by the number of grid crosses during the open field portion of the test (Fig. 2c). The contextual fear exposures took place 4 and 6 weeks, respectively, after social defeat. In this behavioral paradigm, we found that defeated animals exhibited significant freezing behavior during the 5-min span of the test at both time points, indicating that the effects of social defeat are long term [$F(1, 38)=27.5$; $p<0.0001$] (Fig. 3). In the re-exposure to social defeat, there was an expected trend toward extinction [$F(1, 38)=2.97$; $p=0.09$].

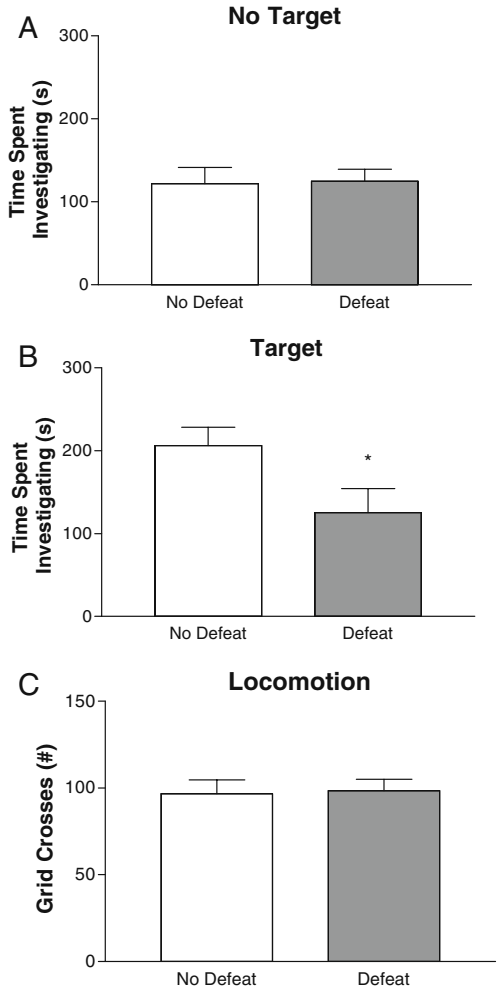

Fig. 2 The effect of repeated defeat on social avoidance behavior. **a** Both defeated and nondefeated rats spent a similar amount of time investigating an empty cage (no target; $p=0.90$). **b** Defeated rats spent significantly less time investigating an unfamiliar caged male (target; *$p<0.05$). **c** Both defeated and nondefeated rats had similar locomotion in the open field, as measured by number of grid crosses ($p=0.87$)

Effects of social defeat on histone modifications

As histone modifications can often underlie behavioral changes, we examined the hippocampus, amygdala, and dorsal prefrontal cortex for evidence of changes in histone acetylation via Western blots. In the hippocampus, we found that repeated exposure to social defeat significantly increased histone H3 acetylation compared to baseline as early as 30 min after exposure [$F(3, 18)=11.0$; $p=0.0002$] (Fig. 4a). This increase persisted 24 h later but was still transient in nature as acetylation levels returned to baseline 72 h following the last exposure to social defeat (Supplementary Figure 1). In the amygdala, repeated defeat however did not affect H3 acetylation in the amygdala at any of the time points that we examined (Fig. 4b). In the dorsal prefrontal cortex, we similarly found no significant changes in histone H3 acetylation levels following repeated social defeat at any of the time points that we investigated (Fig. 4c). To investigate if these changes were specific to H3, we also quantified changes in the acetylation of the N-terminal tail of histone H4 in these three brain regions. We found no significant changes in histone H4 acetylation in any of these brain areas following repeated defeat, indicating that the changes in histone modification following social defeat are specific to histone H3 (Supplementary Table S2).

We investigated the involvement of several pathways in the defeat-induced increase in histone H3 acetylation by

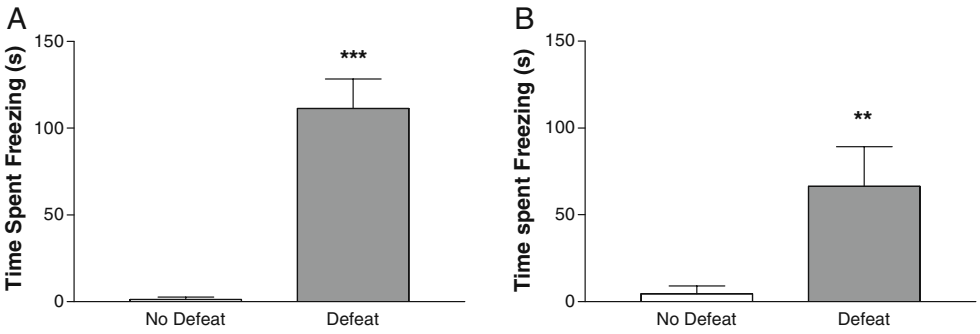

Fig. 3 The effect of repeated defeat on strong emotional memory. **a** Defeated rats spent significantly longer freezing in the contextual fear test 4 weeks after the last exposure to social defeat (***$p<0.0001$). **b** Six weeks after the last exposure to defeat, defeated rats still exhibited strong freezing behavior (**$p<0.01$)

blocking either NMDA-R or GR in the hippocampus prior to each social defeat exposure. Interestingly, we found that injections of the NMDA-R antagonist, MK-801, actually increased histone H3 acetylation regardless of defeat exposure [$F(3, 15)=31.3$ $p<0.0001$] (Fig. 5). Injection of the GR antagonist, mifepristone, had no significant effect on histone H3 acetylation following social defeat, or in nondefeated controls (Fig. 5).

Discussion

Our social defeat paradigm successfully elicits several long-lasting, depressive-like behaviors. Defeated animals exhibited both a shortened latency to immobility and an increased immobility time in the forced swim test. Immobility in the forced swim test has been used as the measure of behavioral despair and efficacy of antidepressant drugs in the reversal of that despair (Lucki 1997; reviewed in Cryan et al. 2005), although some view increased immobility as a heightened adaptation for survival (West 1990; reviewed in Cryan et al. 2005). Repeated defeat also induced social avoidance behavior as exhibited by defeated rats in the social approach and avoidance test. Where the nondefeated rat increased his exploration of an unfamiliar male, the defeated rat did not show an increased interest, representative of social avoidance. This result is in agreement with previous findings in

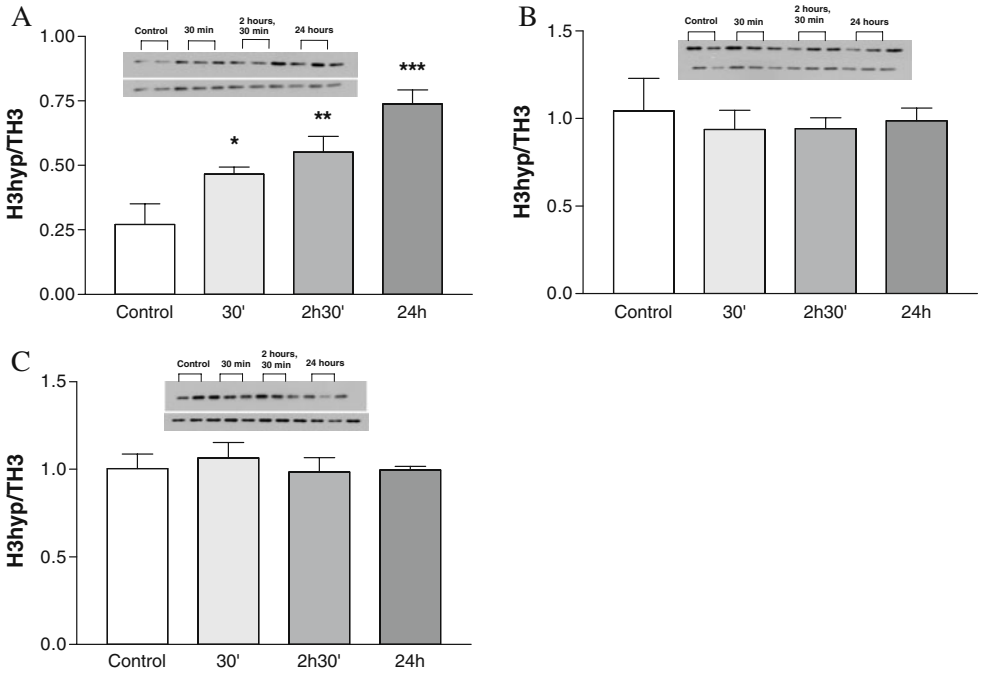

Fig. 4 The effect of repeated defeat on the ratio of histone H3 acetylation (*H3hyp*) to total histone H3 (*TH3*) in the **a** hippocampus. Repeated defeat resulted in an increase in a similar increase in acetylation at 30 min after the last exposure to defeat (*$p<0.05$) that remains sustained 24 h later (***$p<0.0001$); **b** amygdala. Repeated social defeat had no significant effects on histone H3 acetylation in the amygdala ($p=0.89$); **c** dorsal prefrontal cortex. Repeated social defeat had no significant effect on H3 acetylation in the prefrontal cortex ($p=0.84$). A representative blot is shown *above each graph*, with hyperacetylated H3 on the *top blot* and total histone H3 on the *bottom blot*. Lanes are labeled with corresponding treatment groups with each band representing one animal per group: control, defeat group killed *30′* after last defeat exposure, defeat group killed *2h30′* after last exposure, and defeat group killed *24h* after last exposure

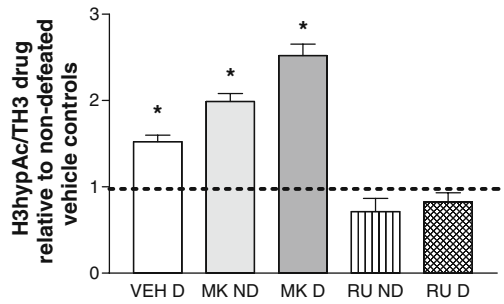

Fig. 5 The effect of repeated defeat on histone H3 hyperacetylation following blockade of either NMDA-R or GR in the hippocampus. The ratio of histone H3 acetylation (*H3hypAc*) to total histone H3 (*TH3*) was calculated for the nondefeat vehicle group for each drug independently. As the ratio of these two groups was not significantly different from one another ($p=0.50$), they were combined into a group-labeled nondefeat vehicle group (indicated on the graph as a *dashed line at 1*). All ratios for other treatment groups were then calculated and normalized to the nondefeat vehicle group. In the hippocampus, regardless of defeat, injection of MK-801 increased H3 acetylation (***$p<0.0001$), but injection of mifepristone had no effect. *VEH D* vehicle defeat, *MK ND* MK-801 nondefeat, *MK D* MK-801 defeat, *RU ND* mifepristone nondefeat, *RU D* mifepristone defeat

mice (Berton et al. 2006; Krishnan et al. 2007; Tsankova et al. 2006) and rats (Kudryavtseva 2003; Razzoli et al. 2009). Notably, we report that upon re-exposure to the defeat context, defeated rats demonstrated a robust and long-lasting freezing behavior that persisted over a month after the last exposure to social defeat. It could thus be argued that these defeated rats possess long-lasting, contextual-dependent emotional memory that might be analogous to memories of patients suffering from posttraumatic stress disorder. To our knowledge, this is the first demonstration of such strong and persistent contextual fear memory following social defeat in rats. Our defeat paradigm, however, did not reduce the rats' preference for sucrose solution over water, and thus did not induce anhedonia in socially defeated rats. Previous reports have found a decreased sucrose preference in chronically defeated rats (Rygula et al. 2005) and mice (Krishnan et al. 2007). The most likely explanation may be due to differences in the social defeat paradigm where the aforementioned groups use paradigms that mimic a more chronic type of social stress, consisting for example, of five consecutive weeks of unpredictable social defeat (Rygula et al. 2005) or 10 days of persistent social defeat (Krishnan et al. 2007). This does not necessarily argue against our repeated defeat paradigm as a good model for depression. Just as humans need show only five of nine major symptoms of depression (DSM IV 2000) to be diagnosed with a major depressive disorder, so too may rats not exhibit every single depressive-like behavior following one specific type of social stress.

We investigated histone acetylation as a potential mechanism behind the depressive-like behaviors induced by social defeat. We found short-term differences in activation-inducing (Agalioti et al. 2002) histone H3 acetylation of lysine residues 9 and 14 in repeatedly defeated rats in the hippocampus. In the hippocampus, rats that had been exposed to repeated defeat showed increased H3 acetylation 30 min following the last exposure to social defeat. This increased acetylation persisted for 24 h, but returned to baseline 72 h later. The timing of this acetylation might seem to argue against this modification as a potential mechanism behind our long-term behavioral data; however, transient changes in acetylation have been previously proposed as a mechanism behind long-term changes in gene activity (Shahbazian and Grunstein 2007; Tsankova et al. 2004; discussed in Tsankova et al. 2007) and behavior (Weaver et al. 2004). Furthermore, this change in acetylation may induce transcription of genes that influence the transcription of other downstream targets that are more long lasting. The work of Nikulina et al. (2008) found that four social defeats were enough to induce long-term increases in mu opioid receptor mRNA. The changes in response to social defeat may be region specific as we found no significant changes in histone H3 or H4 acetylation in the amygdala or dorsal prefrontal cortex at any of the time points that we investigated. This does not necessarily mean that these regions were unaffected by our social defeat paradigm. Since the prefrontal cortex and amygdala are highly heterogeneous regions, a lack of change in histone modifications in the entire region may not reflect individual changes made in specific subregions. Future immunohistochemistry studies investigating histone acetylation changes in the prefrontal cortex and amygdala at the level of the individual subregions may reveal specific histone modifications that were previously undetected in the Western blot studies.

Interestingly enough, we also did not see any significant change in histone H4 acetylation. However, we cannot rule out the possibility that antibody specificity for histone H4 acetylation may not be as precise as that which was used for H3 acetylation. The H3-hyperacetylated antibody specifically recognizes acetylation at lysine residues 9 and 14. The H4 antibody used recognizes global acetylation on all four lysine residues and therefore may be less sensitive to acute changes at specific lysine residues on the H4 terminal tail.

We looked into potential pathways that might mediate this stress-induced increase in histone acetylation for potential mechanistic insight. Previous studies using novelty and forced swim stress have implicated the NMDA-R and GR pathways (Chandramohan et al. 2007, 2008). We found that blocking NMDA receptors actually resulted in a dramatic increase in histone H3 acetylation in both non-defeated and defeated animals. While we are not sure of the

underlying mechanism behind this increase, this is not the first time MK-801 has been associated with increased histone acetylation. Deutsch et al. (2008) found that MK-801's efficacy as an antiseizure treatment is greatly increased when paired with the histone deacetylase inhibitor, sodium butyrate, resulting in increased acetylation in the hippocampus. This finding, however interesting, seems to indicate that the NMDA receptor pathway is not involved in the induction of histone acetylation following social defeat stress. Furthermore, blocking the GR pathway had no effect on H3 acetylation following defeat stress. Both of these findings differ from a previous study that used both of these drugs to successfully block acetylation increases following novelty stress (Chandramohan et al. 2007). One possibility for this discrepancy lies in the method of our detection. Chandramohan et al. (2007) found that both MK-801 and mifepristone blocked novelty stress-induced histone H3 acetylation in various layers of the dentate gyrus via immunohistochemistry. As we examined the entire hippocampus, the ability to detect small changes in very selective regions may have been lost. A more intriguing explanation for the difference in findings is that the pathway involved is specific to the stressor. The aforementioned study focused on novelty stress—a relatively mild stressor when compared to the psychological stress of social defeat. Furthermore, a more recent study by this group found that NMDA-R antagonism but not GR antagonism reversed forced swim-induced immobility and increased histone H3 acetylation (Chandramohan et al. 2008). As social defeat is a more potent psychological stressor, it may rely on an alternative mechanism than the ones investigated here and certainly warrants further investigation into other potential pathways.

Linking chromatin remodeling to environmental stimuli and resulting behavior will require further studies that connect changes in histone modifications to changes in gene expression in response to an external stress, such as social defeat. Studies of other histone modifications such as phosphorylation and methylation, as well as ChIP-on-chip studies to identify which genes are enriched by acetylation following social defeat, will provide additional insight into the epigenetic responses to stress. Our study contributes to the burgeoning field of investigations into the impact of epigenetic mechanisms underlying depressive-like behaviors and emotional behavior as a whole. Such investigations may prove essential in the future development of improved treatment for psychiatric pathologies such as depression.

Acknowledgments This work was supported by two National Institute of Drug Abuse (NIDA) grants (R01 DA019627-01 and R03 DA021554-01-A1) and National Institute of Mental Health (NIMH) grant (R21 MH081046-0182). F. Hollis is supported by the Florida State University Department of Biomedical Sciences Graduate program.

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