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Histone deacetylase and acetyltransferase inhibitors modulate behavioral responses to social stress

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ABSTRACT

Histone acetylation has emerged as a critical factor regulating learning and memory both during and after exposure to stressful stimuli. There are drugs that we now know affect histone acetylation that are already in use in clinical populations. The current study uses these drugs to examine the consequences of acutely increasing or decreasing histone acetylation during exposure to social stress. Using an acute model of social defeat in Syrian hamsters, we systemically and site-specifically administered drugs that alter histone acetylation and measured subsequent behavior and immediate-early gene activity. We found that systemic administration of a histone deacetylase inhibitor enhances social stress-induced behavioral responses in males and females. We also found that systemic administration completely blocks defeat-induced neuronal activation, as measured by Fos-immunoreactivity, in the infralimbic cortex, but not in the amygdala, after a mild social defeat stressor. Lastly, we demonstrated that site-specific administration of histone deacetylase inhibitors in the infralimbic region of the prefrontal cortex, but not in the basolateral amygdala, mimics the systemic effect. Conversely, decreasing acetylation by inhibiting histone acetyltransferases in the infralimbic cortex reduces behavioral responses to defeat. This is the first demonstration that acute pharmacological manipulation of histone acetylation during social defeat alters subsequent behavioral responses in both males and females. These results reveal that even systemic administration of drugs that alter histone acetylation can significantly alter behavioral responses to social stress and highlight the importance of the infralimbic cortex in mediating this effect.

1. Introduction

DNA transcription is necessary for development and maintenance of experience-dependent, long-term memories that elicit subsequent changes in behavior. The removal or addition of acetyl groups to the histones around which DNA is wrapped by histone deacetylases (HDACs) or histone acetyltransferases (HATs) alters the likelihood of gene transcription. Inhibition of Class I HDACs enhances long-term memory at each stage of memory processing (e.g., acquisition, consolidation, reconsolidation, extinction), while HAT inhibition impairs memory (Kilgore et al., 2010; Reolon et al., 2011). For example, acquisition of conditioned fear is enhanced following the administration of a Class I HDAC inhibitor, as is reconsolidation of that memory (Bredy and Barad, 2008), while inhibition of HATs during fear conditioning blocks acquisition and consolidation of that fear memory (Maddox et al., 2013; Monsey et al., 2015).

HDAC inhibitors, including valproic acid (VPA), are already being used clinically to treat a variety of illnesses such as epilepsy and bipolar disorder, but their effects on learning suggest that they may also be useful in a range of neuropsychiatric illnesses, such as posttraumatic stress disorder (PTSD) or specific phobia, wherein fear learning is potentially aberrant (Bredy and Barad, 2008; Parsons and Ressler, 2013). Further investigation into how these drugs impact long-term behavioral and physiological reactions to stress may lead us to the development of more targeted treatments and interventions, many of which could be immediately available for clinical populations. While the initial data are encouraging, most studies completed to date have used physical stressors (e.g., foot/tail shock) and only a few studies have examined the role of histone acetylation in more ethologically relevant models of stress-induced behavioral change (Covington et al., 2015; Espallergues et al., 2012; Hollis et al., 2011). Social defeat models have strong face and construct validity for human anxiety and depressive behavior (Hollis and Kabbaj, 2014; Huhman, 2006; Toth

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and Neumann, 2013), but the majority of these models use relatively severe, repeated exposure to social defeat in male mice. While the study of chronic social stress is important, not all social stressors that humans experience are chronic in nature. Acute social stress or trauma can also lead to sudden and discernable changes in behavior, sometimes leading to psychopathology (e.g., PTSD). Furthermore, using an acute model of social stress allows a much more precise determination of when acquisition and consolidation of stress-related learning are occurring, therefore we can test hypotheses about these processes in a way that is not possible in chronic models.

Our laboratory studies acute social defeat stress in Syrian hamsters. Hamsters provide a unique social stress model because both males and females are highly territorial, and these animals do not require complex housing conditions to elicit conspecific aggression or reliable behavioral responses to defeat in the laboratory. Home cage animals of both sexes will readily attack an intruding conspecific. However, after losing one agonistic encounter hamsters abandon all territorial aggression and, instead, become highly submissive and socially avoidant (Huhman, 2006; McCann et al., 2014; McCann and Huhman, 2012). This behavioral change has been termed conditioned defeat and lasts for at least one month in the majority of hamsters (Huhman et al., 2003). The conditioned defeat model is unique among social defeat models for several reasons. First, the agonistic interactions in hamsters are highly ritualized so that they rarely result in physical injury; thus, it is possible to examine the behavioral and physiological effects of social stress in the absence of physical injury or trauma and the concomitant inflammatory response. In addition, striking behavioral and physiological changes, including social avoidance and elevated cortisol, are observed after even a *single*, relatively mild defeat (Huhman et al., 1991, 2003; McCann and Huhman, 2012). Finally, unlike models using rats or mice, conditioned defeat in hamsters allows examination of defeat-induced behavior in both sexes. Thus, our model of acute social stress provides an excellent opportunity to study the behavioral and physiological responses in both males and females with much more precise temporal specificity compared with chronic defeat models that use only males, that test only extended periods of social stress, or that use species wherein wounding is common during social interactions.

We have made significant progress in delineating the neural circuitry mediating conditioned defeat. It is well established that the amygdala is a crucial site of plasticity necessary for processing and responding to emotional and fearful stimuli (Davis, 1992; Fanselow and Gale, 2003; McGaugh, 2004), and we have demonstrated that the basolateral amygdala (BLA) as well as the medial prefrontal cortex (mPFC) are critical components of the neural circuit mediating conditioned defeat (Jasnow and Huhman, 2001; Jasnow et al., 2005; Markham et al., 2012, 2010). The persistence of the behavioral changes observed after a single social defeat in hamsters suggests that these behavioral changes might be mediated by epigenetic mechanisms. A better understanding of the molecular mechanisms subserving conditioned defeat may lead us to a clearer understanding of how even brief exposure to social stress impacts future social behavior. The purpose of the present study was to test the hypothesis that epigenetic changes within the neural circuit that mediates conditioned defeat contribute to the observed behavioral changes after acute social stress.

2. Material and methods

2.1. Animals

Adult male and female Syrian hamsters (*Mesocricetus auratus*) were obtained from Charles River Laboratories (Wilmington, MA) or bred in-house from animals obtained from Charles River. Subjects (approximately 12 weeks, 120–130 g) were individually housed in a polycarbonate cage (23 × 43 × 20 cm) and were handled daily for at least one week before any behavioral manipulations began. The colony room was temperature-controlled, and animals were kept on a 14:10 light/dark cycle. All cages contained corncob bedding and cotton nesting material, and food and water were available *ad libitum*. Same sex resident aggressors (RAs) were used for social defeat training and for social avoidance testing. RAs are larger, individually housed hamsters that readily attack an intruder placed in their home cage. Female subjects were paired with ovariectomized female RAs because aggression in intact females varies over the estrous cycle and aggression in ovariectomized females is more reliable. Behavioral manipulations were done in a dedicated testing suite within the vivarium during the first 3 h of the dark phase of the daily light/dark cycle. All procedures and protocols were approved by the Georgia State University Institutional Animal Care and Use Committee and are in accordance with the standards outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Social defeat training

For social defeat training, subjects were placed into the home cage of a same-sex RA as described previously (McCann et al., 2014; McCann and Huhman, 2012). Estrous cycles of female subjects were monitored via vaginal swabs for at least two cycles before the experiment, and females were defeated on Diestrus 1 (D1) and tested on Diestrus 2 (D2) because we have previously shown this results in the most pronounced avoidance after social defeat (unpublished observations). A clear cage top was placed on top of the RA's cage to prevent either animal from escaping the cage during a 5 min or 15 min defeat session. The two different training durations were chosen to avoid ceiling and floor effects, respectively, and the choice of which to use was based on *a priori* hypotheses of the directionality of the expected behavioral effect. Shorter defeat was used when submission and avoidance was expected to increase and longer defeat was used when submission and avoidance was expected to decrease in drug-treated animals as compared with vehicle controls. The holding box used for social avoidance testing, described below, was placed in the RA's cage during training. At the end of the defeat, subjects were returned to their home cages. Animals were monitored during defeat to ensure that no injury occurred to either animal. No-defeat controls were placed in a novel cage with soiled RA bedding and a holding box for the same amount of time as the defeat group and were subsequently returned to their home cage until social avoidance testing. Behavior emitted by RAs and by subjects during defeat training was recorded and scored by trained observers that were blind to experimental condition to ensure that pre-training drug infusions did not alter either the amount of aggression displayed by the RAs toward the subjects or the amount of submission shown by the subjects during defeat training.

2.3. Social avoidance testing

Social avoidance testing was conducted as described previously (McCann et al., 2014; McCann and Huhman, 2012) and was recorded for later analysis. In brief, 24 h after social defeat training, subjects were placed in a clean, novel testing arena (23 × 40 × 20 cm) with an unfamiliar RA placed inside a smaller holding box on one end of the arena. The holding box for the unfamiliar RA was constructed of perforated plastic that allowed the subject to see, hear, and smell the unfamiliar stimulus animal but not to come into direct contact with it. For scoring purposes, the testing arena was divided into eight sections (Fig. 1). Time spent in