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Antidepressant Action of HDAC Inhibition in the Prefrontal Cortex

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Abstract

Previous research has demonstrated antidepressant-like effects in rodents upon intracerebral inhibition of histone deacetylases (HDACs). Such effects have been reported for local HDAC inhibition in the nucleus accumbens, hippocampus, and amygdala. However, the effect of HDAC inhibition within the medial prefrontal cortex, which is integral to depression-related symptoms and their treatment, remains unknown. Here we show that local infusion of the highly selective HDAC inhibitor, MS-275, into medial prefrontal cortex exerts robust antidepressant-like effects in the chronic social defeat stress paradigm in mice. These findings provide further impetus for the assessment of HDAC inhibitors for the treatment of depression.

Keywords

MS-275; HDAC inhibitors; depression; gene expression

1. Introduction

There is increasing evidence that epigenetic mechanisms contribute to the lasting changes in gene expression in brain, which are thought to be part of the pathophysiology of depression as well as of the mechanisms of action of antidepressant treatments (Abel and Zukin, 2008; Vialou et al., 2013; Sun et al., 2013; Bagot et al., 2014). Epigenetic mechanisms include a host of post-translational modifications of histone proteins that control the chromatin architecture around specific genes and thereby regulate those genes' transcription (Strahl and Allis, 2000).

The best studied post-translational modification of histones is acetylation, whereby a histone acetyltransferase (HAT) catalyzes the addition of an acetyl group to one of several lysine residues located on the N-terminal tails of several histone subunits (Strahl and Allis, 2000).

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The acetylation of histones is generally associated with transcriptional activation. The removal of these acetyl groups is catalyzed by histone deacetylases (HDACs), which are divided into two main classes. Class I HDACs (including HDACs 1-3 and 8) are responsible for the vast majority of HDAC catalytic activity in the brain, while Class II HDACs (including HDACs 4, 5, 7, and 9) are viewed as regulatory subunits for the Class I enzymes (Haberland et al., 2009; Graff and Tsai, 2013).

The antidepressant potential of HDAC inhibition was first demonstrated by two studies, which reported that systemic administration of sodium butyrate, a highly non-selective HDAC inhibitor, exerts antidepressant-like effects in mice, both in the chronic social defeat stress paradigm (Tsankova et al., 2006) and in the forced swim test in non-stressed animals (Schroeder et al., 2007). Such effects have been replicated in subsequent studies (Zhu et al., 2009; Hollis et al., 2011). The effects of systemic administration of HDAC inhibitors, however, are relatively subtle compared with the far more dramatic effects achieved with direct intra-cerebral administration of more selective HDAC inhibitors. Thus, infusion of MS-275, a highly selective inhibitor of Class I HDACs (Khan et al., 2008), exerts robust antidepressant-like effects upon administration into the nucleus accumbens (Covington et al., 2009). MS-275 administration into either of two other regions—the hippocampus or amygdala—also exerts antidepressant-like effects, but smaller in magnitude compared to those achieved with intra-nucleus accumbens delivery (Covington et al., 2011).

A glaring weakness in the literature is the lack of investigation of HDAC actions in the prefrontal cortex, which is strongly implicated in depression and its treatment (Duman, 2014; Smart et al., 2015). There are conflicting reports of stress regulation of global levels of histone acetylation in the rodent prefrontal cortex (Hollis et al., 2010; Hinwood et al., 2011; Kenworthy et al., 2013). Moreover, there has been no assessment to date of the functional effects of HDAC inhibition within this brain region in depression models. Here we tested the behavioral effects of local administration of MS-275 into the medial prefrontal cortex and found robust antidepressant actions. These findings therefore further justify continued efforts to evaluate the therapeutic potential of HDAC inhibitors in treating depression and related syndromes in humans.

2. Experimental Procedures

2.1. Animals

C57BL/6J male mice (Jackson Laboratories) were used in all experiments. All mice were group housed and maintained on a 12-hr light/dark cycle with *ad libitum* access to food and water for at least one week prior to experimentation. All procedures were carried out in accordance with the Institutional Animal Care and Use (IACUC) guidelines of Mount Sinai.

2.2. Chronic Social Defeat Stress

Chronic social defeat stress was performed on several groups of mice exactly as described previously (Berton et al., 2006; Krishnan et al., 2007). Briefly, 10 week old experimental C57BL/6J mice were exposed to a novel CD1 aggressor for 5 min daily over 10 consecutive days. Each CD1 mouse—11-20 weeks of age—was a retired breeder (Charles River Laboratories). After 5 min of physical contact, experimental mice were removed from

contact with the aggressor and placed on the opposite side of the aggressor's home cage behind a "protective" partition that was perforated with holes to allow for sensory contact during the ensuing 24 hr. Non-defeated control mice were housed as two animals per cage under the same conditions as their experimental counterparts but without the presence of an aggressive CD1 mouse. Experimental mice were relocated to a new cage each day immediately before the commencement of social defeat. Twenty-four hr after the final social defeat, all mice were housed individually. Only mice susceptible to chronic social defeat stress, defined by a reduction in social interaction 24 hr after the last stress episode (Berton et al., 2006; Krishnan et al., 2007) were used in this study. Fewer than 10% of all mice displayed a resilient phenotype (no decrease in social interaction), too few to be studied further.

2.3. Subcutaneous Fluoxetine Pellet Implantation

A subgroup of defeated and control animals (n=12/group) were given fluoxetine by subcutaneous pellet implantation, a treatment which exerts antidepressant-like effects in some but not all defeated animals (Covington et al., 2009). The day after the last of 10 social defeat stress episodes, susceptible mice were implanted subcutaneously with one fluoxetine (or sham) pellet while under brief isoflurane anesthesia. We used custom made pellets (Innovative Research, Sarasota, FL), which delivered fluoxetine (20 mg/kg/day, provided by Eli Lilly) continuously for 20 days. This procedure results in clinically relevant blood levels of fluoxetine (Covington et al., 2009).

2.4. Immunohistochemical Quantification of Acetylated Histone H3

At varying times after chronic social defeat stress, mice were anesthetized and perfused intracardially with 4% paraformaldehyde in 1× PBS. Brains were removed and post-fixed by immersion overnight in 4% paraformaldehyde and cryoprotected in 30% sucrose in 1× PBS. Coronal sections (35 μ m) were cut on a freezing microtome and processed for infrared immunohistochemistry using a Licor system as described previously (Covington et al., 2009). Briefly, brain sections including the prefrontal cortex were pre-incubated in a blocking buffer containing 0.1% triton and 3% normal donkey serum. A cocktail of antibodies raised against acetyl-histone H3 Lys14 (H3K14ac) (Millipore, Billerica, MA) or histone H1 (Millipore) at respective dilutions of 1/30,000 and 1/20,000 were used overnight at 4°C in blocking buffer. After washing, sections were incubated with IRDye 680 donkey anti-mouse antibody and IRDye 800 donkey anti-rabbit (both diluted to 1/5000, Licor Biosciences) in PBS with 0.05% triton for 2 hr at room temperature. Fluorescent immunocomplexes were then detected using a Licor Odyssey Infrared Imager (21 μ m resolution, 1 mm offset at the highest quality). Integrated intensities of acetylated H3 and total H1 were determined using the Odyssey software. Results are given as integrated intensity values/mm² and are presented as mean $\pm$ standard error of the mean (S.E.M.; n=8 to 12 per group). Values for H1, which showed no regulation by chronic social defeat stress or fluoxetine, were used as a normalization control. This approach has previously been shown to provide accurate, reliable measures of H3K14ac in brain (Covington et al., 2009, 2011).

2.5. MS-275 Infusions Into Medial Prefrontal Cortex

The antidepressant potential of MS-275 was examined across several behavioral assays. C57BL/6J mice (n=12/group), previously subjected to chronic social defeat stress or control conditions, were surgically implanted with two subcutaneous Alzet® minipumps (model 1002, Durect Corp., Cupertino, CA) containing MS-275 (100 μ M, provided by Steven Haggerty, Broad Institute), or 5% hydroxypropyl β -cyclodextrin vehicle (Trappsol®, CTD, Inc), connected to bilateral guide cannulae (28 gauge, Plastics One, Roanoke, VA) as described (Covington et al., 2009). Surgery was performed 24 hr after the last defeat episode. This MS-275 dose was selected because it has been shown to increase histone acetylation, including H3K14ac levels, in several brain regions (Covington et al., 2009, 2011; Kennedy et al., 2013). The cannulae targeted medial prefrontal cortex, including aspects of both prelimbic and infralimbic regions (AP +1.8, ML +2.8, and DV -2.0, with a 30° angle relative to bregma).

Mice recovered 5 days after surgery before the initiation of behavioral testing. Mice were assessed across four behavioral domains in the following order using published procedures. *Open field*: Locomotor activity (distance traveled, cm) was assessed in the same arena used for social interaction testing during a 2.5 min trial immediately prior to a test for social interaction. *Social Interaction*: Social interaction was tested as originally originally (Berton et al., 2006), where a novel CD1 aggressor mouse was used as a social target. *Sucrose Preference*: Twenty-four hr later, sucrose preference was calculated as a percentage of sucrose (1%)/water consumed and averaged over 2 days (Krishnan et al., 2007). *Forced Swim Test*: Twenty-four hr after the final sucrose preference assessment, videotracking-based methods were used to record the duration of time spent “immobile” in a beaker of water over a 6-min trial (Krishnan et al., 2007).

Separate groups of non-stressed mice (n=12/group) were assessed in the light-dark test as described (Covington et al., 2009). We used a two sided chamber divided by a small door in the center (20×40×20 cm). One side of the chamber was brightly lit (~300 lux) and the other chamber was protected from light (<5 lux). At the start of each test session, mice were gently placed into the dark compartment, and the door to the lit compartment was opened 2 min later. Movement of the mouse into the lit side was detected by photocells. Mice were allowed to move freely between the lit and dark sides of the chamber for an additional 10 min. The total time spent in the lit side of the chamber was calculated as the dependent measure.

At the completion of all behavioral experiments, cannula placements were visually confirmed for each mouse as described (Covington et al., 2009). In addition, immunohistochemistry as also described by Covington et al. (2009) was carried out on sections of prefrontal cortex directly under the site of infusion to verify increases in H3K14ac after MS-275 infusion.

2.6. Statistics

Ratios of H3K14ac over total H1 were calculated, and 2 × 3 ANOVAs (stress × time) were carried out. The relative density of H3K14ac after 10 days of *in vivo* infusion of vehicle or

MS-275 was analyzed using an ANOVA. For analyses of depressive-like behaviors, groups of non-defeated controls and socially defeated mice were compared using two-way ANOVAs (stress $\times$ drug). For all two-way ANOVAs, Bonferroni post-hocs were used to assess isolated comparisons. All differences were considered significant when $p < 0.05$.

3. Results

3.1. Regulation of Histone Acetylation in Prefrontal Cortex by Chronic Social Defeat Stress and Fluoxetine Administration

As a first step to assess the role of histone acetylation in the prefrontal cortex in depression-related behavioral abnormalities, we studied the effect of chronic (10 days) social defeat stress on levels of H3K14ac in this brain region of mice susceptible to the stress based on a highly validated measure of social avoidance (Figure 1A,B) (Berton et al., 2006; Krishnan et al., 2007). We selected this particular histone acetylation mark as it has been shown in other studies to be altered in other brain regions in stress models (Covington et al., 2009, 2011; Hinwood et al., 2011). As shown in Figure 1C,D, levels of H3K14ac in prefrontal cortex were not affected by chronic social defeat stress 1 hr after the last defeat episode, however, H3K14ac levels were increased in this brain region 24 hr and 10 days later. This effect was seen broadly in medial prefrontal cortex, including both prelimbic and infralimbic regions. These data demonstrate that chronic social defeat stress increases levels of H3K14ac in the prefrontal cortex as seen in certain other brain regions (see Discussion).

We next studied whether chronic administration of fluoxetine to socially defeated or control mice altered levels of histone acetylation in the prefrontal cortex. Mice were first subjected to chronic (10 days) social defeat stress and 24 hr later they were assessed for susceptibility. Susceptible and control mice were then implanted with a fluoxetine or sham pellet and examined 20 days later (Figure 2A). Sham-treated susceptible mice continued to show social avoidance at this 20 day time point, an effect reversed in $\sim 50\%$ of the mice by fluoxetine treatment (Figure 2B). However, levels of H3K14ac in prefrontal cortex were no longer elevated at this more prolonged time point, and fluoxetine had no effect on H3K14ac levels in this brain region either in control or defeated mice (Figure 2C). Previous work has shown that fluoxetine has no effect on H3K14ac in nucleus accumbens or amygdala, although it increases H3K14ac levels slightly in hippocampus (Covington et al., 2009, 2011).

3.2. Behavioral Effects of HDAC Inhibition in Prefrontal Cortex

To assess the functional consequences of stress-induced increases in histone acetylation in the medial prefrontal cortex, mice were subjected to chronic (10 days) social defeat stress, and susceptible and control mice were then implanted with cannulae that delivered MS-275 into this brain region bilaterally. As seen for other regions (Covington et al., 2009, 2011), such infusion increases levels of H3K14ac in medial prefrontal cortex (Figure 3A; $168 \pm 12\%$ of vehicle, $n=12$, $p < 0.05$), confirming the efficacy of the drug as demonstrated previously in other brain regions (Covington et al., 2009, 2011; Kennedy et al., 2013).

As shown in Figure 3B, mice subjected to chronic social defeat stress displayed the expected social avoidance. This effect was completely reversed by intra-prefrontal cortex delivery of MS-275, which had no effect on social interaction behavior in non-defeated control mice.

This reversal of social avoidance, without affecting social behavior in control mice, is similar to effects observed with several standard antidepressant treatments (Berton et al., 2006; Tsankova et al., 2006). Socially defeated mice also displayed increased immobility in the forced swim test and this abnormality too was reversed by MS-275 infusion into the prefrontal cortex (Figure 3C). Interestingly, MS-275 reduced immobility—an antidepressant-like effect—when administered to non-defeated control mice as well. In contrast, MS-275 infusion into the prefrontal cortex did not affect sucrose preference in either control or defeated mice (Figure 3D). We failed to detect a reduction in sucrose preference that is seen in most cohorts of susceptible mice (Krishnan et al., 2007; Covington et al., 2009). MS-275 also did not restore the reduced locomotor activity that defeated mice displayed in an open field (Figure 3E). In a separate study of non-defeated control mice, we found that intra-prefrontal cortex administration of MS-275 for 7 days had no effect on anxiety-like behavior in the light-dark test (Figure 3F).

4. Discussion

In the present study, we demonstrate that exposure to chronic social defeat stress causes a delayed increase in levels of histone acetylation in the medial prefrontal cortex. This increase persisted through 10 days after the last stress episode but returned to control levels by day 20. Chronic administration of fluoxetine, which reverses the behavioral abnormalities induced by chronic social defeat stress in roughly half of defeated animals (Berton et al., 2006; Covington et al., 2009), failed to alter levels of histone acetylation in this brain region. We then went on to demonstrate that selective induction of histone acetylation within the medial prefrontal cortex, via continuous infusion of the highly selective Class I HDAC inhibitor, MS-275, directly into this brain region, exerted antidepressant-like effects in some but not all of the behavioral domains studied. Intra-prefrontal cortex MS-275 completely reversed the profound social avoidance as well as the increase in forced swim immobility induced by chronic social defeat stress, but had no influence on locomotor behavior. We also observed no effect of MS-275 on sucrose preference, although in this experiment we failed to see the expected social defeat-induced decrease in sucrose preference; the reason for this failure is unknown. Finally, intra-prefrontal cortex MS-275 had no effect on baseline anxiety-like behavior as assessed in the light-dark test.

These effects in prefrontal cortex are interesting in light of parallel findings in several other limbic brain regions. Previous studies have shown that chronic social defeat stress causes more transient increases in histone acetylation in hippocampus and amygdala, with effects seen only at 1 or 24 hr after the last defeat episode (Covington et al., 2011), although increases at 10 days are seen in the nucleus accumbens (Covington et al., 2009). It would now be important to study the mechanisms underlying these region-specific and time-dependent effects of social stress on histone acetylation. This is a difficult undertaking given the very large numbers of HATs and HDACs expressed in these various brain regions, each of which might influence histone acetylation levels (Strahl and Allis, 2000; Borrelli et al., 2008; Haberland et al., 2009; Graff and Tsai, 2013). It would also be important to compare stress and fluoxetine regulation of sites of histone acetylation in addition to H3K14ac, although this too is very challenging given the large numbers of such sites on each canonical histone subunit (Strahl and Allis, 2000).

The induction of histone acetylation in the prefrontal cortex would appear to represent an adaptive response to chronic social defeat stress, since our data demonstrate that replicating this effect—via direct inhibition of HDAC activity in this brain region—exerts antidepressant-like effects in the social avoidance and forced swim tests, as noted above. Therefore, the normalization of increased levels of acetylated histones in prefrontal cortex by day 20 after chronic social defeat stress (Figure 2C) might contribute to the behavioral abnormalities that persist at 20 days and beyond (Figure 2B; Krishnan et al., 2007).

The antidepressant-like action of intra-prefrontal cortex HDAC inhibition is similar to the antidepressant-like action of such local inhibition in several other brain regions studied, namely, nucleus accumbens, hippocampus, and amygdala (Covington et al., 2009, 2011). This is a striking finding because the manipulation of most molecules within these brain regions exerts different—in many cases opposite—effects on depression-related behavior, such as cAMP response element binding protein (CREB), BDNF (brain-derived neurotrophic factor (BDNF), and FosB (a Fos family transcription factor), to name just a few (Carlezon et al., 2005; Monteggia and Duman, 2006; Russo and Nestler, 2013; Vialou et al., 2014). However, HDAC inhibition within each of the various brain regions studied to date affects a different range of depression-related behaviors. Whereas HDAC inhibition in the prefrontal cortex reverses abnormalities seen in social avoidance and forced swimming with effects on sucrose preference unclear (see above), such inhibition in the nucleus accumbens robustly reverses abnormalities in all three assays (Covington et al., 2009), whereas HDAC inhibition in hippocampus reverses sucrose preference deficits only and HDAC inhibition in amygdala reverses social avoidance only (Covington et al., 2011). Similarly, HDAC inhibition in hippocampus exerts antianxiety-like effects, with no effect seen for the other three brain regions. These findings provide some insight into the neural circuitry underlying the range of behavioral abnormalities induced by chronic social defeat stress. The findings also highlight the expectation that global inhibition of HDAC activity in the limbic brain would produce the most dramatic therapeutic-like effects.

These observations are interesting in light of the region-specific effects of fluoxetine, which had no effect on histone acetylation in the prefrontal cortex, as seen previously for nucleus accumbens and amygdala as well, although a small induction was seen for hippocampus (Covington et al., 2009, 2011). Given that HDAC inhibition within each of these four brain regions produces some antidepressant-like effects, the inability of fluoxetine to broadly increase histone acetylation in limbic brain regions suggests that HDAC inhibition might offer a novel approach to depression treatment with greater efficacy than standard antidepressants. Indeed, this is borne out by our current and past (Berton et al., 2006; Covington et al., 2009, 2011) observations: MS-275 delivered into the prefrontal cortex or nucleus accumbens appears to exert antidepressant-like effects in a larger percentage of animals (19 out of 24 mice showing reversal of social avoidance) compared with systemic fluoxetine (20 out of 36 showing reversal of social avoidance), suggesting greater intrinsic efficacy of HDAC inhibition, a possibility which now warrants further investigation. Moreover, HDAC inhibition exerts these effects within 7 days, whereas fluoxetine or other standard antidepressants (e.g., imipramine) require 14–20 days for full efficacy (Berton et al., 2006; Tsankova et al., 2006; Covington et al., 2009; Vialou et al., 2010).

Nevertheless, there are two major obstacles for the clinical development of HDAC inhibitors for depression. First, is the difficulty in developing highly selective HDAC inhibitors that efficiently penetrate the brain. MS-275, like all other highly selective inhibitors studied to date, does not readily pass the blood-brain barrier (Hooker et al., 2010). Second, are concerns about the safety of sustained HDAC inhibition in all tissues of the body. HDAC inhibitors have been studied for the treatment of several cancers, but their use is associated with considerable peripheral toxicity (Fraczek et al., 2013). A key question in the field is to determine whether it is possible to inhibit HDACs more selectively in brain, perhaps by targeting HDAC isoforms that are enriched in brain or other brain-enriched proteins that control HDAC activity. An important goal of future research is, therefore, to determine the specific HDAC whose inhibition by MS-275 mediates the antidepressant-like actions observed and to study other ways to achieve such inhibition. As noted earlier, MS-275 inhibits all class I HDACs in vitro, with the highest affinity seen against HDAC1 (Khan et al., 2008), although such selectivity has not been examined in vivo. In any event, results of the present study add impetus for such efforts, given the robust antidepressant-like effects of HDAC inhibition across all brain regions studied to date.

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References

- Abel T, Zukin RS. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. *Curr Opin Pharmacol*. 2008; 8:57–64. [PubMed: 18206423]
- Bagot RC, Labonte B, Peña CJ, Nestler EJ. Epigenetic signaling in psychiatric disorders: stress and depression. *Dialogues Clin Neurosci*. 2014; 16:281–295. [PubMed: 25364280]
- Berton O, McClung CA, DiLeone RJ, Krishnan V, Russo S, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. *Science*. 2006; 311:864–868. [PubMed: 16469931]
- Borrelli E, Nestler EJ, Allis CD, Sassone-Corsi P. Decoding the epigenetic language of neuronal plasticity. *Neuron*. 2008; 60:961–974. [PubMed: 19109904]
- Carlezon WA Jr, Duman RS, Nestler EJ. The many faces of CREB. *Trends Neurosci*. 2005; 28:436–445. [PubMed: 15982754]
- Covington HE III, Maze I, LaPlant QC, Vialou VF, Yoshinori ON, Berton O, Fass DM, Renthal W, Rush AJ III, Wu EY, Ghose S, Krishnan V, Russo SJ, Tamminga C, Haggarty SJ, Nestler EJ. Antidepressant actions of HDAC inhibitors. *J Neurosci*. 2009; 29:11451–11460. [PubMed: 19759294]
- Covington HE 3rd, Vialou VF, Laplant Q, Ohnishi YN, Nestler EJ. Hippocampal-dependent antidepressant-like activity of histone deacetylase inhibition. *Neurosci Lett*. 2011; 493:122–126. [PubMed: 21335060]
- Duman RS. Pathophysiology of depression and innovative treatments: remodeling glutamatergic synaptic connections. *Dialogues Clin Neurosci*. 2014; 16:11–27. [PubMed: 24733968]
- Duman RS, Monteggia LM. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry*. 2006; 59:1116–1127. [PubMed: 16631126]
- Fraczek J, Vanhaecke T, Rogiers V. Toxicological and metabolic considerations for histone deacetylase inhibitors. *Expert Opin Drug Metab Toxicol*. 2013; 9:441–457. [PubMed: 23286281]
- Graff J, Tsai LH. Histone acetylation: molecular mnemonics on the chromatin. *Nat Rev Neurosci*. 2013; 14:97–111. [PubMed: 23324667]

- Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nat Rev Genet.* 2009; 10:32–42. [PubMed: 19065135]
- Hinwood M, Tynan RJ, Day TA, Walker FR. Repeated social defeat selectively increases δ FosB expression and histone H3 acetylation in the infralimbic medial prefrontal cortex. *Cerebral Cortex.* 2011; 21:262–271. [PubMed: 20513656]
- Hollis F, Wang H, Dietz D, Gunjan A, Kabbaj M. 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. *Psychopharmacology.* 2010; 211:69–77. [PubMed: 20454892]
- Hollis F, Duclot F, Gunjan A, Kabbaj M. Individual differences in the effect of social defeat on anhedonia and histone acetylation in the rat hippocampus. *Horm Behav.* 2011; 59:331–337. [PubMed: 20851702]
- Hooker JM, Kim SW, Alexoff D, Xu Y, Shea C, Reid A, Volkow N, Fowler JS. Histone deacetylase inhibitor, MS-275, exhibits poor brain penetration: PK studies of [C]MS-275 using Positron Emission Tomography. *ACS Chem Neurosci.* 2010; 1:65–73. [PubMed: 20657706]
- Kennedy PJ, Feng J, Robison AJ, Maze I, Badimon A, Mouzon E, Chaudhury D, Damez-Werno DM, Haggarty SJ, Han MH, Bassel-Duby R, Olson EN, Nestler EJ. Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. *Nat Neurosci.* 2013; 16:434–440. [PubMed: 23475113]
- Kenworthy CA, Sengupta A, Luz SM, Ver Hoeve ES, Meda K, Bhatnagar S, Abel T. Social defeat induces changes in histone acetylation and expression of histone modifying enzymes in the ventral hippocampus, prefrontal cortex, and dorsal raphe nucleus. *Neuroscience.* 2013; 264:88–98. [PubMed: 23370319]
- Khan N, Jeffers M, Kumar S, Hackett C, Boldog F, Khramtsov N, Qian X, Mills E, Berghs SC, Carey N, Finn PW, Collins LS, Tumber A, Ritchie JW, Jensen PB, Lichenstein HS, Sehested M. Determination of the class and isoform selectivity of small-molecule histone deacetylase inhibitors. *Biochem J.* 2009; 409:581–589. [PubMed: 17868033]
- Krishnan V, Han MH, Graham DL, Berton O, Renthall W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell.* 2007; 131:391–404. [PubMed: 17956738]
- Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. *Nat Rev Neurosci.* 2013; 14:609–625. [PubMed: 23942470]
- Schroeder FA, Lin CL, Crusio WE, Akbarian S. Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biol Psychiatry.* 2007; 62:55–64. [PubMed: 16945350]
- Smart OL, Tiruvadi VR, Mayberg HS. multimodal approaches to define network oscillations in depression. *Biol Psychiatry.* 2015 in press.
- Strahl BD, Allis CD. The language of covalent histone modifications. *Nature.* 2000; 403:41–45. [PubMed: 10638745]
- Sun HS, Kennedy PJ, Nestler EJ. Epigenetics of the depressed brain: Role of histone acetylation and methylation. *Neuropsychopharmacology Rev.* 2013; 38:124–137.
- Tsankova NM, Berton O, Renthall W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in hippocampus in a mouse model of depression and antidepressant action. *Nature Neurosci.* 2006; 9:519–525. [PubMed: 16501568]
- Vialou V, Robison AJ, Laplant QC, Covington HE 3rd, Dietz DM, Ohnishi YN, Mouzon E, Rush AJ 3rd, Watts EL, Wallace DL, Iñiguez SD, Ohnishi YH, Steiner MA, Warren BL, Krishnan V, Bolaños CA, Neve RL, Ghose S, Berton O, Tamminga CA, Nestler EJ. DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. *Nat Neurosci.* 2010; 13:745–752. [PubMed: 20473292]
- Vialou V, Feng J, Robison AJ, Nestler EJ. Epigenetic mechanisms of depression and antidepressant action. *Annu Rev Pharmacol Toxicol.* 2013; 53:59–87. [PubMed: 23020296]
- Vialou V, Bagot RC, Cahill ME, Ferguson D, Robison AJ, Dietz DM, Fallon B, Mazei-Robison M, Ku SM, Harrigan E, Winstanley CA, Joshi T, Feng J, Berton O, Nestler EJ. Prefrontal cortical circuit

for depression- and anxiety-related behaviors mediated by cholecystokinin: role of FosB. *J Neurosci*. 2014; 34:3878–3887. [PubMed: 24623766]

Zhu H, Huang Q, Xu H, Niu L, Zhou JN. Antidepressant-like effects of sodium butyrate in combination with estrogen in rat forced swimming test: involvement of 5-HT(1A) receptors. *Behav Brain Res*. 2009; 196:200–206. [PubMed: 18817816]

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Highlights

We studied the role of histone acetylation in the medial prefrontal cortex in stress models.

Chronic social defeat stress increased levels of histone acetylation in medial prefrontal cortex.

In contrast, chronic administration of fluoxetine had no effect on histone acetylation in medial prefrontal cortex.

Histone deacetylase inhibition in medial prefrontal cortex exerted antidepressant-like effects after chronic social stress.

These findings further support the evaluation of histone deacetylase inhibitors as novel antidepressant treatments.

Figure 1. Regulation of histone acetylation in the prefrontal cortex by chronic social defeat

A. Timeline of experiment. **B.** Social interaction behavior expressed as time in an interaction zone in the absence (no target) or presence (target) of another mouse. Results shown are at the 24 hr time point ($n=10/\text{group}$); equivalent results were seen for 1 hr and 10 days (not shown). **C.** Levels of H3K14ac, and total levels of histone H1 as a control, were measured in brain using immunohistochemistry. Representative sections, obtained 10 days after chronic (10 days) social defeat stress, are shown. The region quantified is marked by the dashed line and arrow. **D.** H3K14ac (normalized to total H1) was increased in the medial prefrontal cortex 24 hr and 10 days after the last defeat, with no change seen at 1 hr. Differences between non-stressed controls and chronically defeated mice at each time point are denoted by # to indicate significance at $p < 0.05$ ($n=8/\text{group}$ at 1 hr, $10/\text{group}$ at 24 hr, and $12/\text{group}$ at 10 days). Error bars indicate SEM.

Figure 2. Regulation of histone acetylation in the prefrontal cortex by chronic fluoxetine administration

A. Timeline of experiment. **B.** Social interaction behavior expressed as time in an interaction zone in the presence of another mouse. The same mice were studied at two time points: day 11, 24 hr after 10 days of chronic social defeat stress or control conditions; and day 31, after 20 subsequent days of fluoxetine (FLU) or sham treatment (n=12/group). **C.** Levels of H3K14ac, and total levels of histone H1 as a control, were measured in brain using immunohistochemistry. No effect of fluoxetine was seen in either control or defeated animals. Differences between non-stressed controls and chronically defeated mice at each time point are denoted by # to indicate significance at $p < 0.05$. Error bars indicate SEM.

Figure 3. Behavioral effects of HDAC inhibition in the prefrontal cortex

Mice (n=12/group) were subjected to chronic (10 days) social defeat stress and, beginning 24 hr later, were infused intra-prefrontal cortex with MS-275 (100 μ M). Mice were then analyzed in a battery of behavioral tests starting 5 days after initiation of MS-275 infusion.

A. MS-275 infusion increased H3K14ac levels within the infused region (see text for quantification of this effect). **B.** Intra-prefrontal cortex MS-275 infusion reversed the deficit in social interaction seen in defeated mice, without affecting social interaction in non-stressed control animals. **C.** Likewise, MS-275 infusion reversed the increase in immobility in the forced swim test induced by social defeat, with a similar reduction in immobility seen in non-stressed control animals. **D.** In contrast, MS-275 infusion had no effect on sucrose preference in stressed or control mice, however, the expected reduction in this measure in defeated animals was not significant. **E.** MS-275 infusion also had no effect on sucrose preference in stressed or control mice. Note the reduction in locomotor activity in stressed mice with or without MS-275 infusions. **F.** In a separate experiment (n=12/group), non-stressed control mice were assessed for anxiety-like behavior in the light-dark test, a measure which was not affected by intra-prefrontal cortex MS-275 infusion. Differences between MS-275-infused animals and vehicle-infused animals are denoted by asterisks (*), and differences between non-stressed controls and defeated mice are denoted by #, in both cases to indicate significance at $p < 0.05$. Error bars indicate SEM.