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Histone deacetylase inhibition reduces ventral tegmental area dopamine neuronal hyperexcitability involving AKAP150 signaling following maternal deprivation in juvenile male rats

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Abstract

Traumatic early life stress (ELS) is linked to dopamine (DA) dysregulation which increases the probability of developing psychiatric disorders in adolescence and adulthood. Our prior studies demonstrated that a severe early life stressor, a 24hr maternal deprivation (MD) in juvenile male rats, could lead to altered DA signaling from the ventral tegmental area (VTA) due to impairment of GABAergic synaptic plasticity (promoting GABAergic long-term depression, LTD) with concomitant changes in the abundance of synaptic regulators including A-kinase anchoring protein (AKAP150). Importantly, these MD-induced synaptic changes in the VTA were accompanied with upregulation of histone deacetylase 2 (HDAC2), histone hypoacetylation, and were reversible by HDAC inhibition (Authement et al., 2015; Shepard et al., 2018). Using cell-attached and whole cell patch clamp recordings, we found that MD stress also increased spontaneous VTA DA neuronal activity and excitability in juvenile male rats without affecting intrinsic excitability. Postsynaptic chemical disruption of AKAP150 and protein kinase A (PKA) interaction increased VTA DA neuronal excitability in control non-MD rats mimicking the effects of MD on DA cell excitability with similar changes in membrane properties. Interestingly, this disruption decreased MD-induced VTA DA hyperexcitability. This MD-induced DA neuronal hyperexcitability could also be normalized at 24hrs after injection of the class 1 HDAC inhibitor, CI-994. Altogether, our data suggest that AKAP150 plays a critical role in regulation of VTA DA neuronal excitability and that HDAC-mediated targeting of AKAP150 signaling could normalize VTA DA dysfunction following ELS thereby providing novel therapeutic targets for prevention of later-life psychopathology.

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Author Contributions

RS, LL, MA, and FN designed the experiments. RS, LL, MA, and FN performed electrophysiology experiments. RS, LL, MA, and FN analyzed the data and prepared the figures. RS and FN wrote the manuscript.

Conflict of Interest Statement

The authors declare no competing financial interests.

Data Availability Statement

All relevant data is contained within the manuscript. All datasets [GENERATED/ANALYZED] for this study are included in the manuscript.

Keywords

AKAP; dopamine; epigenetics; histone acetylation; HDAC; VTA

Introduction

Early life stress (ELS) has been shown to increase the risk of developing stress-related disorders ranging from depression to substance abuse disorders in adulthood (Nemeroff, 2016; Targum & Nemeroff, 2019). Monoaminergic dysregulation has been implicated to be central in the development of these psychiatric disorders. Specifically, dysregulation of DA neurotransmission from the VTA has been observed in a multitude of clinical observations, as well as animal models, related to neuropsychiatric illnesses (Ellenbroek, Derks, & Park, 2005; Pruessner, Champagne, Meaney, & Dagher, 2004). ELS-induced effects on DA signaling could result in critical gene expression changes involving epigenetic modifications which ultimately affect VTA DA cellular signaling, synaptic plasticity, and behavior (Nestler, Pena, Kundakovic, Mitchell, & Akbarian, 2016; Sultan & Day, 2011). One such epigenetic modification that can be targeted by ELS is histone acetylation (Bannister & Kouzarides, 2011; Kouzarides, 2007). In brief, alterations in histone acetylation are governed by two classes of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). Generally, histone acetylation by HATs leads to chromatin relaxation and promotes gene expression; histone deacetylation by HDACs is associated with chromatin condensation resulting in gene repression (Sun, Kennedy, & Nestler, 2013). Histone hyperacetylation of chromatin by HDAC inhibition using HDAC inhibitors (HDACi) has been shown to improve learning and memory by impacting synaptic plasticity (Graff et al., 2014; Graff & Tsai, 2013). Additionally, the novel use of HDAC inhibitors has been proposed in a wide variety of neurological conditions such as post-traumatic stress disorder (Whittle & Singewald, 2014), depression (Misztak, Panczyszyn-Trzewik, & Sowa-Kucma, 2018), spinal cord injury (S. Zhang, Fujita, Matsuzaki, & Yamashita, 2018) and addiction (Anderson et al., 2019; Kennedy et al., 2013; Kennedy & Harvey, 2015) due to the ability of HDAC inhibitors to penetrate the central nervous system and attenuate the negative symptoms associated with these conditions through epigenetic and non-epigenetic mechanisms.

Consistently, our lab has shown that administration of HDAC inhibitors ameliorate ELS- as well as morphine-induced changes in histone acetylation and synaptic plasticity in the VTA (Authement et al., 2015; Authement et al., 2016; Shepard et al., 2018). In our ELS studies, we use a model of child neglect, maternal deprivation (MD), where pups are isolated from the dam for a 24hr period. MD preferentially targeted GABAergic synapses onto VTA DA neurons and impaired the ability of these synapse to exhibit Hebbian spike timing dependent plasticity, STDP through disruption of AKAP150 (Authement et al., 2015). AKAP150 scaffold protein (AKAP150 in rodents, the human ortholog being AKAP79/AKAP5) plays a pivotal role in synaptic plasticity through anchoring of PKA, calcineurin and other signaling molecules such as PKC to their substrates, such as synaptic receptors including AMPA, NMDA, and GABA_A receptors, and ion channels (Sanderson & Dell'acqua, 2011; Wild & Dell'Acqua, 2017). We previously found that AKAP150 selectively regulates GABA_A

receptor trafficking and plasticity in VTA DA neurons and displays impaired interactions with PKA (Dacher, Gouty, Dash, Cox, & Nugent, 2013). Additionally, MD-induced changes in AKAP150 favored signaling through calcineurin and promoting LTD at GABAergic synapses in VTA DA neurons through changes in its localization at synapses (Authement et al., 2015; Shepard et al., 2018). We also showed that mBDNF in the VTA is downregulated by MD in parallel with decreased histone H3 acetylation at lysine 9 (Ac-H3K9) and upregulation of HDAC2 in VTA DA neurons. Importantly, we demonstrated that *in vivo* HDAC inhibition reversed these epigenetic modifications and altered AKAP150 and BDNF expression associated with MD in the VTA (Shepard et al., 2018), suggesting that AKAP150, BDNF and HDAC2 represent potential targets for later life reversal of ELS-induced neuroplasticity in VTA reward circuits.

To expand our earlier studies, here we show that MD increases spontaneous neuronal activity of VTA DA neurons which may involve altered AKAP150 and HDAC signaling; *in vivo* HDAC inhibition can reduce MD-induced VTA DA neuronal hyperexcitability for up to 24hrs. These data along with our previous studies contribute to our understanding of how AKAP150 can influence VTA DA function through its synaptic effects and the potential use of HDACi as a novel class of drug for prevention of ELS-induced DA dysfunction.

Materials and Methods

All experiments were carried out in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals and were approved by the Uniformed Services University Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

Housing and Husbandry

Experiments were conducted on juvenile male rats, postnatal day 14 to postnatal day 21 (P14-P21). For this study 127 animals across 16 litters were used. Upon arrival to the research institution, animals were allowed to acclimate in the laboratory for animal research for 48hrs. Juvenile male rats were housed with the dam for the entire study minus the maternal deprivation procedure. Rats were maintained on a 12 hour light/dark cycle and provided food and water ad libitum. Both the dam and juvenile rats were checked daily for illness or distress. Any male rats displaying illness were not used in the study.

Maternal Deprivation Procedure

At age P9, half of each litter of male Sprague–Dawley rat (Taconic Farms) pups were isolated at 10:00 a.m. from the dam and their siblings for 24hr (MD group). The isolated rats were placed in a separate quiet room and kept on a heating pad (34° C) and not disturbed until being returned to their home cage. The remaining non-separated male rat pups received the same amount of handling and served as the non-maternally deprived control group (non-MD group). Each day, two MD and non-MD rats (age-matched) of the same litter were sacrificed over P14–21 for electrophysiology recordings as previously described (Authement et al., 2015; Shepard et al., 2018).

Slice preparation for Electrophysiology

Rats were briefly anesthetized with isoflurane (3–5%) and decapitated. The brains were quickly dissected and placed into ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 21.4 NaHCO₃, 2.5 KCl, 1.2 NaH₂PO₄, 2.4 CaCl₂, 1.00 MgSO₄, 11.1 glucose, 0.4 ascorbic acid, saturated with 95% O₂–5% CO₂. Horizontal slices containing the VTA were cut at 250 µm and incubated in ACSF at 34°C for at least 1 hr. Slices were then transferred to a recording chamber and perfused with ascorbic acid-free ACSF at 28°C.

Electrophysiology

Whole cell and cell-attached recordings were performed on midbrain slices using a patch amplifier (Multiclamp 700B) under infrared-differential interference contrast microscopy. Data acquisition and analysis were carried out using DigiData 1440A, pClamp 10 and Clampfit (Molecular devices, Union City, CA). Signals were filtered at 3 kHz and digitized at 10 kHz. The recording ACSF was the same as the cutting solution except that it was ascorbic acid-free. Spontaneous activity was monitored using cell-attached voltage-clamp recordings of action potentials (APs) at $V = 0$ mV for 5 minutes with intact synaptic transmission. Cells were categorized as either “spontaneously active” or “silent”. The appearance of APs during the 5 min recording was used to classify cells as spontaneously active or silent. Number of APs was counted over 5 min and spike frequency was calculated. At the end of each recording, whole cell patch clamp configuration was established to measure hyperpolarization-activated currents (I_h). The appearance of an I_h current (50 pA) in response to stepping cells from –50 mV to –100 mV was used to identify putative VTA DA neurons as previously described (Dacher et al., 2013). Neuronal excitability recordings in response to depolarization were performed in whole cell current-clamp mode with intact synaptic transmission. The patch pipettes (3–6 MΩ) were filled with 130 mM K-gluconate, 15 mM KCl, 4 mM ATP-Na⁺, 0.3 mM GTP-Na⁺, 1 mM EGTA, and 5 mM HEPES (pH adjusted to 7.28 with KOH, osmolarity adjusted to 275–280 mOsm). To measure AP generation in response to membrane depolarization, VTA DA neurons were given increasingly depolarizing current steps from +50 pA to +500 pA (+50 pA intervals, 5 sec duration each). Current injections were separated by a 20 s inter-stimulus interval and neurons were kept at –65 mV with manual direct current injection between pulses. The number of APs induced by depolarization at each intensity was counted and averaged for each experimental group. AP threshold, medium after hyperpolarization (mAHPs) and fast after hyperpolarizations (fAHP) amplitudes were measured at the current step that was sufficient to generate the first AP/s as previously described (Authement et al., 2018). Briefly, AP threshold was measured at the beginning of the upward rise of the AP. mAHPs were measured as the difference between AP threshold and the peak negative membrane potential at the end of the current step. fAHPs were calculated as the difference between AP threshold and the peak negative potential following the AP. Input resistance (R_{in}) was determined in recordings at 50 pA depolarizing current pulse (5 s) and calculated using Ohm’s law by dividing the steady-state voltage response by the current pulse amplitude. All of these measurements were taken from the same set of recorded cells. The cell series resistance was monitored through all the experiments and if this value changed by more than 10%, data were not included.

Drug treatment

Fast synaptic transmission was blocked by adding 6,7-dinitroquinoxaline-2,3-dione (DNQX; 10 μ M), picrotoxin (100 μ M) and APV (50 μ M) to block AMPA, GABA_A and NMDA receptor-mediated synaptic transmission, respectively. Synaptic blockers were present from the start of each recording in the ACSF perfusion. AKAP150 inhibitor, Ht31 and Ht31p (control peptide) (1 μ M) were obtained from Promega. Ht31p and Ht31 were included in the patch pipette internal solution for delivery via intracellular dialysis.

For HDAC inhibitor treatment, Sprague–Dawley male rats (P14–P21) (non-MD or MD) received either one intraperitoneal (i.p.) injection of CI-994 (10mg/kg) dissolved in 1% Tween80 (vehicle) or an injection of comparable volumes of 1% Tween80 (vehicle) 24hr prior to sacrifice for electrophysiological recordings. Synaptic blockers, HDAC inhibitor, CI-994, were purchased from Tocris.

Data analysis

Data analysis was performed blindly to ensure reproducibility of results. Since only one cell was recorded per animal for electrophysiological recordings, the n-value represents both the total number of cells as well as the total number of animals used in the study. Values are presented as means $\pm$ SD. Statistical significance was determined using unpaired two tailed Student's t-test, one-way or two-way ANOVA with Bonferroni post hoc analysis. Two-way ANOVA was used with AP number as the dependent variable repeated at each step of depolarizing current for stress, drug treatment or combined stress+drug to reveal whether stress or drug had a significant effect on the membrane excitability in VTA DA neurons. The threshold for significance was set at $*p < 0.05$ for all analyses. All statistical analyses were performed using GraphPad Prism 7 or Origin 2016.

Results

MD increased VTA DA neuronal activity via changes in synaptic transmission.

Given our previous study showed that MD does not affect glutamatergic synaptic transmission and plasticity, but favors LTD at GABAergic synapses onto VTA DA neurons (Authement et al., 2015), we made the assumption that this MD-induced GABAergic LTD should lead to increased DA cell excitability. To test this assumption, we first recorded VTA DA spontaneous neuronal activity using cell-attached recordings in voltage-clamp mode to determine the percentage of DA neurons that were spontaneously active while synaptic transmission was intact. Consistently, we observed that the proportion of spontaneously active DA neurons was larger in MD rats compared to non-MD rats, although DA neurons displaying spontaneous activity did not differ in spike frequencies between non-MD and MD rats (Figure 1A). Although induction of GABAergic LTD in VTA DA neurons by MD could underlie MD-induced DA neuronal hyperexcitability, it was also possible that MD induced an increase in intrinsic excitability (through changes in active and passive membrane properties) independent of synaptic transmission. However, we found no significant change in the number of APs produced in response to depolarizing current injection (50–500pA depolarizing current steps) between non-MD and MD rats with fast synaptic transmission

blocked (Figure 1B, $n=8$ non-MD rats, $n=14$ MD rats, MD stress: $F(1, 200) = 1.248$, $P=0.2653$, current steps: $F(9, 180) = 3.628$, $P=0.0003$; interaction: $F(9, 180) = 0.4282$, $P=0.9187$; two-way ANOVA).

Inhibition of AKAP-PKA interaction alters DA cell excitability differently in non-MD and MD rats.

In our previous studies, we found that AKAP150 signaling selectively regulates GABAergic synaptic transmission and plasticity in VTA DA neurons as disruption of AKAP150-PKA association with the AKAP inhibitor (intra-pipette inclusion of Ht31, a small peptide inhibitor which disrupts the binding of PKA-RiIB to its binding domain on AKAP150, resulting in intracellular dialysis) does not affect AMPAR-mediated glutamatergic synaptic transmission (Dacher et al., 2013). Instead, AKAP150-PKA disassociation by Ht31 induces a significant rundown of GABA_A transmission in VTA DA neurons in a calcineurin-dependent manner (induction of a chemical GABAergic LTD) (Dacher et al., 2013). Moreover, MD-induced impairment GABAergic STDP is dependent on PKA-AKAP150-Calcineurin signaling (Authement et al., 2015). In order to assess the role of AKAP150-PKA signaling on DA cell excitability, we tested how disruption of AKAP150-PKA binding would affect VTA DA neuronal firing in response to depolarization in slices from non-MD and MD rats while synaptic transmission is intact. Using intra-pipette Ht31 and Ht31p (control peptide) in recordings from DA neurons in midbrain slices from non-MD and MD rats, we measured DA neuronal excitability in response to 50–500pA depolarizing current steps. VTA DA neuronal excitability was evaluated at 30 min after the initiation of whole cell patch clamp recordings to allow sufficient time for Ht31 intracellular diffusion and maximal rundown of IPSC as previously described (Authement et al., 2015; Dacher et al., 2013). We detected a significant increase in the number of APs triggered in response to depolarization at 30 min after initiation of the whole cell recordings in DA neurons with Ht31-filled pipettes compared to recordings with control Ht31p-filled pipettes at the same time point in slices from non-MD rats. Statistical analysis was performed between Ht31p and Ht31 experiments (Figure 2A, Ht31p: $n=7$ non-MD rats, $n=10$ non-MD rats, drug: $F(1,150)=48.13$, $P=0.0001$, current steps: $F(9, 150) = 4.781$; $P=0.0001$; interaction: $F(9, 150) = 0.6628$, $P=0.7415$; two-way ANOVA). Given that AKAP150 can affect neuronal excitability through changes in subsets of postsynaptic ion channel conductance and trafficking (such as L-type Ca²⁺ channels (Murphy et al., 2014b) or A-type voltage gated K⁺ channels (Lin, Sun, Kung, Dell'Acqua, & Hoffman, 2011), we also analyzed Ht31's effects on different characteristics of APs and intrinsic neuronal excitability including fAHPs, mAHPs, AP threshold potential and Rin. Of these, Ht31 treatment significantly lowered AP threshold and increased Rin compared to those from Ht31p experiments (Figure 2B, measurements were derived from AP recordings in Figure 1A, Rin: $t(15)=1.77$, $p=0.0485$; AP threshold: $t(14)=2.31$, $p=0.0183$; fAHP: $t(14)=0.4289$, $p=0.3372$; mAHP: $t(14)=0.4133$, $p=0.3428$, unpaired Student's *t* test). Although, we expected that MD-induced hyperexcitability in VTA DA neurons should occlude the increase in VTA DA excitability by AKAP150-PKA dissociation as observed with Ht31 experiments in non-MD rats, we observed that intra-pipette administration of Ht31 decreased VTA DA neuronal excitability in VTA DA neurons in MD slices with respect to Ht31p control experiments (Fig 3A, Ht31p: $n=7$ MD rats, Ht31: $n=8$ MD rats, drug: $F(1,130)=15$, $P=0.0002$; current steps: $F(9, 130) = 3.039$, $P=0.0025$;

interaction: $F(9, 130) = 0.9397$; $P=0.4933$, two-way ANOVA). It should be noted that Ht31p experiments in non-MD and MD rats still showed the effect of stress in increasing VTA DA excitability (comparison was between the Ht31p data from Fig 2A and Fig 3A in non-MD and MD rats, respectively; Ht31p: $n=7$ non-MD rats, $n=10$ MD rats, stress: $F(1,120)=8.201$, $P=0.0049$, current steps: $F(9, 120) = 2.138$, $P=0.0312$; interaction: $F(9, 120) = 0.994$, $P=0.4488$; two-way ANOVA). We then evaluated intrinsic properties to account for this decreased excitability; however, there was no statistical significance in Rin, AP threshold, fAHP, or mAHP. Although not statistically significant, it is important to note that Ht31 decreased the Rin (Figure 3B, measurements were derived from AP recordings in Figure 3A, Rin: $t(13)=1.049$, $p=0.1566$; AP threshold: $t(13)=0.117$, $p=0.4543$; fAHP: $t(13)=0.4107$, $p=0.344$; mAHP: $t(13)=0.2645$, $p=0.3978$, unpaired Student's t test).

HDAC inhibition reversed MD-induced DA hyperexcitability in the VTA.

Our most recent work shows that MD induces histone hypoacetylation at H3K9 and was associated with an increase in HDAC2 in VTA DA neurons (Shepard et al., 2018). *In vivo* administration of CI-994, a class I HDAC inhibitor, is sufficient to recover MD-induced changes in histone acetylation, AKAP150 and mBDNF levels up to 24hrs. To test the effects of HDAC inhibition on VTA DA cell excitability, we first assessed VTA DA neuronal activity in response to injection of depolarizing current steps in slices from MD rats compared to those from control non-MD rats with intact synaptic transmission. Consistent with cell-attached recordings of basal neuronal firing, we detected a significant increase in the number of APs triggered in response to depolarization in MD rats compared to those from non-MD rats. We then injected non-MD and MD rats with vehicle or CI-994 and evaluated DA neuronal excitability in response to depolarization at 24hr-post injection. Vehicle treatment did not affect DA cell excitability between non-MD and MD rats; therefore, we pooled the data from non-injected and vehicle-injected rats in future comparisons. CI-994 treatment significantly reduced AP generation in response to depolarization in DA neurons from MD rats injected with CI-994 compared to vehicle+MD rats (Figure 4B, $n=17$ cells from 17 non-MD and vehicle+non-MD rats; $n=18$ cells from 18 MD and vehicle+MD rats; $n=9$ cells from 9 non-MD+CI-994 rats; $n=10$ cells from 10 MD +CI-994 rats, stress+drug: $F(3,500)=17.77$, $P=0.0001$; current steps: $F(9, 500) = 3.648$, $p=0.0002$, interaction: $F(27, 500) = 0.2002$, $P=0.9999$, two-way ANOVA). When evaluating intrinsic membrane properties, MD was also associated with a significant increase in Rin and a lower AP threshold. CI-994 treatment reversed MD-induced increases in Rin while the treatment was ineffective in normalizing the effects of MD on AP threshold (Figure 4C, measurements were derived from recordings in Figure 4B, Rin: $F(3, 47) = 5.01$, $p=0.0043$; AP threshold: $F(3, 45) = 3.428$, $p=0.0248$; fAHP: $F(3, 45) = 0.4539$, $p=0.7158$; mAHP: $F(3, 45) = 0.691$, $p=0.5623$, one-way ANOVA).

Discussion

It has been shown that repeated or prolonged exposure to stress increases DA levels in the projection targets of the VTA, such as NAc and PFC, and this increased dopaminergic activity is linked to heightened impulsivity and anxiety-like behaviors from adolescence and adulthood (Pruessner et al., 2004; Rentesi et al., 2013). Specifically, changes in VTA DA

signaling have been shown to contribute to a myriad of behavioral phenotypes associated with ELS (Authement et al., 2015; Jahng et al., 2010; Pena et al., 2017; Whitaker, Degoulet, & Morikawa, 2013). Epigenetic processes play a role in driving these ELS-induced behavioral adaptations involving reward-seeking behaviors and motivation (Klengel & Binder, 2015). Previously we have found that MD engages an epigenetic mechanism involving HDAC2 and histone hypoacetylation at H3K9 that may play an important role in GABAergic synaptic plasticity in VTA DA neurons through localization of AKAP150 signaling (Authement et al., 2015; Shepard et al., 2018). Here, we investigated whether AKAP150 also regulates DA cell excitability and found that the association of PKA and AKAP150 significantly influenced cell excitability and intrinsic membrane properties of VTA DA neurons. Interestingly, we observed that AKAP150-PKA disruption increased AP generation in non-MD animals whereas the opposite effect was observed in MD animals (less APs produced). Consistent with our previous studies, we found that MD-induced increases in DA cell excitability were also reversed by *in vivo* HDAC inhibition. Therefore, targeting AKAP150 and HDACs may present a novel pharmacotherapy that can potentially be used as treatment and/or intervention by recovering changes in histone acetylation at possible critical windows of development to prevent later psychopathology.

AKAP150 has a critical role in synaptic receptor trafficking and plasticity of glutamatergic and GABAergic synapses. Specifically, AKAP150 can modulate neuronal excitability not only through synaptic transmission, but also by direct interaction and modulation of ion channel activity and trafficking of postsynaptic ion channels that regulate intrinsic neuronal excitability (Lin et al., 2011; Murphy et al., 2014a; Welch, Jones, & Scott, 2010). Interestingly, individuals carrying AKAP5 polymorphisms show altered emotional processing and behavioral responses including aggression, expression of anger and impulsivity associated with alterations in function in limbic regions (Richter et al., 2013; Richter et al., 2011; Suryavanshi, Jadhav, & McConnell, 2018). Moreover, copy number variations in AKAP5 have been found in DNA samples of schizophrenia patients but not control subjects (Suturala et al., 2007), suggesting the possible involvement of AKAP5 in the pathogenesis of schizophrenia, a neurodevelopmental disorder also linked to DA dysfunction, ELS, and high rates of addiction (Scheller-Gilkey, Moynes, Cooper, Kant, & Miller, 2004; Scheller-Gilkey, Thomas, Woolwine, & Miller, 2002; Winklbaur, Ebner, Sachs, Thau, & Fischer, 2006). Given these observations, drugs targeting the AKAP150 complex could provide efficacy in treating psychiatric disorders where AKAP signaling is dysregulated. Our previous work shows that in response to MD, there is upregulated AKAP150 expression in VTA synaptosomal fractions, but decreased PKA-RIIB abundance indicating possible disrupted AKAP150-PKA signaling (Shepard et al., 2018). Here we found that disruption of AKAP150-PKA association by intra-pipette Ht31 increased neuronal excitability of DA neurons in non-MD while synaptic transmission was intact. Although reduction of GABAergic inhibition (through chemical induction of GABAergic LTD here) could promote DA cell excitability (Johnson & North, 1992; Theile, Morikawa, Gonzales, & Morrisett, 2011), the Ht31-induced increase in DA cell excitability was also associated with significant increases in membrane resistance and a lower AP threshold. These membrane property changes suggest that AKAP150-PKA anchoring may be essential in trafficking or gating of a subset of K^+ leak channels that are open at rest and maintain the

membrane potential of DA neurons. Interestingly, we also found that DA neurons from MD rats fired more APs in response to depolarization compared to those from non-MD control rats. Similar changes to Ht31-induced hyperexcitability of DA neurons in membrane resistance and threshold potential were detected in DA neurons in MD rats compared to non-MD rats (i.e., higher R_{in} with lower AP threshold). Consistently, *in vivo* HDAC inhibition reduced DA cell excitability of DA neurons in MD rats at 24hr after a single injection of CI-994 and reversed the effects of MD on membrane resistance, but not on AP threshold. We suggest that in addition to MD-induced changes in AKAP-dependent GABAergic plasticity, an AKAP150-dependent dysregulation of neuronal excitability through changes in activity or trafficking of postsynaptic K^+ channels may contribute to hyperexcitability of DA neurons in MD rats. Furthermore, normalization of AKAP150 expression by *in vivo* HDAC inhibition may be sufficient to reverse MD-induced changes in synaptic and neuronal function of DA neurons through proper anchoring and targeting of signaling enzymes (kinases and phosphatases) to ion channels and synaptic receptors. For example, AKAP150 has been shown to regulate hippocampal CA1 neuronal excitability through PKA-anchoring to A-type voltage-gated K^+ (Kv) channels (Lin et al., 2011). Other interesting candidates for AKAP150 signaling includes members of the family of KCNQ voltage-gated K^+ channels (Hoshi et al., 2003) and the family of two-pore-domain (KCNK) K^+ Channels (Sandoz et al., 2006). It is also worth mentioning that AKAP150 plays a direct role in transcriptional expression of K^+ channels and control of neuronal excitability through organization and targeting of signaling complexes that include calcineurin/nuclear factor of activated T-cells (NFAT) signaling pathway (Murphy et al., 2014a; J. Zhang & Shapiro, 2012). Another interesting premise is the role of G-Protein Gated Inwardly Rectifying Channels (GIRK) within the context of modulation of potassium channel activity. VTA DA neurons express solely GIRK2c and GIRK3 (Cruz et al., 2004). GIRKs control the excitability of VTA DA neurons by hyperpolarizing neurons when activated and thus preventing AP generation (Rifkin, Moss, & Slesinger, 2017). The activation/inactivation of GIRKs has been shown to be modulated by phosphorylation of PKA (Rusinova et al., 2009). Dephosphorylation of GIRKs has been shown to be mediated by protein phosphatase 2A (PP2A) and Phospholipase C (PLC) (Glaaser & Slesinger, 2015). Given that PLC can activate PKC which directly interacts with AKAP150, GIRK activity could be altered through AKAP150 interactions with PKA and PKC.

In juxtaposition to our findings of intra-pipette disruption of AKAP150-PKA in non-MD animals, we also found that Ht31 decreased the excitability and input resistance of DA neurons in MD rats; however, this decrease in input resistance was not significant. While we expected that MD would occlude Ht31's effect on VTA excitability and intrinsic properties, we were surprised to find that intra-pipette dialysis of Ht31 in DA cells mirrors the effects observed by *in vivo* HDACi administration in MD rats. We have previously shown that PKA activity rather than changes in PKA expression can be increased by MD and this is associated with increased neuronal excitability in the lateral habenula during early adolescence (Authement et al., 2018). It would be interesting to test whether the enzymatic activity of PKA has been altered following MD in VTA DA neurons and that disruption of the AKAP150-PKA association normalizes VTA DA cell excitability through AKAP150-PKA-dependent alterations in potassium channel modulation and/or trafficking.

Another possibility would be that AKAP150-PKA disruption affects glutamatergic plasticity in MD rats. In hippocampus, AKAP150 has been shown to be a requirement for both NMDARs in LTP/LTD (Jurado, Biou, & Malenka, 2010; Sanderson & Dell'acqua, 2011) as well as homeostatic synaptic plasticity through insertion and maintenance of calcium-permeable AMPARs (Sanderson, Scott, & Dell'Acqua, 2018). Both observations involve the interplay of PKA and calcineurin that are docked to the AKAP150 complex. Therefore, it is also possible that MD affects glutamatergic plasticity involving AKAP150 signaling and this disruption by Ht31 produces the dual effects seen when synaptic transmission is intact. The contribution of changes to glutamatergic plasticity and the involvement of AKAP150 and HDACs within the VTA warrants additional study within the context of promoting VTA DA dysregulation.

In our recent study, we showed that mBDNF expression changed by MD and this was reversible by HDAC inhibition (Shepard et al., 2018) possibly through lowering HDAC2 activity and thus increasing acetylation of H3K9. Given that BDNF is known as a key regulator for synaptic plasticity and neuronal excitability, it is possible that epigenetic recovery of BDNF signaling impacted by ELS recovers normal VTA synaptic and cellular function (Fuchikami, Yamamoto, Morinobu, Takei, & Yamawaki, 2010; Roth, Lubin, Funk, & Sweatt, 2009). Quite possibly, there is a cross-talk between BDNF and AKAP150 signaling that merits further investigation.

Due to a lack of understanding of the etiology of ELS-induced changes to VTA DA circuitry, there are only a few treatment options that provide symptomatic relief. Our previous studies provided data that there is a role for increased HDAC activity within the VTA which results in a loss of GABAergic inhibition onto DA neurons involving both AKAP150 and BDNF signaling. This study highlights that ELS-induced changes in synaptic plasticity as well as VTA DA neuronal excitability involving AKAP150 signaling could be normalized by HDAC inhibitors. One study limitation is the non-specificity of Ht31 which impairs all AKAP-PKA interactions. To further elucidate the mechanisms behind impaired AKAP150/PKA signaling following MD, the use of AKAP mutant mice (Murphy, Crosby, Dittmer, Sather, & Dell'Acqua, 2019; Murphy et al., 2014a; Sanderson et al., 2012) would be complementary to confirm the role of AKAP150 signaling in VTA DA excitability following MD. The other study limitation is that the effect of HDAC inhibition in normalizing local AKAP150 signaling to reduce VTA DA neuronal excitability was observed only in juvenile male rats. Therefore, this warrants the question of whether MD induces the same changes in histone acetylation in females and whether this epigenetic regulation of AKAP150 impacts VTA physiology and plasticity. Overall, our data suggests that targeting HDACs and AKAP150 can potentially be used for as early pharmacological intervention for ameliorating DA cell dysfunction associated with early life adversities via restoration of AKAP150 signaling complexes.

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Significant statement

Histone acetylation and deacetylation are epigenetic modifications that play an important role in chromatin remodeling and regulation of gene expression. Changes in HDAC levels and its enzymatic activity have a potent role in pathogenesis and progression of neuropsychiatric disorders and drug addiction. Shepard et al. demonstrate that scaffold protein AKAP150 plays an important role in VTA DA excitability and that HDAC inhibition can normalize VTA DA neuronal hyperexcitability associated with a severe early life stress (maternal deprivation) possibly through AKAP150 signaling. Therefore, HDAC inhibitors and peptides targeting the AKAP150 complex could represent promising compounds for prevention of later-life psychopathology associated with early life stress.

Figure 1. MD increases activity of VTA DA neurons that is dependent on synaptic transmission. (A) Sample cell-attached voltage clamp AP recordings from VTA DA neurons depicting silent and spontaneously active neurons with proportional distribution of these neurons in non-MD and MD rats displayed as pie charts (57% vs 73% active neurons, respectively) and spike frequency bar graph. (B) AP recordings in response to depolarizing current steps in the presence of fast synaptic transmission antagonists for AMPAR, NMDAR, and GABA_AR with representative AP traces in response to 200pA depolarizing current step. In all recordings in this graph and the subsequent graphs only one cell/rat was recorded, therefore the n-value represents total number of animals used.

Figure 2. Disruption of AKAP150-PKA complex increases VTA DA neuronal excitability in non-MD rats.

(A) All recordings in this graph were performed with intact synaptic transmission. Graph shows AP recordings in response to depolarizing step currents with representative AP traces (in response to a 200pA depolarizing current step) with intra-pipette inclusion of either control peptide Ht31p (1 μ M) or active AKAP150 inhibitor peptide Ht31 (1 μ M). (B) Average bar graphs of Rin, threshold potential, and the amplitude of fAHP, and mAHP derived from recordings in Figure 2A.

Figure 3. Disruption of AKAP150-PKA complex decreases VTA DA neuronal excitability in MD rats.

(A) All recordings in this graph were performed with intact synaptic transmission. Graph shows AP recordings in response to depolarizing step currents with representative AP traces (in response to a 200pA depolarizing current step) with intra-pipette inclusion of either control peptide Ht31p (1μM) or active AKAP150 inhibitor peptide Ht31 (1μM). (B) Average bar graphs of Rin, threshold potential, and the amplitude of fAHP, and mAHP derived from recordings in Figure 3A.

Figure 4. *In vivo* HDAC inhibition reverses MD-induced increases in VTA DA neuronal excitability.

(A) Representative timeline of experimental design for HDACi experiments. (B) AP recordings in response to depolarizing current steps with representative AP traces (in response to a 200pA current step) in intact synaptic transmission from non-MD or MD rats that were either: naïve, injected with vehicle, or CI-994. Rats were sacrificed 24hr after the injection. Data from naïve and vehicle-injected rats were pooled and averaged. (C) Average bar graphs of Rin, threshold potential, and the amplitude of fAHP, and mAHP derived from recordings in Figure 4B.