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Synaptic plasticity at inhibitory synapses in the ventral tegmental area depends upon stimulation site

Robyn St. Laurent[1,2] and Julie Kauer[1]

[1]Department of Psychiatry & Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305

[2] Gladstone Institutes, 1650 Owens Street, San Francisco, CA 94158

Correspondence should be addressed to:
Julie A. Kauer, Ph.D.
Department of Psychiatry & Behavioral Sciences
Stanford University School of Medicine, MSLS P-104
Stanford, CA 94305
jkauer@stanford.edu

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ABSTRACT

Drug exposure induces cell and synaptic plasticity within the brain reward pathway that could be a catalyst for progression to addiction. Several cellular adaptations have been described in the ventral tegmental area (VTA), a central component of the reward pathway that is the major source of dopamine release. For example, administration of morphine induces long-term potentiation (LTP) of excitatory synapses on VTA dopamine cells and blocks LTP at inhibitory synapses. Drug-induced synaptic changes have a common endpoint of increasing dopamine cell firing and dopamine release. However, gaining a complete picture of synaptic plasticity in the VTA is hindered by its complex circuitry of efferents and afferents. Most studies of synaptic plasticity in the VTA activated a mixed population of afferents, potentially yielding an incomplete and perhaps misleading view of how drugs of abuse modify VTA synapses. Here, we use midbrain slices from mice and find that electrical stimulation in two different regions induces different forms of plasticity, including two new forms of LTP at inhibitory synapses. High frequency stimulation induces LTP independently of NMDA receptor activation, and surprisingly, some inhibitory inputs to the VTA also undergo NMDAR-independent LTP after a low frequency stimulation (LFS) pairing protocol.

SIGNIFICANCE STATEMENT

Synaptic plasticity of inhibitory inputs onto dopamine cells in the ventral tegmental area has a major influence on the circuits implicated in addictive behaviors. The location of electrical stimulation in an acute midbrain slice dictated the response of inhibitory inputs to plasticity induction protocols. We describe a new form of synaptic strengthening that occurs at an opioid-sensitive input to the ventral tegmental area.

INTRODUCTION

The ventral tegmental area (VTA) contains dopaminergic cells that receive inhibitory innervation from γ-aminobutyric acid (GABA)-ergic cell bodies originating within the VTA and from many other brain regions (Watabe-Uchida et al., 2012, Beier et al., 2015). Despite a wealth of anatomical and behavioral studies investigating the diversity of VTA afferents, plasticity at inhibitory synapses was historically described without identification of the presynaptic partner (Melis et al., 2002, Liu et al., 2005, Nugent et al., 2009, Nugent et al., 2007, Niehaus et al., 2010, Dacher and Nugent, 2011, Padgett et al., 2012, Kodangattil et al., 2013, Graziane et al., 2013, Polter et al., 2014). For example, nitric-oxide dependent long-term potentiation (LTP_{GABA}) can be triggered using electrical stimulation within the VTA (Nugent et al., 2007), however, when specific afferents were isolated using optogenetics, induction of LTP_{GABA} was found to depend upon the presynaptic partner (Simmons et al., 2017, Polter et al., 2018). Specifically, LTP_{GABA} is expressed at nucleus accumbens and VTA $GABA_A$ synapses, but not rostromedial tegmental nucleus (RMTg)-originating $GABA_A$ synapses. These observations demonstrate that all GABAergic synapses cannot be assumed to share a common plasticity mechanism. The idea that plasticity is segregated to specific populations is not a new one, and in fact many reports segregate experiments by postsynaptic cell identity. For example, long-term depression (LTD) induced by low frequency afferent stimulation is only expressed in putative dopamine cells in the VTA that express large H currents (I_h) (Dacher and Nugent, 2011). With local electrical stimulation in acute slices, it is possible to isolate synapses of one neurotransmitter type pharmacologically, but the identity of the presynaptic source is not always as easy to manipulate or determine. Although the location of the postsynaptic VTA cell (e.g. medial vs. lateral VTA) can sometimes predict output site, inputs from over 20 brain regions contact dopamine cells in

all VTA subregions (Beier et al., 2015). It is possible that other plasticity mechanisms have yet to be uncovered because their expression is limited to a subset of inputs, and therefore not apparent with global activation of all inputs. Here we use different electrical stimulation sites, and report two ways of inducing LTP at inhibitory synapses in the VTA with a mechanism(s) that is non-overlapping with that of LTP_{GABA} or other known forms of LTP at inhibitory synapses in the VTA.

METHODS

1. *Animals:*

All procedures were carried out in accordance with the guidelines of the National Institutes of Health for animal care and use and were approved by the [Author University] Institutional Animal Care and Use Committee. This study used VGAT::IRES-Cre (RRID:IMSR_JAX:028862, strain code: B6J.129S6(FVB)-Slc32a1$^{tm2(cre)Lowl}$), DAT::IRES-Cre (RRID:IMSR_JAX:006660, strain code: B6.SJL-Slc6a3$^{tm1.1(cre)Bkmn/J}$) (Zhuang et al., 2005), Ai14 Cre-reporter mice (RRID:IMSR_JAX:007908, strain code: B6;129S6-Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze/J}$), VGAT-ChR2(H134R)-EYFP (RRID:IMSR_JAX:014548, strain code: B6.Cg-Tg(Slc32a1-COP4*H134R/EYFP)8Gfng/J; (Zhao et al., 2011), and C57BL/6 (RRID:IMSR_JAX:000664) male and female mice bred in-house. Mice were maintained on a 12-h light/dark cycle and provided food and water ad libitum.

2. *Preparation of brain slices*

Horizontal brain slices (220 μm) were prepared from deeply anesthetized mice. Briefly, anesthetized mice were perfused with ice-cold oxygenated artificial cerebrospinal fluid (ACSF)

98 (in mM): 126 NaCl, 21.4 $NaHCO_3$, 2.5 KCl, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 1.0 $MgSO_4$, 11.1 glucose, 5
99 sodium ascorbate. Following perfusion, the brain was rapidly dissected and horizontal slices
100 (220 µm) were prepared using a vibratome. Slices recovered for 1 h at 34°C in oxygenated
101 HEPES holding solution (in mM): 86 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 35 $NaHCO_3$, 20 HEPES, 25
102 glucose, 5 sodium ascorbate, 2 thiourea, 3 sodium pyruvate, 1 $MgSO_4.7H_2O$, 2 $CaCl_2.2H_2O$
103 (Ting et al., 2014), and then were held in the same HEPES solution at room temperature until
104 use. Slices were then transferred to a recording chamber where they were submerged in ACSF
105 without sodium ascorbate.

106

107 *3. Electrophysiology*

108 Electrophysiological experiments were performed in horizontal midbrain slices containing the
109 VTA that were continuously perfused with ACSF containing 10 µM 6,7-dinitroquinoxaline- 2,3-
110 dione (DNQX) and 1 µM strychnine, AMPA and glycine receptor antagonists, respectively.
111 Except where noted, recordings also included the NMDA receptor antagonist d-APV (50 or 100
112 µM). Whole-cell recordings were performed from neurons in the lateral VTA with KCl pipette
113 solution and voltage-clamped at -70mV. Patch pipettes were filled with (in mM): 125 KCl, 2.8
114 NaCl, 2 $MgCl_2$, 2 ATP-Na^+, 0.3 GTP-Na^+, 0.6 EGTA, and 10 HEPES. In some experiments
115 EGTA was increased to 15 mM, or GDP-β-S (1 mM) was included in the pipette solution as
116 noted. The presence of a large hyperpolarization-activated inward current was used to select
117 postsynaptic cells for recording, although we are aware that this metric can allow inclusion of a
118 subset of non-dopamine neurons. If the steady-state h-current was greater than 25 pA during a
119 step from -50 mV to -100 mV, the cell was included in analyses. In a subset of recordings,
120 dopamine cells were also identified via fluorescence imaging using a DAT::IRES-cre x
121 TdTomato reporter line. All experiments were performed at 30°C, maintained by an automatic

temperature controller. The series resistance was monitored continuously during the experiment and cells were discarded for deviations >15%.

4. Stimulation protocols

For electrical stimulation, a bipolar stainless steel stimulating electrode was placed caudal to the VTA approximately 500 μm from the recorded cell; for rostral placement the stimulating electrode was placed within the VTA at 200-500 μm from the recorded cell (Figure 1A). Inhibitory postsynaptic currents (IPSCs) were evoked at 0.1 Hz using 100μs current pulses. We used input-output curves to identify the stimulation intensity used for plasticity experiments for both rostral and caudal afferents, and the baseline amplitude was at 50% of this generated curve. No correlation was observed between stimulation intensity and LTP magnitude. This stimulation protocol did not produce action potentials escaping voltage-clamp, but in the rate cases that cells began spiking later in the recording, they were excluded from analysis. Channelrhodopsin-induced synaptic currents were evoked at 0.033 Hz using 0.1-5 ms light pulses from a white LED (Mightex) controlled by driver (ThorLabs) and reflected through a 40x water immersion lens. When feasible, IPSCs were shown to be $GABA_A$ receptor-mediated by bath application of 10 μM bicuculline at the end of recordings. For all stimulus frequencies, intensity remained constant throughout the experiment.

5. Statistical analysis

Results are expressed as mean +/- standard error of the mean (S.E.M.). Significance was determined using a two-tailed paired Student's t-test or one-way analysis of variance (ANOVA) with significance level of $p < 0.05$. LTP values are reported as averaged IPSC amplitudes for 10 min just before LTP induction compared with averaged IPSC amplitudes during the 10-min

period from 10–20 min after manipulation. Paired-pulse ratios (50 ms interstimulus interval) and coefficient of variation were measured over 10 min epochs of 10-30 IPSCs each. The paired pulse ratio was calculated using the average value for all IPSC2 amplitudes divided by the average value for the corresponding IPSC1 amplitudes and reported as the mean paired pulse ratio for that epoch. Coefficient of variance analysis of $1/CV^2$ values were determined by dividing the mean amplitude of IPSCs squared recorded over 10 minute epochs by the mean variance of these IPSCs.

6. Materials

6,7-Dinitroquinoxaline-2,3[1H,4H]-dione (DNQX) were obtained from Sigma-Aldrich. D-2-Amino-5-phosphonopentanoic acid (APV), bicuculline, [D-Ala^2, NMe-Phe^4, Gly-ol^5]-enkephalin (DAMGO), forskolin, and naloxone were obtained from Tocris. Strychnine was obtained from Tocris or Abcam.

RESULTS

Location of electrical stimulation determines expression of synaptic plasticity

Most reports examining synaptic plasticity in the VTA have used a stimulating electrode placed within the VTA 200-500 μM rostral to the recorded cell in a horizontal slice (Figure 1A). This approach has been assumed to randomly sample the synaptic inputs onto cells within the VTA. We hypothesized that stimulating caudal to and outside of the VTA, approximately 500 μM from the recorded cell, might bias the inputs differently than with a rostral placement (Figure 1A). We refer to this as “caudal” stimulation. We recorded inhibitory postsynaptic potentials (IPSCs) in putative dopamine cells identified by a large I_h and compared synaptic properties using either

169 caudal or rostral electrical stimulation. We did not detect any differences between IPSCs evoked
170 by rostral or caudal stimulation in the onset delay, rise slope, or time of peak amplitude of IPSCs
171 (Figure 1B-D; paired t test of rostral vs. caudal onset delay: $p = 0.82$ [a]; rise slope: $p = 0.74$[b]; time
172 of peak: $p = 0.98$ [c]; rostral: n = 15 cells, caudal: n = 18 cells). Opioids depress GABAergic
173 inhibition in the VTA, therefore we compared the opioid-sensitivity of caudal and rostral-
174 stimulated IPSCs. IPSCs from both stimulating locations were depressed by 1 μM [D-Ala^2, *N*-
175 MePhe^4, Gly-ol]-enkephalin (DAMGO) to the same degree (Figure 1E-F; rostral = -55 ± 8%,
176 n = 6 cells; caudal = -58 ± 9%, n = 13 cells; rostral vs. caudal: $p = 0.86$[d]). Thus, synaptic
177 properties were similar when stimulating either the rostral or caudal site.

178

179 We next used a stimulation protocol known to induce the nitric oxide-dependent LTP_{GABA}: high
180 frequency stimulation (HFS) consisting of two 100 Hz tetani separated by 10 seconds (Nugent
181 et al., 2007). LTP_{GABA} is dependent on NMDA receptor (NMDAR) activation that leads to the
182 release of nitric oxide and activation of a signaling cascade that increases presynaptic GABA
183 release (Nugent et al., 2007, Nugent et al., 2009). Instead, HFS of the caudally stimulated site
184 resulted in LTP, even with the NMDAR antagonist, APV (100 μM), in the bath solution (Figure
185 2A,B,E; 157 ± 23% of baseline value; paired t test: $p = 0.018$[e], n = 16 cells). Conversely and
186 consistent with prior results, the same tetanus of a rostrally-placed electrode did not potentiate
187 IPSCs in APV ((Nugent et al., 2007); Figure 2C-E; 90 ± 11% of baseline value; paired t test: $p =$
188 0.73[f], n = 6 cells). Potentiation after HFS of the caudally-stimulated electrode was correlated
189 with a decrease in paired pulse ratio for cells that potentiated by at least by 10% (Figure 2F;
190 baseline baseline: 1.1 +/- 0.2, after HFS: 0.9 +/- 0.1), without a change in $1/CV^2$ values (Figure
191 2G; baseline: 7.5 ± 2.1, 10-20 min after HFS: 8.1 ± 1.4; paired t test: $p = 0.048$[g] and $p = 0.70$ [h],
192 respectively, n = 12 cells). These findings supported our hypothesis that electrode placement

193 may activate different subsets of afferents and lead to a different outcome when performing
194 protocols to induce synaptic plasticity.

195

196 **Low frequency stimulation potentiates caudal-evoked inhibitory inputs**

197 Inhibitory synapses can be regulated bidirectionally by different afferent stimulation patterns. In
198 an earlier study, low frequency stimulation (LFS) of afferents by a stimulating electrode placed
199 rostral to the VTA cell being recorded was used to elicit long term depression (LTD). LTD is
200 induced by LFS, 6 minutes of 1 Hz stimulation while voltage clamping the postsynaptic cell at -
201 40 mV (LFS-LTD; (Dacher and Nugent, 2011)). LFS-LTD occurs independently of NMDAR
202 activation and is partially blocked by a dopamine D2 receptor antagonist (Dacher and Nugent,
203 2011). Given the surprising result with HFS of a caudally-placed electrode, we asked whether
204 LFS of a caudally-placed stimulating electrode would also induce LTD. Instead, LFS of caudally-
205 evoked IPSCs triggered LTP both in the absence or presence of APV (LFS-LTP_{GABA})(Figure 3A-
206 D; 131 ± 10% of baseline value; $p = 0.026$ [i], n = 38 cells). PPR was not significantly altered in
207 cells potentiating by at least 10% after LFS (Figure 3E; baseline: 1.0 ± 0.1, 10-20 min after
208 LFS: 0.9 ± 0.1; $p = 0.058$ [j], n = 22 cells) and neither were the normalized $1/CV^2$ values (Figure
209 3F; baseline: 5.7 ± 1.1, 10-20 min after LFS: 7.8 ± 1.8; $p = 0.09$[k], n = 19 cells). This surprising
210 finding led us to conclude that as with HFS-induced LTP, previous observations of LTD
211 following LFS were likely dependent upon activation of a subset of VTA afferents.

212

213 **LFS of optically-evoked inhibitory inputs in the VTA does not induce plasticity**

214 There are many sources of GABAergic inhibition in the VTA and given that LFS can induce
215 either LTD or LTP, depending on stimulation site, we wondered which form of plasticity was
216 predominant when activating GABAergic synapses more globally. We hypothesized that just a

subset of VTA synapses express LFS-LTP$_{GABA}$, so that when using optical stimulation of VGAT+ inputs in a VGAT-ChR2 transgenic mouse line, both forms of plasticity might occur at different synapses on the same dopamine cell. We used a BAC transgenic mouse line, VGAT-ChR2(H134R)-EYFP, to activate multiple GABAergic inputs in the VTA (Zhao et al., 2011), and used whole field LED illumination of the slice to activate inhibitory inputs. After generating a stable 10 minute baseline of light-evoked IPSCs, we delivered optical LFS while depolarizing the postsynaptic cell to -40 mV. In contrast to what we observed with caudal electrical stimulation, the mean light-evoked IPSC amplitude was unchanged after optical LFS (Figure 4A-C; 102 ± 10% of baseline value; $p = 0.83^{l}$, n = 7 cells). PPR was not significantly altered after LFS (data not shown; baseline: 0.72 ± 0.07, 10-20 min after LFS: 0.72 ± 0.05, $p = 0.97^{m}$, n = 7 cells) and neither were $1/CV^2$ values (data not shown; baseline: 20.0 ± 2.4, 10-20 min after LFS: 20.2 ± 3.6, $p = 0.95^{n}$, n = 7 cells).

Forskolin potentiation does not occlude LFS-induced LTP

Forskolin is known to potentiate many synapses. Forskolin activates adenylyl cyclase which potentiates GABAergic synapses in the VTA (Melis et al., 2002, Nugent et al., 2009) as well as at many excitatory and inhibitory synapses throughout the CNS (Briggs et al., 1988, Greengard et al., 1991, Cameron and Williams, 1993, Chavez-Noriega and Stevens, 1994, Huang and Kandel, 1994, Weisskopf et al., 1994, Bonci and Williams, 1996, Salin et al., 1996, Bonci and Williams, 1997, Huang and Kandel, 1998, Castro-Alamancos and Calcagnotto, 1999, Linden and Ahn, 1999, Mellor et al., 2002). Both prior VTA studies using forskolin and stimulating GABAergic VTA afferents used rostral electrical stimulation. We wondered if forskolin would also potentiate inputs evoked with caudal afferent stimulation or whether these synapses would prove distinct again. We found that 10 μM forskolin potentiated IPSCs stimulated with a caudally-placed electrode (Figure 5A-C; 183 ± 19% of baseline value; $p = 0.003^{o}$, n = 14 cells),

although PPR was not significantly altered after forskolin (data not shown; baseline: 0.9 ± 0.1, 10-20 min after LFS: 0.9 ± 0.1; $p = 0.65^{p}$, n = 13 cells). Forskolin potentiation occludes NMDAR-dependent LTP_{GABA} (Nugent et al., 2009). Therefore, we performed occlusion experiments to ask if forskolin potentiation occludes caudal LFS-induced LTP. However, forskolin-induced potentiation did not occlude further potentiation by LFS (Figure 5D-F; 131 ± 10% of baseline value; $p = 0.046^{q}$, n = 10 cells). These data suggest that the mechanism underlying LFS-induced LTP is distinct from that of forskolin potentiation.

LFS-induced LTP does not require postsynaptic GPCR signaling and is not prevented by postsynaptic EGTA

Potentiation after LFS was not associated with a significant change in the paired pulse ratio or coefficient of variation (see Figure 3E-F), suggesting that the mechanism may reflect an increased GABAergic sensitivity of the postsynaptic cells. Most forms of LTP are triggered by increases in calcium concentration in the postsynaptic cell, and so we tested whether LFS-LTP requires a rise in postsynaptic calcium. When the concentration of the calcium chelator, EGTA, was raised to 15mM in the patch pipette, LFS still resulted in potentiation of caudally-evoked IPSCs (Figure 6A-C; 155 ± 24% of baseline value; n = 8 cells). An alternative postsynaptic mechanism might require activation of receptors on the postsynaptic cell other than $GABA_A$ receptors. For example, the report of LFS-LTD found that depression was partially dependent upon dopamine D2 receptors (Dacher and Nugent, 2011), which are coupled to the G_i subtype of G-protein coupled receptors (GPCR). When we included an inhibitor of GPCR activity (1 mM GDP-βS) in the patch pipette to block all postsynaptic GPCR signaling, LFS still potentiated caudally-evoked IPSCs (Figure 6D-F; 135 ± 8% of baseline value; n = 8 cells). The magnitude of LTP after LFS was not significantly different for high EGTA or GDP-β-S conditions than experiments with normal KCl internal solution ($F(2,27) = 0.77$, $p = 0.48^{r}$, n = 8 cells high EGTA,

n = 8 cells GDP-β-S, n = 14 cells normal KCl). Together our results suggest a mechanism that does not require postsynaptic GPCRs or Ca^{2+} influx. Future experiments will be needed to understand this novel form of LTP.

Statistical Table.

Data Structure	Type of Test	95% confidence interval
[a] normal distribution	two-tailed unpaired t-test	-0.79 to 0.98
[b] normal distribution	two-tailed unpaired t-test	-85.83 to 119.6
[c] normal distribution	two-tailed unpaired t-test	-0.99 to 1.01
[d] normal distribution	two-tailed unpaired t-test	-27.21 to 32.06
[e] normal distribution	two-tailed paired t-test	18.20 to 162.8
[f] normal distribution	two-tailed paired t-test	-78.82 to 59.01
[g] normal distribution	two-tailed paired t-test	-0.43 to -0.002
[h] normal distribution	two-tailed paired t-test	-2.47 to 3.56
[i] normal distribution	two-tailed paired t-test	5.89 to 85.91
[j] normal distribution	two-tailed paired t-test	-0.17 to 0.0029
[k] normal distribution	two-tailed paired t-test	-0.38 to 4.43
[l] normal distribution	two-tailed paired t-test	-87.36 to 104.7
[m] normal distribution	two-tailed paired t-test	-0.15 to 0.15
[n] normal distribution	two-tailed paired t-test	-9.54 to 10.06
[o] normal distribution	two-tailed paired t-test	37.01 to 147.8
[p] normal distribution	two-tailed paired t-test	-0.21 to 0.13
[q] normal distribution	two-tailed paired t-test	1.22 to 110.4
[r] normal distribution	one-way ANOVA	High EGTA 99.09 to 210.7; GDP-β-S 115.9 to 153.4

DISCUSSION

Most reports describing synaptic plasticity in the VTA used electrical stimulation, which can miss the possibility of circuit-specificity that can now be probed using optogenetic tools. For example,

277 LTP_{GABA} (Nugent et al., 2007) was found to vary depending on presynaptic source (Simmons et
278 al., 2017, Polter et al., 2018). Here we report that inhibitory synapses in the VTA have different
279 requirements for inducing LTP depending on the placement of the stimulating electrode.

280

281 **Synaptic plasticity induction**

282 Using the same stimulation protocol but with the electrode at a site that deviated from the usual
283 placement, we serendipitously discovered that we could induce NMDAR-independent LTP using
284 either HFS or LFS. Pairing postsynaptic cell depolarization with afferent stimulation, to
285 substitute for a strong tetanus, is a classic approach used to induce LTP in the hippocampus
286 (Nicoll, 2017). However, this method for inducing LTP is generally due to NMDAR activation.
287 Instead, robust caudal LFS-induced LTP in the VTA was elicited in the presence of an NMDAR
288 antagonist. Previously described LFS-LTD in the VTA is also NMDAR-independent (Dacher and
289 Nugent, 2011). How might the same pattern of afferent stimulation result in opposite synaptic
290 plasticity outcomes? One likely explanation is that different electrode locations preferentially
291 activate different subsets of afferents in the VTA slice. The VTA dopamine cells are innervated
292 both by local GABA neurons and by GABA projections originating in regions throughout the
293 brain that may differ in protein expression leading to different forms of synaptic plasticity.
294 Another possibility is that the timing of inputs differs when using the two stimulating electrode
295 locations; however, we did not observe a significant difference in onset delay, rise slope, or time
296 of peak amplitude of IPSCs from caudal vs. rostral. We speculate that LFS with mild
297 depolarization may lead to release of a signaling molecule from the post- or pre-synaptic cell. If
298 different synapses express different receptor subtypes for that signaling molecule, then release
299 via LFS could result in distinct synaptic strength changes. Future experiments will be needed to
300 determine if HFS-LTP and LFS-LTP result e.g. from activation by metabotropic glutamate,
301 endocannabinoid, or dopamine receptors.

Low frequency stimulation and synaptic plasticity

Numerous studies have shown that LFS induces LTD, often when paired with modest postsynaptic depolarization (Bear and Malenka, 1994, Gutlerner et al., 2002). There are fewer instances where LFS induces LTP, and these generally required pairing with strong depolarization to activate NMDA receptors (Bonci and Malenka, 1999, Ikeda et al., 2007, Dringenberg et al., 2014, Lante et al., 2006). Here we found that LFS potentiates VTA GABA synapses with mild depolarization and LFS that did not require NMDAR activation, an apparently rare mechanism at CNS synapses. One other example is at the excitatory synapse from lateral perforant path to dentate gyrus cells, where LFS also potentiates synapses independently of NMDAR activation (Gonzalez et al., 2014). However, to our knowledge, ours is the first report of LTP elicited by LFS at GABAergic synapses.

GABAergic afferents innervating the VTA

Exposure to drugs of abuse causes long-term potentiation (LTP) at excitatory synapses on VTA DA cells (Ungless et al., 2001, Saal et al., 2003). Many drugs of abuse also block LTP_{GABA} in the VTA (Nugent et al., 2007, Guan and Ye, 2010, Niehaus et al., 2010, Graziane et al., 2013). The net result of these drug-induced changes in synaptic strength is thought to be increased dopamine cell firing via enhanced excitatory drive and disinhibition. However, if more types of synaptic plasticity exist than previously suspected, differential effects of drugs of abuse on VTA afferents may produce a more nuanced effect on dopamine cell firing. LTP_{GABA} is expressed at VTA_{GABA}→VTA but not at $RMTg_{GABA}$→VTA synapses; by analogy, it is likely that the HFS- and LFS- induced LTP we report here are expressed only at a subset of inputs. It is difficult to be certain precisely which afferents are sufficiently close to the caudal stimulation site to be

activated by our stimulus protocol. Regions other than the RMTg that are located caudal to the VTA with reported GABAergic innervation include: the dorsal raphe, periaqueductal gray, pedunculopontine nucleus, and laterodorsal tegmentum (Beier et al., 2015, Faget et al., 2016, Omelchenko and Sesack, 2010, Ntamati et al., 2018). Given the placement of the caudal electrode in our experiments, it is possible that the presynaptic source of those inputs is from one of these caudal brain regions, although it is alternatively possible that regions located elsewhere in the brain send projections that pass through the caudal stimulation location.

In conclusion, depending upon stimulation site, HFS and LFS can induce LTP at GABAergic synapses in the VTA via a mechanism that does not require NMDAR activation. These results support the recent findings that some forms of plasticity, like LTP_{GABA} (Simmons et al., 2017, Polter et al., 2018), are selectively expressed at some synapses but not others. Together, this points towards a specificity of synaptic plasticity based upon presynaptic partner and postsynaptic cell identity. Furthermore, this study highlights the fact that there may be plasticity mechanisms in the VTA still to be identified.

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460

FIGURES & LEGENDS

Figure 1. Electrical stimulation in horizontal midbrain slices

A. Recording setup illustrating caudal or rostral placements of the bipolar stimulating electrodes. Analysis of caudal vs. rostral IPSC: **B.** onset delay, **C.** rise slope, and **D.** time to peak amplitude. **E.** Example IPSCs illustrating control IPSCs (black) and in the μ-opioid receptor agonist, DAMGO (1 μM)(green), for caudal or rostral inputs, and **F**. Mean IPSC amplitude depression after DAMGO (1 μM), for each input.

Error bars represent S.E.M.

Figure 2. Location of electrical stimulation determines expression of synaptic plasticity

A. Representative experiment showing LTP induction by HFS with a caudal electrode placement. Inset: baseline (black traces) and 10-20 min after HFS (red traces). **B.** Mean IPSC amplitudes from a 10 min baseline and 10-20 min after caudal HFS (n = 16 cells). In this and subsequent figures, thicker black symbols/lines represent the mean response across all cells. **C.** Representative experiment with HFS of a rostral electrode. Inset: baseline (black traces) and 10-20 min after HFS (red traces). **D.** Mean IPSC amplitudes from a 10 min baseline to 10-20 min after rostral HFS (n = 6 cells). **E.** Time course of averaged IPSC amplitudes before and after HFS. (closed symbols = caudal, open symbols = rostral) **F.** Paired pulse ratios before and after caudal HFS from each cell that potentiated >10% of basal values (n = 12 cells). **G.** $1/CV^2$ values before and after caudal HFS from each cell that potentiated >10% of basal values (n = 12 cells).

*$p < .05$, paired t-test of amplitude of 10 min baseline vs.10-20 min after HFS.

Error bars represent S.E.M.

Figure 3. Low frequency stimulation of caudal electrode induces LTP

A. Representative experiment with LFS with a caudal electrode placement without APV, or **B.** with APV. Insets: baseline (black traces) and 10-20 min after LFS (red traces). **C.** Time course of averaged IPSC amplitudes before and after LFS. **D.** Mean IPSC amplitudes from a 10 min baseline to 10-20 min after caudal LFS (n = 38 cells). **E.** Paired pulse ratios before and after caudal LFS from each cell that potentiated >10% of basal values (n = 22 cells). **F.** $1/CV^2$ values before and after caudal LFS from each cell that potentiated >10% of basal values (n = 19 cells). **D., E., F.** Grey symbols/lines no APV, black symbols/lines, with APV present.

*p < .05, paired t-test of amplitude of 10 min baseline vs.10-20 min after LFS.

Error bars represent S.E.M.

Figure 4. No effect with low frequency optical stimulation of $VGAT^+$ synapses

A. Representative experiment with optical LFS. Inset: baseline (black traces) and 10-20 min after LFS (red traces). **B.** Time course of averaged IPSC amplitudes before and after LFS. **C.** Mean IPSC amplitudes from a 10 min baseline to 10-20 min after optical LFS (n = 7 cells).

Error bars represent S.E.M.

Figure 5. Forskolin potentiates GABAergic synapses evoked with caudal stimulation but does not prevent subsequent potentiation by caudal LFS

A. Representative experiment with 10 μM forskolin. Inset: baseline (black traces) and in forskolin (gray traces). **B.** Time course of averaged IPSC amplitudes before and during forskolin. **C.** Mean IPSC amplitudes from a 10 min baseline to 10-20 min after forskolin addition (n = 14 cells). **D.** Representative experiment with caudal LFS after potentiation by 10 μM

509 forskolin. Inset: baseline in forskolin (gray traces) and 10-20 min after LFS (red traces). **E.** Time
510 course of averaged IPSC amplitudes before and after caudal LFS after forskolin-induced
511 potentiation was established`1. **F.** Mean IPSC amplitudes from a 10 min baseline to 10-20 min
512 after forskolin (n = 10 cells).
513 *$p < .05$, paired t-test of amplitude of 10 min baseline vs.10-20 min after forskolin or LFS.
514 Error bars represent S.E.M.

515

516 **Figure 6. LFS-induced LTP does not require postsynaptic calcium elevation or GPCR**
517 **activation**
518 **A.** Representative experiment with caudal LFS when 15 mM EGTA was included in the patch
519 pipette. Inset: baseline (black traces) and 10-20 min after LFS (red traces). **B.** Time course of
520 averaged IPSC amplitudes before and after caudal LFS with 15 mM EGTA. **C.** Mean IPSC
521 amplitudes from a 10 min baseline to 10-20 min after caudal LFS with 15 mM EGTA (n = 8
522 cells). **D.** Representative experiment with caudal LFS when 1 mM GDP-β-S was included in the
523 patch pipette intracellular solution. Inset: baseline (black traces) and 10-20 min after LFS (red
524 traces). **E.** Time course of averaged IPSC amplitudes before and after caudal LFS with 1 mM
525 GDP-β-S. **F.** Mean IPSC amplitudes from a 10 min baseline to 10-20 min after LFS with 1 mM
526 GDP-β-S (n = 8 cells).
527 *$p < .05$, paired t-test of amplitude of 10 min baseline vs.10-20 min after LFS.
528 Error bars represent S.E.M.