



Short communication

Opioid self-administration results in cell-type specific adaptations of striatal medium spiny neurons



Alex S. James^{a,1}, Jane Y. Chen^{b,1}, Carlos Cepeda^b, Nitish Mittal^c, James David Jentsch^a, Michael S. Levine^b, Christopher J. Evans^c, Wendy Walwyn^{c,*}

^a Department of Psychology, University of California, Los Angeles, Los Angeles, CA, USA

^b IDDR, Semel Institute for Neuroscience and Human Behavior, and Brain Research Institute, University of California, Los Angeles, Los Angeles, CA, USA

^c Department of Psychiatry and Biobehavioral Sciences, Semel Institute for Neuroscience and Human Behavior, and Brain Research Institute, University of California, Los Angeles, Los Angeles, CA, USA

HIGHLIGHTS

- We examined the effect of repeated contingent opioid exposure on striatal medium spiny neurons (MSNs) in D1- and D2-GFP expressing mice.
- Mice were trained to self-administer remifentanyl, a mu opioid receptor agonist.
- Once stable self-administration was obtained, electrophysiology recordings were done.
- Mu receptors pre-synaptic to D1-, but not D2-, MSNs demonstrated reduced sensitivity.
- This finding suggests a specific adaptation of D1-MSNs after opiate self-administration.

ARTICLE INFO

Article history:

Received 25 June 2013

Received in revised form 2 August 2013

Accepted 5 August 2013

Available online 19 August 2013

Keywords:

Striatum

Medium spiny neurons

Intravenous self-administration

Mu opioid receptor

Electrophysiology

Mice

ABSTRACT

Medium-sized spiny neurons (MSNs), the predominant neuronal population of the striatum, are an integral component of the many cortical and limbic pathways associated with reward-related behaviors. A differential role of the D1 receptor-enriched (D1) MSNs of the striatonigral direct pathway, as compared with the D2 receptor-enriched (D2) MSNs of the striatopallidal indirect pathway, in mediating the addictive behaviors associated with cocaine is beginning to emerge. However, whether opioids, well-known analgesics with euphoric properties, similarly induce dissociable signaling adaptations in these neurons remains unclear. Transgenic mice expressing green fluorescent protein (GFP)-labeled D1 or D2 neurons were implanted with intravenous jugular catheters. Mice learned to self-administer 0.1 mg/kg/infusion of the opioid remifentanyl during 2 h sessions over 13 contiguous days. Thereafter, the electrophysiological properties of D1- and D2-MSNs in the shell region of the nucleus accumbens (NAc) were assessed. We found that prior opioid exposure did not alter the basic membrane properties nor the kinetics or amplitude of miniature excitatory postsynaptic currents (mEPSCs). However, when challenged with the mu opioid receptor (μ OR) agonist DAMGO, the characteristic inhibitory profile of this receptor was altered. DAMGO inhibited the frequency of mEPSCs in D1-MSNs from control mice receiving saline and in D2-MSNs from mice exposed to remifentanyl or saline, but this inhibitory profile was reduced in D1-MSNs from mice receiving remifentanyl. Remifentanyl exposure also altered the probability of glutamate release onto D1-, but not D2-MSNs. Together these results suggest a D1-pathway specific effect associated with the acquisition of opioid-seeking behaviors.

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Opioids are potent analgesics of important clinical value but can also induce euphoria. This can lead to abuse behaviors by those taking prescription opioids and contribute to the increasing diversion of these readily available pharmaceutical compounds for non-medical use [1]. As occasional opioid use transitions to habitual use, this progression is often accompanied by tolerance and dependence, which contribute to an escalating, prolonged pattern of opioid abuse. However, in contrast to our knowledge of the neuroadaptations induced by the traditional psychostimulants, much less is known of the adaptations of specific neural cells, and

* Corresponding author at: UCLA-Semel Institute, Box 77, 760 Westwood Plaza, Los Angeles, CA, 90095, USA. Tel.: +1 3102063231; fax: +1 3108257067.

E-mail address: wwalwyn@ucla.edu (W. Walwyn).

¹ denotes equal contribution.

therefore circuits that mediate the transition from occasional to chronic opioid use and abuse.

The mesolimbic dopamine pathway is undoubtedly involved in the initial phase of opioid reward and reinforcement [2,3], with cooperative signaling through the D1 and D2 receptors expressed in striatal medium-sized spiny neurons (MSNs), mediating aspects of the unconditioned effects of the drug and subsequent conditioning [4–6]. As drug-taking progresses, the role of these receptors appears to diverge, with D2 receptor activation playing a more prominent role in cue-associated behaviors whereas the role of D1 receptors appears to diminish [7]; both receptors, however, appear to be involved in opioid-seeking after prolonged abstinence [8].

Though an understanding of the effects of psychostimulants on the neurophysiology of D1- and D2-enriched MSNs and their role in the initial psychomotor and reinforcing effects of psychostimulants is coming to light [9–12], there is little known of how prolonged voluntary opioid exposure alters these properties. To fill this gap, we have combined several techniques, including GFP-expressing mice, intravenous self-administration and electrophysiological recordings, to examine how the acquisition and maintenance of opioid-seeking behavior alters signaling properties of the D1-enriched, direct-pathway, and D2-enriched, indirect-pathway MSNs of the striatum.

Fully back-crossed male and female mice expressing D1- or D2-GFP [13] were bred from hemizygous mice crossed with C57Bl/6J mice to obtain hemizygous and wildtype littermates. Initially, both sexes were used to examine the effect of the GFP transgene on opioid sensitivity, which may alter cellular function and/or behavior. This was assessed by the studying the locomotor effect of an opioid agonist, fentanyl, which was used for these experiments as it is μ OR-specific and, unlike remifentanyl (according to pilot data not shown), induces a hyperlocomotion response. We found an effect of transgene insertion in female but not male mice (SFig. 1, Supplemental Information). Male D1- and D2-GFP mice were therefore used for the subsequent intravenous self-administration experiments and electrophysiological analyses.

An intravenous catheter (0.2 mm i.d., 0.4 mm o.d.; Cathcams, Oxford, UK) was implanted in the right jugular vein of male mice under sterile conditions as previously described [14]. After 7 days of recovery, and on a daily basis thereafter, mice were trained to self-administer remifentanyl (0.1 mg/kg/infusion) or had access to saline in operant conditioning chambers (Med-Associates Georgia, VT) in which the active versus inactive lever location was assigned in a counterbalanced manner across groups. Remifentanyl was chosen for these self-administration experiments as it is highly μ OR-specific and potent, has abuse-liability, and its short half-life facilitates the association of the active lever with the opioid infusion, thereby enhancing acquisition of self-administration behavior [15–17]. A response on the designated active, but not inactive, lever resulted in an intravenous drug infusion (0.67 μ l/g body weight), presentation of a 20 s visual cue and a 30 s 'timeout' period. The mice underwent a minimum of 10 days of acquisition training at Fixed Ratio 1 (FR1), followed by 3 to 4 days at Fixed Ratio 3 (FR3) where 1 or 3 lever presses respectively, resulted in an infusion, up to a maximum of 50 infusions/session. Self-administration behavior was considered established if a minimum of 20 infusions/session were obtained with no more than 30% variation in the number of infusions earned across the last 3 days.

Statistical Analysis: Over-dispersed count data gathered from self-administration sessions (counts of infusions earned and lever presses) were analyzed using Generalized Estimating Equations (GEE; see supplemental information) with a negative binomial distribution and a log link function along with an unstructured covariance matrix. Training day and lever (for analysis of active versus inactive pressing) were entered as repeated measures,

whereas genotype and self-administration drug were between-subjects factors.

Mice which had self-administered remifentanyl or saline were sacrificed 24 h after the last session for subsequent electrophysiological analysis. Slice preparation, cell identification, recording parameters and excitatory post-synaptic currents (EPSC) analysis were conducted as previously described [18,19]. Miniature EPSCs (mEPSCs) were recorded in the presence of tetrodotoxin (TTX, 1 μ M) and with or without DAMGO; [D-Ala², NMe-Phe⁴, Gly-o⁵]-enkephalin (1 μ M, 8–10 min).

Statistical Analysis: As there were no significant differences between labeled and non-labeled MSNs from the other genotype, data were pooled. High-frequency electrical noise was filtered in Clampfit and root mean square (rms) noise was calculated at 1–2 pA. Events with peak amplitudes between 10 and 50 pA were grouped, aligned by half-rise time, and normalized by peak amplitude. The effect of DAMGO was assessed during the last 2 min of application, the time of maximal effect [19]. Differences in average mEPSC amplitude and frequency between groups were analyzed with GEE using a gamma distribution and a log link function along with an exchangeable covariance structure and DAMGO application entered as a repeated factor. Amplitude-frequency histograms were also analyzed using the same GEE model, with amplitude bin and DAMGO application as repeated factors. Because many of these data equaled zero, a constant was added to all values to allow analysis with a gamma distribution (which best fit the data). Differences in inter-event interval (IEI) distributions, interpreted as probability of release, were analyzed using the Kolmogorov–Smirnov (KS) test.

We found that, when compared to saline mice (D1-GFP $n=4$, D2-GFP $n=5$), mice with contingent access to remifentanyl (D1-GFP $n=9$, D2-GFP $n=6$) acquired self-administration behavior and earned significantly more infusions at FR1 and FR3 schedules (both Wald $\chi^2 > 43.127$, p 's < 0.0001 , $df=1$, Fig. 1A). While no significant effects of or interactions with genotype were found for FR1 (all Wald $\chi^2 < 10.543$, p 's > 0.308), we did detect interactions with genotype during FR3 (day \times genotype: Wald $\chi^2 = 41.606$, $p < 0.001$, $df=2$; day \times drug \times genotype: Wald $\chi^2 = 39.191$, $p < 0.001$, $df=2$). However, pairwise comparisons between genotypes across FR3 days did not reveal any significant differences in infusions earned (remifentanyl mice: all p 's > 0.677 ; saline mice: all p 's > 0.246). As shown in Fig. 1B, mice self-administering remifentanyl learned to discriminate the active from inactive lever during FR1 (day \times lever: Wald $\chi^2 = 22.404$, $p = 0.008$, $df=9$) and maintained this discrimination during FR3 (lever: Wald $\chi^2 = 122.261$, $p < 0.001$, $df=1$; day \times lever: Wald $\chi^2 = 2.390$, $p = 0.303$, $df=2$), whereas saline mice did not (data not shown). Lever discrimination learning during FR1 and maintenance during FR3 did not differ as a function of genotype (all Wald $\chi^2 < 7.798$, p 's > 0.144 ; see supplemental information for further statistical analyses).

The basic cell membrane properties – capacitance (C_m), resting membrane potential (R_m), time constant or tau – were not different in D1- or D2-MSNs from mice receiving saline or remifentanyl. This indicates that chronic opioid self-administration did not produce major changes in MSN morphology or resting membrane potential (Table 1). There were also no dissociable effects of opioid self-administration on the amplitude, frequency or kinetics of spontaneous EPSCs in D1 and D2 neurons (data not shown).

As we have found that DAMGO inhibits miniature EPSC frequency in both types of MSNs in the NAc shell [19], we examined this effect following self-administration. We found that opioid self-administration specifically altered this profile in D1-MSNs (Fig. 2; cell type \times self-administration drug \times DAMGO application: Wald $\chi^2 = 4.251$, $p = 0.039$, $df=1$). Post hoc analysis, shown in Fig. 2A, demonstrated that DAMGO inhibited mEPSC frequency in both D1- and D2-MSNs from mice receiving saline (D1-MSNs: $p = 0.003$; D2-MSNs: $p = 0.040$). This inhibitory effect of DAMGO

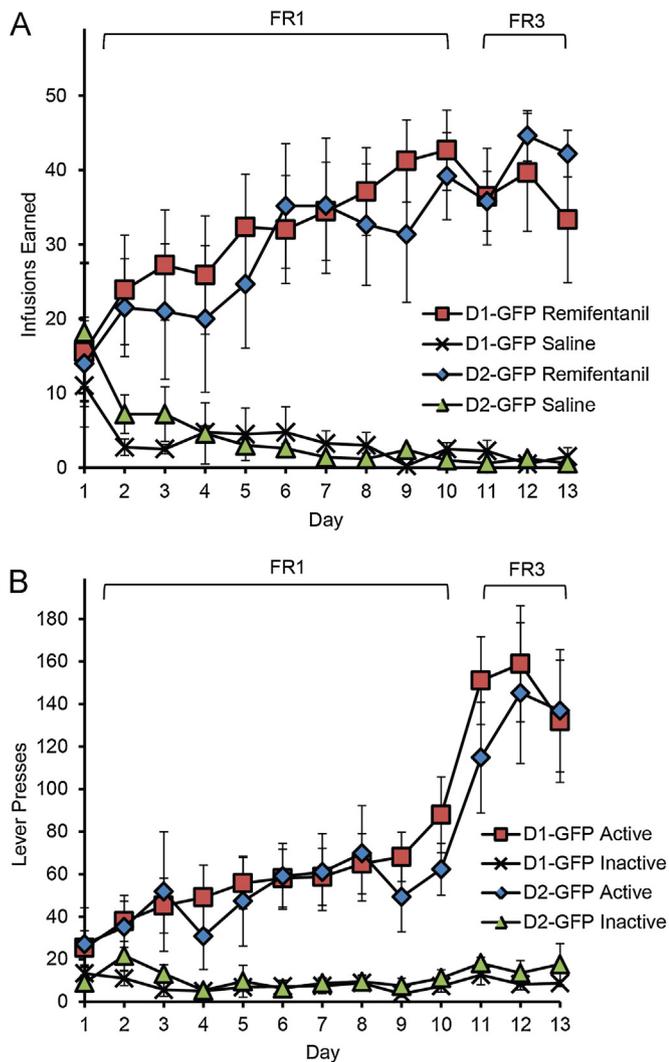


Fig. 1. Both D1- and D2- GFP mice acquire remifentanil self-administration. A. D1- and D2-GFP mice increased drug self-administration over a period of 13 days, reaching >35 infusions of remifentanil per session. Thus, while remifentanil self-administering animals escalated infusions earned across days, saline animals did not (all Wald $\chi^2 > 7.935$, p 's < 0.019). B. Both D1- and D2-GFP mice acquired remifentanil self-administration behavior at similar levels and showed no differences in their ability to discriminate between active and inactive levers across training days (all Wald $\chi^2 < 7.798$, p 's > 0.555); see main text and supplement for additional statistical analyses.

was also seen in D2-MSNs from animals self-administering remifentanil ($p=0.001$). In contrast, there was no detectable effect of DAMGO on the mEPSC frequency of D1-MSNs from mice self-administering remifentanil ($p=0.277$). Further in-depth

Table 1
Chronic remifentanil self-administration did not alter the membrane properties of D1 and D2 MSNs.

	Cm (pF)	Rm (M Ω)	Tau (ms)
D1			
Saline (n = 11)	68.0 \pm 5.7	144.3 \pm 62.5	1.9 \pm 0.1
Remifentanil (n = 10)	368.9 \pm 5.7	94.5 \pm 17.5	2.2 \pm 0.1
D2			
Saline (n = 12)	73.1 \pm 6.3	141.0 \pm 71.3	1.8 \pm 0.1
Remifentanil (n = 13)	70.8 \pm 5.7	163.2 \pm 35.5	2.0 \pm 0.1

There was no effect of treatment on cell capacitance (Cm), resting membrane potential (Rm) or time constant (Tau) of D1 and D2 MSNs.

examination of these recordings, examples of which are shown in Fig. 2B, showed that the frequency modulation by DAMGO was amplitude-specific in D2-MSNs of remifentanil self-administering animals (DAMGO application \times amplitude bin, D2-MSNs: Wald $\chi^2 = 16.461$, $p = 0.021$, $df = 7$; D1-MSNs: Wald $\chi^2 = 2.311$, $p = 0.941$, $df = 7$). As shown in Fig. 2C, DAMGO inhibited the frequency of small-amplitude events in D2- (5–10 pA: $p < 0.001$; 10–15 pA: $p = 0.002$), but not D1-MSNs (all p 's > 0.157); all other amplitudes were not significantly affected by DAMGO, regardless of cell type (all p 's > 0.288). DAMGO also inhibited (significantly or at a trend level; see supplemental results) the frequency of low amplitude events in both cell types from saline animals. Note that all amplitudes, including those of in the 5–10 pA range, were above our 1–2 pA rms noise level. There was no effect of DAMGO or remifentanil exposure on mEPSC amplitude (i.e., magnitude) or kinetics (Table 2).

Using KS tests, analysis of the inter-event interval (IEI) curves, which depict the cumulative probability of glutamate release, further revealed differential effects of remifentanil on MSN subtypes. While baseline (i.e., TTX only) IEI curves did not differ between D1- and D2-MSNs in saline mice ($p = 0.277$), remifentanil self-administration shifted the baseline IEI curve of D1-MSNs to the left ($p < 0.001$), as depicted in Fig. 2D. There was, however, no effect of remifentanil exposure on the baseline IEI distribution of D2-MSNs ($p > 0.999$). In contrast to the average frequency data shown in Fig. 2A, the cumulative IEI curves demonstrated a graded profile of DAMGO-induced inhibition in D1- vs. D2-MSNs. Specifically, in remifentanil self-administering animals, DAMGO induced some inhibition in D1-MSNs ($p = 0.017$), but caused significantly more inhibition in D2-MSNs (DAMGO IEI data expressed as a percent of baseline, in D1- vs. D2-MSNs, $p < 0.001$; data not shown). Moreover, D2-MSN DAMGO IEI curves were significantly rightward shifted relative to D1-MSN DAMGO IEI curves in remifentanil animals ($p < 0.001$).

By combining bacterial artificial chromosome genetic engineering approaches with mouse intravenous self-administration to allow for cell-type specific electrophysiological analysis of synaptic function, we have demonstrated a specific adaptation in MSNs from mice with a history of opioid self-administration. This adaptation, a reduction in the normal inhibitory profile of μ OR activation in D1-, but not D2-MSNs and an increase in the probability of presynaptic glutamate release onto D1-, but not D2-MSNs, suggests that repeated opioid exposure during the acquisition of opioid-self-administration behaviors had altered the function of D1-MSNs and their inputs.

The striatum expresses μ ORs in extensively diverse neuronal populations. In D1- and D2-MSNs, μ ORs are found in synaptic vesicles and on the extrasynaptic plasma membrane of both the dendrites and axon terminals of these neurons [20,21]. μ ORs are also found on the cholinergic interneurons of the dorsal striatum [22] and on corticostriatal terminals [20]. As remifentanil self-administration altered μ OR inhibition of D1-MSN, but not D2-MSN, mEPSC frequency in the NAc, it is possible that the function of μ ORs presynaptic to D1-MSNs, perhaps those on the glutamatergic corticostriatal neurons, was affected. Recent findings revealing differential afferents onto different MSN subtypes provide a possible neuroanatomical basis for the dissociable presynaptic adaptations we observed [23].

Although much remains to be determined, these initial findings suggest that the function of D1-, but not D2-MSNs, was altered during the acquisition of opioid self-administration. This adaptation, associated with the reinforcing properties of this opioid, is likely to differ substantially from the cellular adaptations associated with dependence and withdrawal observed once the behavior is established. Although opioids and psychostimulants induce different neuroadaptations, our findings of an MSN-specific effect of

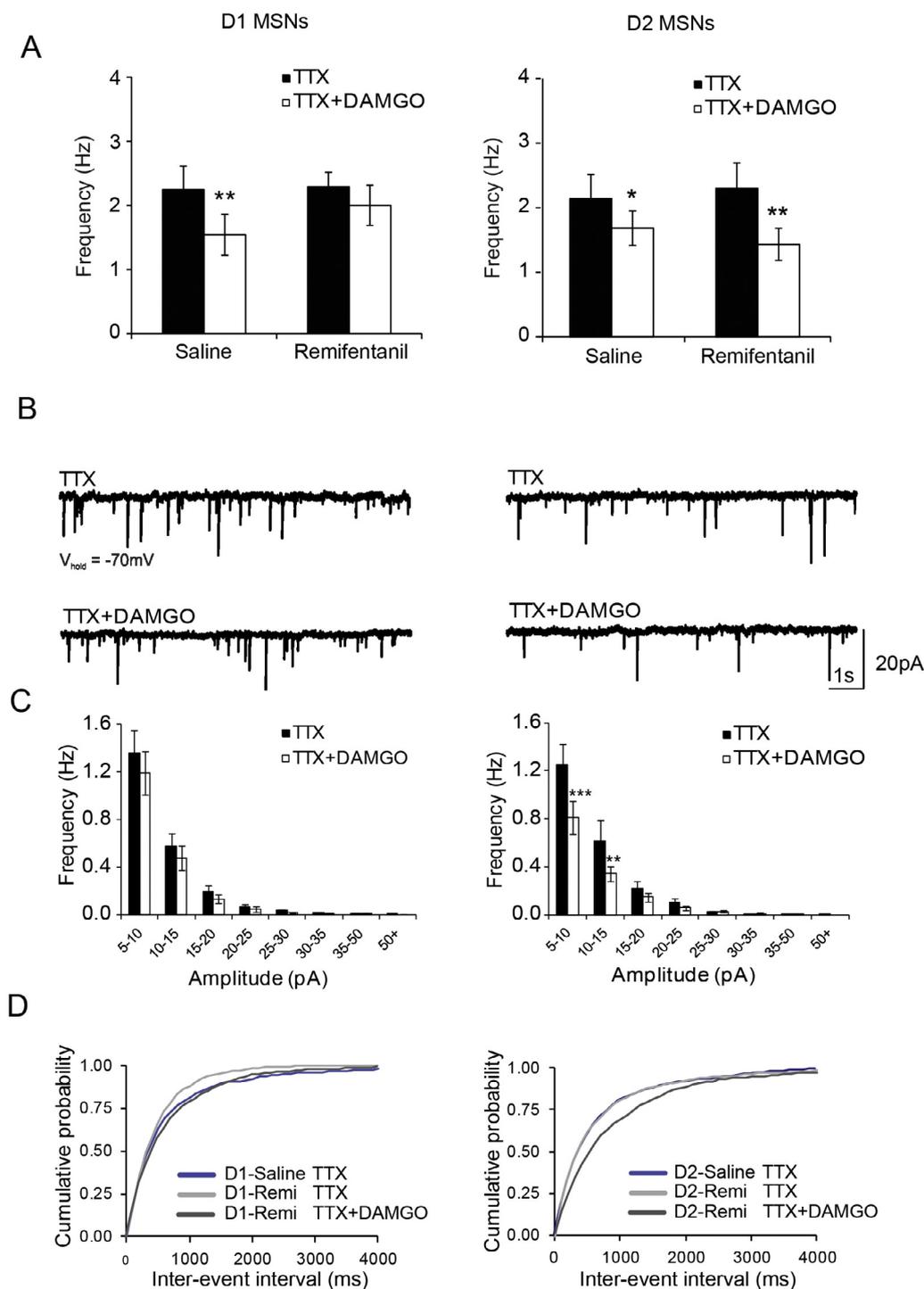


Fig. 2. Opioid self-administration reduces the sensitivity of direct (D1) but not indirect (D2) pathway neurons to μ OR stimulation. **A.** The specific and potent μ OR agonist, DAMGO, resulted in its characteristic profile of inhibition of mEPSC frequency, in both D1- and D2-enriched MSNs from mice that had received saline (D1-MSNs: $p = 0.003$, $n = 11$; D2-MSNs: $p = 0.040$, $n = 12$). This inhibition was also seen in D2-MSNs from mice that had self-administered remifentanil ($p = 0.001$, $n = 10$), but was not detected in D1-MSNs from these animals ($p = 0.277$, $n = 9$). **B.** Sample traces of mEPSCs recorded from D1- and D2-MSNs before and after DAMGO in mice that self-administered remifentanil. **C.** Amplitude–frequency histograms demonstrate a significant reduction in small-amplitude (5–10 pA and 10–15 pA) events exclusively in D2- (p 's < 0.002), but not D1-MSNs (p 's > 0.157) in mice that self-administered remifentanil. **D.** Cumulative TTX (base-line) IEI distributions demonstrate a leftward shift in D1-MSNs following remifentanil self-administration (left panel; $p < 0.001$). This was not seen in D2-MSNs (right panel; $p > 0.999$). In contrast to Fig. 2A, there was an effect of DAMGO on release probability in D1-MSNs of remifentanil animals, depicted by the rightward shift in IEI curve ($p = 0.017$). In D2-MSNs, DAMGO also shifted the IEI curve rightwards ($p = 0.008$). This shift was greater in magnitude in D2- vs. D1-MSNs (see main text). Abbreviation: remi – remifentanil. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

chronic opioid exposure on mEPSC frequency are similar to the effect of chronic cocaine on NAc MSNs [10], and are generally consistent with prior observations of greater involvement of D1- than D2-MSNs in the initial drug effects and drug reinforcement, relative

to withdrawal [11,12,24,25]. However, to our knowledge, our data are the first demonstration of a dissociable effect on the function of D1- versus D2-MSNs following the acquisition and maintenance of opioid self-administration behavior.

Table 2
DAMGO, a μ OR agonist, did not alter the amplitude or kinetics of mEPSC events.

DAMGO (1 μ M)	Amplitude (pA)		Rise (ms)		Decay (ms)		Half-width (ms)	
	–	+	–	+	–	+	–	+
D1								
Saline (n = 11)	13.7 \pm 0.2	14.3 \pm 0.2	1.1 \pm 0.1	1.3 \pm 0.1	3.8 \pm 0.2	3.7 \pm 0.2	5.3 \pm 0.2	5.5 \pm 0.3
Remifentanyl (n = 9)	12.5 \pm 0.8	12.7 \pm 0.4	1.2 \pm 0.1	1.2 \pm 0.1	3.6 \pm 0.1	3.9 \pm 0.2	5.2 \pm 0.2	5.5 \pm 0.3
D2								
Saline (n = 12)	15.2 \pm 0.7	15.3 \pm 0.6	0.9 \pm 0.2	1.1 \pm 0.1	3.5 \pm 0.1	3.8 \pm 0.2	4.94 \pm 0.1	5.3 \pm 0.2
Remifentanyl (n = 10)	13.3 \pm 0.8	13.9 \pm 0.2	1.2 \pm 0.1	1.0 \pm 0.1	3.5 \pm 0.1	3.5 \pm 0.1	4.88 \pm 0.2	4.9 \pm 0.2

mEPSCs were assessed in the presence (+) or absence (–) of DAMGO. There was no effect of DAMGO on the amplitude, the time required to reach maximal amplitude (rise time), time of decay (decay), or time at which the mEPSC width had decayed by 50% (half-width).

Acknowledgements

Funding Sources: NIH grants DA05010 (CC, JYC, CJE, DJ, MSL, WMW), DA30866 (WMW), DA027309 (ASJ) and HD004612 (CC, JYC, MSL). NM and WMW are supported in part by Hatos Scholarships and NM is supported by the Gates Millennium Scholars program.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bbr.2013.08.009>.

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