

SHORT COMMUNICATION

Decreased firing frequency of midbrain dopamine neurons in mice lacking mu opioid receptors

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Keywords: addiction, dopaminergic, electrophysiology, impulse activity, reward

Abstract

Dopamine neurons originating in the midbrain and projecting to cortico-limbic and motor structures are one of the major neuronal substrates implicated in the reinforcing properties of drugs of abuse. The output of this system is largely determined by its impulse activity (amount and pattern of firing activity). Several intrinsic and synaptic factors can influence dopamine neuronal activity and, consequently, addiction liability. Pharmacological studies indicate that μ -opioid receptors and their activation by endogenous opioids may play an important role. In the present study, we use a genetic approach to better understand the role of μ -opioid receptors in modulating dopamine neuronal activity *in vivo*. Using *in vivo* extracellular single-unit recordings, we show that mice lacking μ -opioid receptors exhibit lower firing rates of dopamine neurons compared with their wild-type littermates. Although we observed no overall changes in bursting activity compared with wild-type mice, animals lacking μ -opioid receptors exhibited a higher proportion of regular-spiking cells that lacked bursting activity. These findings are the first to emphasize the critical role of μ -opioid receptors in modulating action potential output of dopamine neurons *in vivo* using a genetic approach. They also provide a possible underlying mechanism for the decreased reinforcing properties of drugs of abuse that was previously observed in mice lacking μ -opioid receptors.

Introduction

Dopamine neurons originating in the midbrain (the ventral tegmental area, VTA, and substantia nigra pars compacta, SNc) and projecting to cortico-limbic and motor structures are one of the major neuronal substrates implicated in the reinforcing properties of drugs of abuse (White & Kalivas, 1998; Wise, 1998; Everitt & Wolf, 2002). The output of this system is largely determined by its impulse activity, i.e. amount and pattern of firing activity (Grace & Bunney, 1984b; Overton & Clark, 1997; Cooper, 2002; Mathon *et al.*, 2003). Our lab has previously demonstrated that rats with elevated basal dopamine cell impulse activity self-administer cocaine more readily compared with rats with low action potential activity (Marinelli & White, 2000). Furthermore, paradigms known to increase future drug self-administration, such as prior exposure to drugs of abuse or to stress, also enhance dopamine cell activity (Marinelli *et al.*, 2001, 2002, 2003) and the proportion of cells exhibiting high levels of bursting activity (Moore *et al.*, 2001). Conversely, experimentally induced decrease in dopamine neuron firing and bursting dampens drug seeking behavior (Marinelli *et al.*, 2003). Collectively, these data suggest that dopamine neuron activity plays a key role in modulating addiction liability.

Several intrinsic and synaptic factors can influence dopamine neuronal activity and, consequently, addiction liability (Mathon *et al.*, 2003). Amongst these, μ -opioid receptors (MORs) and their activation by endogenous opioids fulfill a key modulatory role in regulating both neuronal activity and vulnerability to drugs (Wise *et al.*, 1986; Gerrits *et al.*, 2003). Activation of MORs, through inhibition of local γ -aminobutyric acid (GABA)ergic neurons, disinhibits dopamine cells, thereby enhancing dopamine output (Johnson & North, 1992). Mice lacking MORs display reduced responses to the reinforcing properties of a wide range of drugs of abuse, indicating decreased addiction liability (Kieffer & Gaveriaux-Ruff, 2002; Becker *et al.*, 2002). In addition, we recently demonstrated that dopamine neurons in MOR knockout (KO) mice receive increased GABAergic input compared with wild-type (WT) mice (Mathon *et al.*, 2005). Based on these behavioral and neurophysiological observations, we hypothesized that MOR KO mice might display reduced impulse activity of dopamine neurons.

Materials and methods

Animals

Homozygous MOR KO mice (Schuller *et al.*, 1999) and their WT littermates were obtained from heterozygous breeding following 10 generations of back-crossing to C57Bl6/J. Mice arrived in the animal room at 3–4 months old (24–33 g) and were tested 7–21 days

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Received 6 December 2004, revised 22 March 2005, accepted 24 March 2005

after arrival. During this time, mice were kept at $21 \pm 1^\circ\text{C}$ on a 12 : 12 h light : dark schedule (lights on at 08.00 h). At the end of the experiments, animals were genotyped by polymerase chain reaction on genomic DNA isolated from tail tips (Schuller *et al.*, 1999). Mice were handled according to the 'Guide for the Care & Use of Laboratory Animals' (1996), and the Institutional Animal Care and Use Committee of The Chicago Medical School approved all procedures (protocol 04-21).

Extracellular single-unit recordings

Methods were similar to those reported previously (Henry *et al.*, 1989). Briefly, mice were anesthetized with chloral hydrate (650–720 mg/kg, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Anesthesia was maintained throughout the recording with i.p. chloral hydrate injections (70–100 mg/kg i.p., every 40–50 min). A hole was drilled in the skull, and the dura-matter was retracted from the area overlying the midbrain. A glass electrode filled with a 2 M NaCl solution saturated in 1% fast green dye (Fisher Scientific, Houston, TX, USA) was positioned 2 mm above the dopamine cell region and slowly advanced with a hydraulic microdrive (David Kopf Instruments). The coordinates (in mm, from interaural line) were: 0.4–1.2 AP; 0.2–1.0 L; and 4.0–5.0 ventrally from the surface of the brain. *In vitro* impedance of the electrodes was 1.8–2.5 M Ω , measured at 135 Hz (Winston Electronics BL1000-B, San Francisco, CA, USA).

Electrical signals were fed into a high-impedance amplifier (Fintronics, Orange, CT, USA), displayed on an oscilloscope (Tektronix R5110, Chicago, IL, USA), and monitored with a window discriminator and audio monitor (Grass AM8, Quincy, MA, USA). Digital outputs were fed through an interface (Digidata 1200 series, Axon Instruments, Foster City, CA, USA) to a computer running AxoScope software (Axon Instruments).

After the recording, the site of recording was determined by locally ejecting (–28 nA) fast green dye, the location of which was subsequently established using routine histochemical techniques (Henry *et al.*, 1989). Dopamine cells were identified by anatomical location in the midbrain and by known electrophysiological characteristics (Grace & Bunney, 1984a, 1984b). These neurons had: (i) low spontaneous firing rates of 0.5–10 Hz; (ii) either a slow irregular firing pattern or a slow bursting pattern with decreasing spike amplitude and increasing interspike interval within the burst; and (iii) a characteristic triphasic (+/-/+) waveform lasting 2.5–4.5 ms when filtered between 0.4 and 0.5 kHz (Grace & Bunney, 1983), or 4.0–6.9 ms when filtered between 0.3 and 5.0 kHz (Ungless *et al.*, 2004).

Neuronal activity was determined for 1–3 cells/animal (WT $n = 15$ cells, $n = 7$ mice; KO $n = 13$ cells, $n = 5$ mice) by an

experimenter that was blind with respect to the mouse genotype. Recordings were performed for 3–5 min to establish a mean baseline firing rate. Only cells with stable activity (< 5% variation) were included. Data were analysed with a custom-made visual basics program (Burst, M. Marinelli) that measured both firing rates (number of spikes/s) and bursting activity. A burst was initiated by two spikes with an interspike interval < 80 ms and ended with two spikes having an interspike interval > 160 ms. We evaluated both burst quantity (percentage of spikes emitted in bursts, percentage time spent bursting and burst event frequency) and burst quality (number of spikes/burst, burst duration and frequency of spikes within bursts). We also evaluated 'non-bursting activity', by subtracting all burst events from the record of total activity.

Throughout our recordings, we maintained body temperature at 36.1 – 37.4°C with a thermostatically controlled heating pad (Fintronics); both WT and MOR KO mice showed similar body temperatures throughout the recordings (WT: $36.7 \pm 0.1^\circ\text{C}$; KO 36.6 ± 0.1 ; $t = 0.20$, $P > 0.8$). Animals also did not differ for sensitivity to anesthesia, as both groups required a similar amount of chloral hydrate to induce anesthesia (data not shown, $t = -0.94$, $P > 0.3$).

Statistical analyses

Data were first analysed with an analysis of variance (ANOVA) considering Genotype (WT vs. KO) and Structure (VTA vs. SNc) as between factors. There were no effects of Structure, or any Genotype–Structure interaction for any parameter ($F_s = 0.01$ – 3.0 , n.s.). Therefore, we pooled data obtained from the VTA and SNc and analysed data using two-tailed Student's *t*-tests comparing WT and MOR KO animals. We used χ^2 analysis to compare the proportion of cells exhibiting any given parameter between groups.

Results

Firing rates of midbrain dopamine neurons (action potentials/ s) were lower in mice lacking MORs compared with those in WT controls (WT $n = 15$ cells, $n = 7$ mice; KO $n = 13$ cells, $n = 5$ mice; $t = 2.2$, $P < 0.03$, Fig. 1). Absence of MORs also modified the distribution of firing rates across neurons; thus, whereas most (60%) WT neurons exhibited fast firing rates (> 4 Hz), only a small subpopulation (23%) of cells fired > 4 Hz in MOR KO animals ($\chi^2 = 3.88$, $P < 0.05$).

To examine which component of firing rate was modified by lack of MORs, we analysed dopamine cell firing rates after having subtracted bursting activity (bursts are spikes clustered at high frequency, see Materials and methods) from overall spiking activity. As Fig. 1C

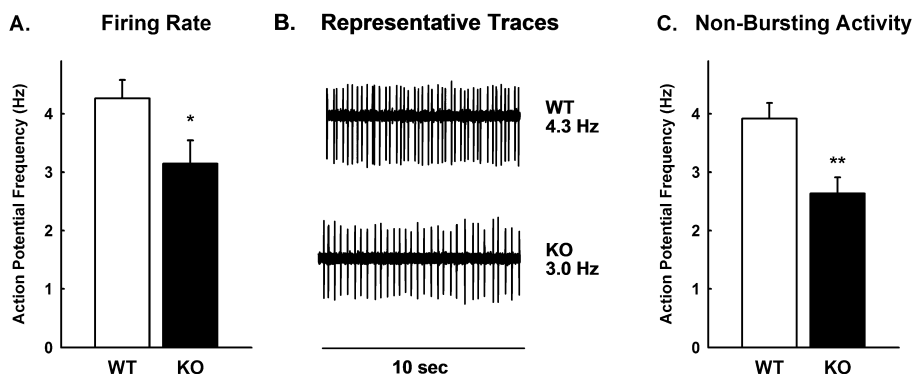


FIG. 1. *In vivo* firing rate of midbrain dopamine neurons from wild-type (WT) and MOR knockout (KO) mice. (A) The firing rate of dopamine neurons (action potential frequency) is lower in MOR KO compared with WT controls. (B) Representative traces from a WT and MOR KO mouse. (C) The non-bursting firing rate (action potential frequency after having subtracted all burst events) is lower in MOR KO compared with WT controls (WT: $n = 15$ cells, $n = 7$ mice; KO: $n = 13$ cells, $n = 5$ mice; * $P < 0.03$, ** $P < 0.01$).

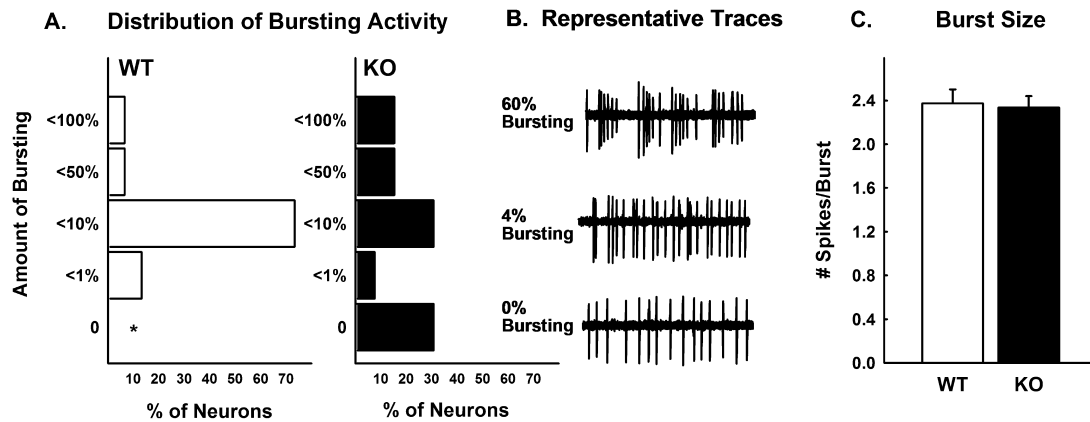


FIG. 2. *In vivo* bursting activity of midbrain dopamine neurons from wild-type (WT) and MOR knockout (KO) mice. (A) Bursting distribution of dopamine neurons shows different degrees of bursting activity in cells from WT and KO mice. The x-axis represents the percentage of cells bursting at a particular rate, the y-axis shows the different bursting rates: 0% indicates no bursting at all, <1% represents 0.1–1.0% bursting, 10% represents 1.1–10.0% bursting, <50% represents 10.1–50.0% bursting, <100% represents 50.01–100.0% bursting. In WT mice, all neurons showed some degree of bursting; in MOR KO mice, however, approximately 30% of neurons fired no bursts at all ($*P < 0.05$, χ^2 at 0% bursting). (B) Representative 10 s traces of neurons firing 0%, 4% and 60% bursting. (C) The average number of spikes within each burst did not differ between genotypes (WT: $n = 15$ cells, $n = 7$ mice; KO: $n = 9$ cells, $n = 5$ mice; $P > 0.8$, analysed only for those cells exhibiting bursting activity).

TABLE 1. Impulse activity parameters of midbrain dopamine neurons in WT and MOR KO mice

Group	Firing rate (Hz)	Non-bursting frequency (Hz)	Bursting spikes (%)	Time spent bursting (%)	Burst event frequency (Hz)	Spikes per burst	Burst duration (ms)	Intraburst frequency (Hz)
WT	4.26 ± 0.32	3.81 ± 0.26	8.18 ± 3.84	2.27 ± 1.30	0.15 ± 0.07	2.37 ± 0.13	94.15 ± 12.63	17.35 ± 1.24
KO	3.14 ± 0.40*	2.64 ± 0.28**	13.56 ± 5.86	3.15 ± 1.74	0.23 ± 0.12	2.34 ± 0.10	103.09 ± 12.99	14.44 ± 0.95

* $P < 0.03$; ** $P < 0.01$.

shows, non-bursting activity was lower in MOR KO vs. WT mice ($t = 3.1$, $P < 0.01$), suggesting that differences in firing rate can be explained by the non-bursting component of neuronal impulse activity.

To determine whether lack of MOR had any effects on bursting activity of dopamine neurons, we analysed cell firing patterns in our two groups. We did not observe any changes in average bursting activity (Fig. 2, Table 1). Concerning the quantity of bursting (percentage of spikes emitted in bursts, percentage time spent bursting, burst event frequency), WT and MOR KO mice showed similar group averages. Interestingly, however, although all WT neurons showed some degree of bursting (ranging from about 1% to 55% of bursting spikes), about one-third of MOR KO neurons displayed no bursting at all ($\chi^2 = 5.4$, $P < 0.05$). With regard to the quality of bursting (burst size, burst duration, frequency of spikes within bursts), both WT and MOR KO showed similar group averages (Fig. 2, Table 1) and comparable sample variance distribution (data not shown).

Discussion

We examined the impulse activity of midbrain dopamine neurons in mice lacking MORs. Absence of MORs is associated with low firing activity of dopamine neurons. An analysis of the firing pattern revealed that MORs preferentially regulate non-bursting activity of these cells, without modifying overall bursting activity. Despite the lack of a general change in the level of bursting activity, we did observe a difference in the proportion of cells showing bursting spikes: whereas in WT mice all cells displayed some degree of bursting, in MOR KO mice about one-third of the neuronal population displayed

no bursting at all. Concerning intraburst characteristics, no differences were found between the genotypes, indicating that the intrinsic burst-regulating properties of the cells are not influenced by MORs.

MOR activation increases the activity of midbrain dopamine neurons by inhibiting local GABAergic interneurons (Gysling & Wang, 1983; Johnson & North, 1992). We have recently shown that midbrain dopamine neurons of MOR KO mice receive increased GABAergic input *in vitro*, as indicated by increased frequency of spontaneous inhibitory postsynaptic currents (Mathon *et al.*, 2005). GABA, acting both through ionotropic GABA_A and metabotropic GABA_B receptors, has been demonstrated to decrease the firing frequency *in vivo* (Engberg *et al.*, 1993; Paladini & Tepper, 1999; Erhardt *et al.*, 2002; Floresco *et al.*, 2003). The observed decreased firing frequency of dopamine neurons in MOR KO mice is therefore likely to be the result of increased GABAergic inhibition to these neurons.

In addition to modulating the firing frequency, GABAergic input can also affect bursting activity of midbrain dopamine neurons. Activation of GABA_A receptors inhibits bursting by decreasing neuronal input resistance (Paladini *et al.*, 1999); activation of GABA_B receptors located on dopamine neurons showed decreased bursting activity, probably by acting on GIRK channels (Slesinger *et al.*, 1997; Erhardt *et al.*, 2002; Cruz *et al.*, 2004). The greater proportion of non-bursting neurons in MOR KO mice is therefore also likely to be due to the previously observed increase in GABAergic input (Mathon *et al.*, 2005).

Decreased impulse activity of dopamine neurons could be expected to lead to a decrease in dopamine release in target areas such as the nucleus accumbens. Such changes in firing rate are only partly

associated with detectable changes of dopamine levels (Gonon, 1988; Floresco *et al.*, 2003). In the nucleus accumbens, MOR KO mice have a decreased dopamine reuptake whereas extracellular dopamine levels are unaltered compared with control mice (Chefer *et al.*, 2003). As extracellular dopamine levels are determined by both release and reuptake, this provides indirect evidence for a decreased dopamine release in MOR KO and further corroborates our findings that lack of MOR decreases dopaminergic transmission.

Under a functional point of view, the greater proportion of non-bursting neurons in MOR KO mice suggests that dopamine neurons in MOR KO mice might not be capable of making the switch from regular to burst firing, which is associated with the presentation of unexpected rewarding stimuli (Schultz, 1998). This, together with the association between dopamine action potential output and addiction liability (Marinelli & White, 2000; Marinelli *et al.*, 2003), provides a possible underlying mechanism for the decreased vulnerability to drugs of abuse observed in MOR KO mice (Becker *et al.*, 2002; Kieffer & Gaveriaux-Ruff, 2002).

In summary, we demonstrate that MOR KO mice display lower impulse activity of midbrain dopamine neurons compared with WT controls. This is the first report using KO mice to understand the role of MOR in regulation of dopamine neuron activity *in vivo*. Despite the well-known shortcomings of developmental KO models in studying receptor function, these findings emphasize the critical role of MORs in modulating action potential output of dopamine neurons. They also provide a possible underlying mechanism for the decreased reinforcing properties of drugs of abuse in MOR KO and support the hypothesis that dopamine neuronal firing modulates vulnerability to drugs of abuse.

Acknowledgements

D.S.M. was supported by NWO Travel Grant R 93-454; J.E.P. was supported by DA-09040 and DA-15537. The authors wish to thank C.N. Rudick for performing the histology and K. Philibert for doing the genotyping.

Abbreviations

GABA, γ -aminobutyric acid; KO, knockout; MORs, μ -opioid receptors; SNc, substantia nigra pars compacta; VTA, ventral tegmental area; WT, wild-type.

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