

# No Effect of Morphine on Ventral Tegmental Dopamine Neurons during Withdrawal

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Substantial evidence indicates that the ventral tegmental area (VTA) of the mesocorticolimbic dopaminergic (DA) system has a key role in mechanisms of opiate dependence. Although DA neurons have been studied extensively, little is known about their activity and their response to acute morphine during morphine dependence. We recorded the activity of VTA DA neurons in five groups of anesthetized rats: drug-naïve (naïve) rats, morphine-dependent [(MD) implanted with pellets] rats, and three groups of withdrawn rats. Withdrawals either were precipitated by naltrexone or occurred spontaneously 24 h or 15 d after pellet removal. We confirmed that acute morphine in naïve rats produced a marked increase in the firing of VTA DA neurons. We also found that the basal firing rate of VTA DA neurons was markedly higher in MD than in naïve rats; however, in MD rats, acute morphine failed to increase DA activity. We confirmed inhibition of VTA DA activity in MD rats in response to precipitated withdrawal; however, this inhibition resulted only in a normalization of the firing rate to that of naïve animals. In rats that had spontaneous withdrawal after 24 h or 15 d, the activity of VTA DA neurons was similar to that of naïve rats, and an acute injection of morphine failed to alter their activity. Our results indicate that VTA DA neurons show long-lasting tolerance to the acute effect of morphine after withdrawal. These findings show that VTA DA neural activity is unlikely to be a factor in the altered behavioral responses that occur with acute morphine or naltrexone administration after chronic opiate exposure.

**Key words:** addiction; withdrawal; opiates; ventral tegmental area; naltrexone; extracellular recording

## Introduction

Dopamine (DA)-containing neurons originating in the ventral tegmental area (VTA) project primarily to the nucleus accumbens and the prefrontal cortex, forming the mesocorticolimbic system (Carr and Sesack, 2000; for review, see Onn et al. 2000; O'Donnell, 2003). All drugs of abuse influence DA-mediated neurotransmission by affecting directly or indirectly the activity of these cells (Spanagel and Weiss, 1999; Marinelli et al., 2003). These and other observations indicate that drug abuse is a DA-dependent disorder in which the positive reinforcing value of the drug is mediated through the activation of the mesocorticolimbic DA system (Di Chiara, 1999). Adaptive changes in cellular and synaptic function in the mesocorticolimbic DA system after prolonged or repeated opiate administration have been shown (for review, see Williams et al., 2001). These adaptations are believed to play an important role in the development of physiological tolerance to and dependence on opiates and to contribute to additional changes underlying the complex neurobehavioral syndrome of opiate withdrawal (Nestler, 2001). This withdrawal

syndrome has short-, long-, and very long-term features, including altered hedonic processing with increased drug reward and decreased rewarding properties of natural reinforcers long after acute withdrawal has ended (Harris and Aston-Jones, 2003).

The above observations indicate that opiate dependence or withdrawal may substantially alter the activity of VTA DA neurons to mediate these prolonged effects of exposure; however, although DA neurons have been studied extensively during opiate withdrawal (Diana et al., 1995, 1999), little is known about their activity during morphine dependence or their response to acute morphine during dependence. Here, we examined the effects of chronic morphine on VTA DA neuronal activity, as well as the response of these neurons to acute morphine during chronic morphine treatment or during morphine abstinence. Our results show that chronic morphine produces a prolonged tolerance in the activity of DA neurons to subsequent acute morphine injections, indicating that these neurons are unlikely to mediate many long-term effects of opiate dependence (e.g., craving and sensitization).

## Materials and Methods

**Animals.** One hundred eighty-five Sprague Dawley rats (Taconic, Germantown, NY; Iffa-CREDO, Lyon, France; 200–225 g) were used. Rats were housed three or four per cage under controlled conditions (22–23°C; 40% relative humidity; 12 h light/dark illumination cycle; lights on from 7 A.M. to 7 P.M.) and were allowed *ad libitum* access to commercial chow and tap water.

**Chronic drug treatment.** Two 75 mg morphine pellets (provided by the

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National Institute on Drug Abuse, National Institutes of Health, Rockville, MD) were implanted subcutaneously under halothane anesthesia for the chronic morphine treatment. This procedure has been shown to produce the following results: (1) a consistent plasma morphine concentration beginning a few hours after the implantation of the pellets (Yoburn et al., 1985) and (2) a “full” withdrawal syndrome, including overt somatic signs after an acute injection of opiate antagonist (Frenois et al., 2002). Full behavioral dependence on morphine was achieved 24 h after implantation of the morphine pellets and remained relatively constant for 15 d (Gold et al., 1994). On the basis of these physiological and behavioral findings, we used the term “morphine dependent” (MD) to denote rats chronically implanted with two pellets of morphine. The drug-naïve rats (naïve) received placebo pellets containing the excipient without morphine ( $n = 12$ ) or were not implanted ( $n = 52$ ). Electrophysiological experiments in MD rats were performed 6 d after the implantation of morphine pellets ( $n = 88$ ). Precipitated withdrawal (PW) was induced by intravenous injection of naltrexone hydrochloride (NAL) (Sigma, St. Louis, MO; 0.1 mg/kg dissolved in isotonic saline) in anesthetized MD rats placed in a stereotaxic frame. Electrophysiological experiments were performed within 1 h after NAL injection (1H-PW) ( $n = 10$ ). Spontaneous withdrawal (SW) was induced by removing the pellets after 6 d, and electrophysiological experiments were performed 24 h (24H-SW) ( $n = 9$ ) or 15 d (15D-SW) ( $n = 14$ ) after removal. To avoid exposing the other rats to the stress of a rat undergoing withdrawal, all rats in a cage received the same treatment.

**Intravenous drug injection.** The jugular vein was cannulated for intravenous administration of pharmacological agents. NAL (0.1 mg/kg) and morphine hydrochloride (1 mg/kg) were prepared in isotonic saline. The DA receptor agonist apomorphine (APO) (0.1 mg/kg; Sigma) and the D<sub>2</sub> DA receptor antagonist eticlopride (0.1 mg/kg; Sigma) were prepared in isotonic saline and used to pharmacologically characterize VTA DA neurons.

**Surgery.** Animals were initially anesthetized with 3% halothane in air administered through a face mask. A tracheotomy was performed, and 2% halothane was delivered through a tracheal cannula via spontaneous respiration for surgical procedures. During recording experiments, the concentration of halothane was kept at 1.0–1.2%. Animals were placed in a stereotaxic frame, and body temperature was maintained at 36–38°C with a thermistor-controlled electric heating pad. All incision points were infiltrated with a long-lasting anesthetic (sensorcaine, 0.5%). The skull was exposed, and a hole was drilled above the VTA (5.3 mm caudal to bregma; 0.8 mm lateral to the midline).

**VTA recordings.** A glass micropipette (tip diameter, 2–3  $\mu\text{m}$ ; 4–6 M $\Omega$ ) filled with a 2% Pontamine Sky Blue solution in 0.5 M sodium acetate was lowered into the VTA. DA neurons were identified according to well established electrophysiological features (Guyenet and Aghajanian, 1978; Grace and Bunney, 1983, 1984; Tepper et al., 1984; Chiodo, 1988; Ungless et al., 2004). These included the following: (1) action potential (AP) with biphasic or triphasic waveform and a half AP width >1.1 ms in duration; (2) single and/or burst spontaneous firing patterns (characterized by spike-amplitude decrement; and (3) inhibition of spontaneous activity by DA receptor agonists and subsequent reversal by DA receptor antagonists. Signals were amplified and filtered (0.1–5 kHz bandpass) with conventional electronics. Spikes of single neurons were discriminated, and digital pulses were sent to a computer for on-line data collection with a laboratory interface and software (CED 1401, Spike2; Cambridge Electronic Design, Cambridge, UK).

**Histology.** At the end of each recording experiment, the electrode placement was marked with an iontophoretic deposit of Pontamine Sky Blue dye (–20  $\mu\text{A}$ ; continuous current for 12–15 min). After the experimental procedures, the animals were deeply anesthetized with halothane (5%) and decapitated. Brains were removed and snap-frozen in a solution of isopentane at –70°C.

**Data analysis.** Three parameters of VTA impulse activity were computed over 200 s epochs before and after drug administration: the basal firing rate, the proportion of spikes that occurred in bursts, and the regularity of firing. The onset of a burst was defined as the occurrence of two spikes with an interspike interval <80 ms (Grace and Bunney, 1983). The percentage of spikes in bursts was calculated by dividing the number

of spikes occurring in bursts (first spike of the burst not included) by the total number of spikes occurring in the same period of time. Variations of burst firing are reported as the percentage of spikes in bursts before the drug minus the percentage after the drug. We also evaluated the amount of bursting activity by calculating the burst-event frequency (number of burst events over time), the burst size (number of spikes within each burst), and the duration of each burst. Regularity of firing was measured with the coefficient of variation, i.e., the SD and mean value of instantaneous interspike intervals (Georges and Aston-Jones, 2002). Results are expressed throughout as mean  $\pm$  SEM. When two means were compared, the statistical significance of their difference was assessed by two-tailed paired Student's *t* tests. For multiple comparisons, values were subjected to a one-way ANOVA followed by *post hoc* Dunnett or Newman–Keuls tests.

## Results

Data are reported for 520 histologically verified VTA neurons that were identified as DA neurons according to well established electrophysiological features (see Materials and Methods). All DA neurons reported here have an AP width of  $\geq 1.1$  ms (Ungless et al., 2004), and no difference for this criterion was observed among naïve, dependent, or withdrawn rats (Fig. 1B) (ANOVA;  $F_{4,515} = 2.140$ ;  $p = 0.075$ ). The DA receptor agonist APO was administered for the last DA neuron recorded in each experiment (0.1 mg/kg, i.v.). As reported previously (Grace and Bunney, 1983, 1984), this drug consistently inhibited spontaneous impulse activity of DA neurons within 30 s of injection. A subsequent injection of the D<sub>2</sub> DA receptor antagonist eticlopride (0.1 mg/kg, i.v.) consistently restored impulse activity (Fig. 1C,D).

### The activity of VTA DA neurons is higher in morphine-dependent rats

The basal firing rate of VTA DA neurons was higher in MD than in naïve or withdrawn rats (Fig. 2A) (ANOVA;  $F_{4,515} = 12.42$ ;  $p < 0.0001$ ). A typical recording of a fast-firing VTA DA neuron (8.5 Hz) is shown in Figure 1C. In contrast, the firing rates for the withdrawn groups did not differ from each other or from those in naïve rats. The mean firing rate of VTA DA neurons in MD rats was  $5.5 \pm 0.2$  Hz compared with  $3.9 \pm 0.2$  Hz in naïve,  $4.5 \pm 0.2$  Hz in 1H-PW,  $4.1 \pm 0.2$  Hz in 24H-SW, and  $4.0 \pm 0.2$  Hz in 15D-SW (Fig. 2A). A typical recording of a pharmacologically identified VTA DA neuron firing at 5.5 Hz in a 24H-SW rat is shown in Figure 1D. The scatterplot in Figure 1A reports the bursting activity as a function of firing rate for individual neurons. For clarity, only VTA DA neurons of naïve and MD rats are shown on this graph. There were small but significant positive correlations between bursting activity (percentage spikes in bursts) and firing rate for VTA DA neurons in naïve, MD, 1H-PW, 24H-SW, and 15D-SW rats (naïve,  $r = 0.10$ ,  $p < 0.0001$ ; MD,  $r = 0.25$ ,  $p < 0.0001$ ; 1H-PW,  $r = 0.50$ ,  $p < 0.0001$ ; 24H-SW,  $r = 0.31$ ,  $p < 0.0001$ ; 15D-SW,  $r = 0.16$ ,  $p < 0.0001$ ; Pearson's correlation). Firing rates for the five groups were normally distributed (Fig. 3) (range, 0.2–10.5 Hz), indicating similar neuronal populations in all groups of rats; however, the Gaussian curve was shifted  $\sim 1.5$  Hz to the right in MD compared with naïve and withdrawn rats (Fig. 3F).

Morphine dependence increased the amount of burst events emitted over time (Fig. 2B) (ANOVA;  $F_{4,527} = 10.19$ ;  $p < 0.0001$ ) as well as the duration of the bursts (Fig. 2E) (ANOVA;  $F_{4,527} = 17.50$ ;  $p < 0.0001$ ). The mean burst-event frequency of VTA DA neurons in MD rats was  $1.7 \pm 0.1$  Hz compared with  $1.2 \pm 0.1$  Hz in naïve,  $1.2 \pm 0.1$  Hz in 1H-PW,  $1.3 \pm 0.1$  Hz in 24H-SW, and  $1.0 \pm 0.1$  Hz in 15D-SW (Fig. 2B). The average burst duration of VTA DA neurons in MD rats was  $108.6 \pm 2.0$  ms compared with

89.3 ± 2.8 ms in naive, 94.9 ± 2.3 ms in 1H-PW, 84.9 ± 2.3 ms in 24H-SW, and 84.1 ± 3.1 ms in 15D-SW (Fig. 2E). The means for spikes in bursts were similar in naive, MD, and withdrawn rats (Fig. 2C) (ANOVA;  $F_{4,515} = 0.66$ ;  $p = 0.62$ ). The percentage of spikes occurring in bursts was 20.2 ± 1.4% in naive, 19.7 ± 1.4% in MD, 22.5 ± 1.5% in 1H-PW, 21.0 ± 1.4% in 24H-SW, and 19.6 ± 1.4% in 15D-SW (Fig. 2C). The burst sizes (spikes per burst) were similar in naive, MD, and withdrawn rats (Fig. 2D) (ANOVA;  $F_{4,527} = 1.65$ ;  $p = 0.16$ ). The number of spikes per burst was 2.2 ± 0.04 in naive, 2.2 ± 0.03 in MD, 2.1 ± 0.03 in 1H-PW, 2.1 ± 0.04 in 24H-SW, and 2.1 ± 0.02 in 15D-SW (Fig. 2D).

The regularity of firing of VTA DA neurons was higher in MD than in naive or withdrawn rats (Fig. 2F) (ANOVA;  $F_{4,515} = 2.7$ ;  $p < 0.03$ ). Regularity of firing was measured from the coefficient of variation (see Materials and Methods) and was 0.70 ± 0.02 in naive, 0.63 ± 0.02 in MD, 0.72 ± 0.02 in 1H-PW, 0.70 ± 0.02 in 24H-SW, and 0.70 ± 0.02 in 15D-SW (Fig. 2F).

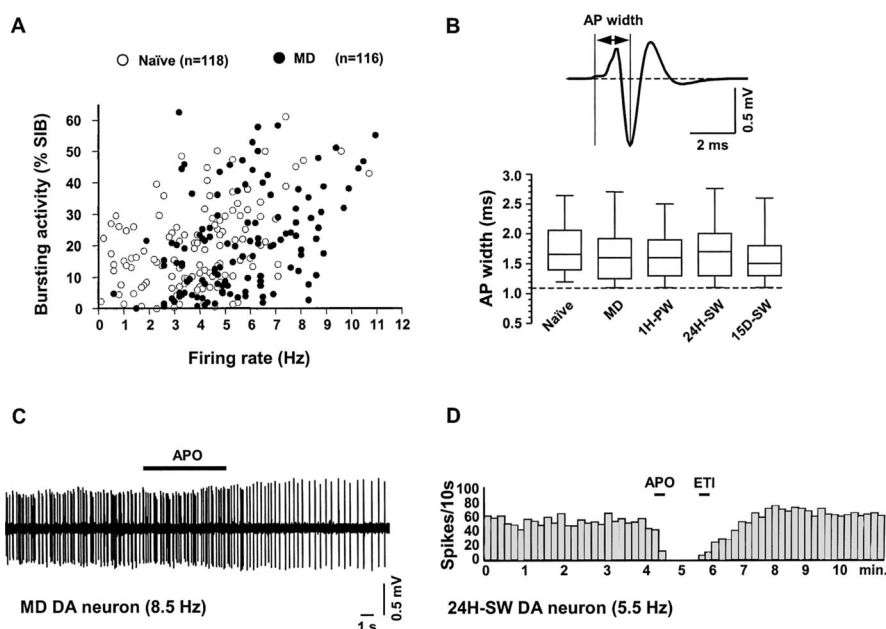
### Acute morphine did not modify the activity of VTA DA neurons in morphine-dependent or withdrawn rats

Administration of morphine (1 mg/kg, i.v.) to naive rats increased the impulse activity of VTA DA neurons to ~40% and the burst firing to ~80% of the pre-drug baseline within 3 min (Fig. 4A) (ANOVA;  $F_{5,66} = 10.03$ ;  $p < 0.0001$ ) (Table 1) ( $p < 0.001$ , Student's *t* test for firing rate;  $p < 0.01$ , Student's *t* test for burst firing;  $n = 13$ ; typical example in Fig. 4C). This activation is maximal 3 min after the injection, and all cells returned to baseline activity 6–8 min after the injection (Fig. 4B).

In contrast to this activation in naive rats, intravenous injection of morphine to morphine-dependent or spontaneously withdrawn rats (24 h or 15 d after removal of morphine pellets) had no significant effect on the firing rate, burst firing, or regularity of firing of VTA DA neurons. Administration of morphine (1 mg/kg, i.v.) 1 h after precipitating withdrawal with NAL (0.1 mg/kg, i.v.) elicited a small but significant decrease of firing rate (–10%) and burst activity (–8.5%) (Table 1) ( $p < 0.01$ ; Student's *t* test for firing rate and burst firing;  $n = 6$ ). This effect did not reach significance when the five groups were compared together in an ANOVA (Fig. 4A). Administration of saline had no effect on firing rate, burst firing, or regularity of firing of VTA DA neurons in naive, MD, 1H-PW, 24H-SW, or 15D-SW rats (all groups pooled together; ANOVA;  $F_{4,27} = 0.31$ ;  $p = 0.870$ ).

### Acute NAL normalized activity of VTA DA neurons in morphine-dependent rats

Administration of NAL (0.1 mg/kg, i.v.) precipitated a withdrawal syndrome in our anesthetized morphine-dependent rats. The following somatic signs were observed for each morphine-pelleted rats injected with NAL: body shake, mastication, and rhinorrhea. These observations confirmed that our morphine treatment produced dependence.



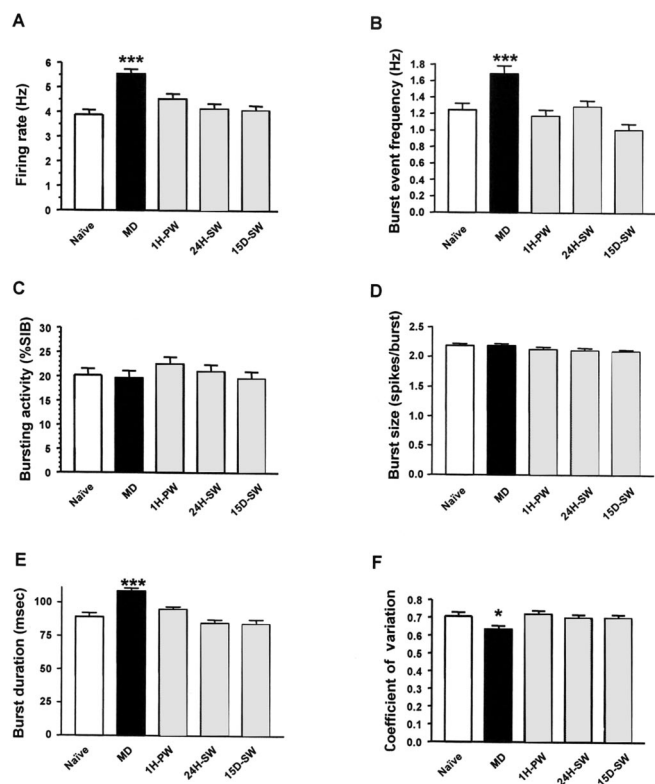
**Figure 1.** Electrophysiological and pharmacological identification of VTA DA cells. **A**, Scatterplot showing the bursting activity [percentage spikes in bursts (%SIB)] as a function of firing rate of individual neurons from naive rats ( $n = 64$  rats, 118 cells) and MD rats ( $n = 88$  rats, 116 cells). Note the small number of VTA DA neurons in MD rats with a slow-firing and low-bursting activity. **B**, Typical example of average extracellular waveform of DA neuron. AP widths were measured from the start of the action potential to the negative trough (top). An AP width ≤ 1.1 ms was used to exclude all non-DA neurons. AP widths were similar and normally distributed (range, 1.1–2.7 ms) in naive rats ( $n = 64$  rats, 118 cells), MD rats ( $n = 88$  rats, 116 cells), and in both withdrawal conditions [precipitated: 1H-PW ( $n = 10$  rats, 87 cells); spontaneous: 24H-SW ( $n = 9$  rats, 93 cells); and 15D-SW ( $n = 14$  rats, 106 cells)]. **C**, Oscilloscope traces of impulses from a VTA DA neuron showing a typical recording of a fast-firing VTA DA neuron (8.5 Hz) in an MD rat. Note the characteristic inhibition of spontaneous activity by APO (0.1 mg/kg, i.v.). The APO injection is designated by the line above the trace. **D**, Firing rate histograms showing the firing activity in a typical VTA DA neuron (5.5 Hz) in a spontaneously withdrawn rat 24 h after removal of the pellet. Note the characteristic inhibition of spontaneous activity by APO (0.1 mg/kg, i.v.) and subsequent reversal by eticlopride (ETI) (0.1 mg/kg, i.v.).

Administration of NAL (0.1 mg/kg, i.v.) to morphine-dependent rats decreased the tonic firing rate (–40%;  $n = 10$ ) (Fig. 4D) (ANOVA;  $F_{5,64} = 15.21$ ;  $p < 0.0001$ ) and the burst firing of VTA DA neurons (–55%;  $n = 10$ ) (Table 1) ( $p < 0.001$ ; Student's *t* test) without affecting the regularity of firing. This reduction of activity was biphasic: (1) the first rapid inhibition was maximal 30 s after the injection and was followed by a return to baseline activity (Fig. 4F, trace); and (2) a second inhibition started 3 min after the injection and persisted for several hours [as reported previously (Georges and Aston-Jones, 2003)] (Fig. 4E). Analyses of impulse activity after NAL revealed that this second inhibition during withdrawal resulted only in a normalization of the firing rate to that of naive animals. Thus, the tonic firing rate of VTA DA neurons in naive rats ( $4.5 ± 0.2$  Hz) was similar to their firing rate in morphine-dependent rats after NAL ( $4.2 ± 0.6$  Hz). NAL administered to naive or withdrawal rats had no significant effect on firing rate, burst firing, or regularity of firing of VTA DA neurons (Table 1).

## Discussion

Here we confirmed that acute morphine in naive rats produced a marked increase in the firing of VTA DA neurons (Gysling and Wang, 1983; Matthews and German, 1984). Morphine dependence also increased the firing rate of VTA DA neurons compared with naive animals and produced a shift to the right in the firing rate distribution; however, our results also showed that, in dependent or withdrawal rats, an acute injection of morphine failed to increase the activity of DA neurons. We also confirmed that



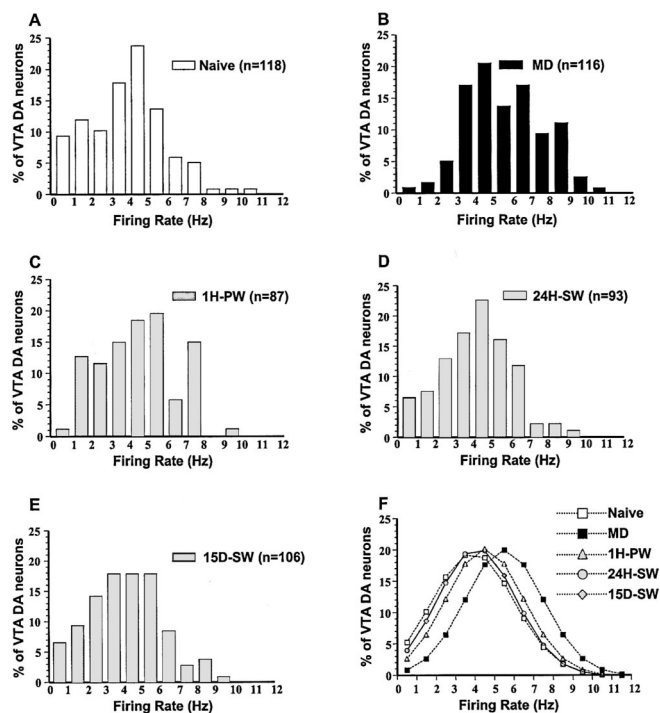


**Figure 2.** Analysis of the firing rate (**A**), burst firing characteristics (**B–E**), or regularity of firing (coefficient of variation) (**F**) of VTA DA neurons in naive rats ( $n = 64$  rats, 118 cells), in MD rats ( $n = 88$  rats, 116 cells), and in both withdrawal conditions [precipitated: 1H-PW ( $n = 10$  rats,  $n = 87$  cells); spontaneous: 24H-SW ( $n = 9$  rats,  $n = 93$  cells); and 15D-SW ( $n = 14$  rats, 106 cells)]. **A**, MD rats exhibited a higher basal firing rate than did naive or morphine-withdrawn rats (1H-PW; 24H-SW; 15D-SW). **B**, MD rats exhibited a higher burst-event frequency than did naive or morphine-withdrawn rats (1H-PW; 24H-SW; 15D-SW). **C**, Means of bursting activity were similar in naive, MD, and withdrawn rats. **D**, Mean burst sizes were similar in naive, MD, and withdrawn rats. **E**, MD rats exhibited a higher duration of the bursts than did naive or morphine-withdrawn rats (1H-PW; 24H-SW; 15D-SW). **F**, A decreased coefficient of variation implies regularization of firing. The regularity of firing of VTA DA neurons was higher in MD than in naive or withdrawn rats. ANOVAs followed by Newman–Keuls tests for pairwise comparisons were performed for firing rate (**A**), bursting activity characteristics (**B–E**), and regularity of firing (**F**). \*\*\* $p < 0.001$  versus VTA DA activity in naive rats; \* $p < 0.05$  versus VTA DA activity in naive rats.

VTA DA impulse activity is decreased in response to precipitated withdrawal in dependent rats; however, this withdrawal-induced inhibition resulted only in a normalization of the firing rate to that of naive animals rather than a true inhibition, e.g., below the rates in naive rats.

### Morphine induced a change in the firing pattern in VTA DA neurons

Our results indicate that administration of morphine to naive rats increased the impulse activity and, perhaps more importantly, the burst firing of VTA DA neurons to ~50% above the pre-drug baseline. To our knowledge, this is the first report showing that morphine elevates the bursting activity of VTA DA neurons, although it has been shown that acute injection of morphine increases the firing rate activity of VTA DA neurons in naive animals (Gysling and Wang, 1983; Matthews and German, 1984). Previous studies emphasize that the firing pattern rather than the mean discharge rate of DA neurons is of critical importance for the control of DA release in terminal areas (Gonon, 1988). Thus, our findings are consistent with the increase in DA



**Figure 3.** Analysis of the firing rate distributions of VTA DA neurons in naive rats ( $n = 64$  rats, 118 cells), in MD rats ( $n = 88$  rats, 116 cells), and in both withdrawal conditions [precipitated: 1H-PW ( $n = 10$  rats, 87 cells); spontaneous: 24H-SW ( $n = 9$  rats, 93 cells), and 15D-SW ( $n = 14$  rats, 106 cells)]. **A–E**, Firing rate was measured for each cell and plotted as a proportion of total cells recorded. **F**, Firing rates for the five groups of cells were normally distributed (range, 0.2–10.5 Hz). Morphine dependence induced a shift to the right of the Gaussian curve of ~1.5 spikes per second, indicating hyperactivity of the VTA DA neuronal population.

release observed with microdialysis after acute morphine administration (Marinelli et al., 1998; Acquas et al., 1991; Pothos et al., 1991; Pontieri et al., 1995). Opiates interact with the DA system by suppressing GABA inhibitory input to DA neurons in the VTA (Johnson and North, 1992). Based on the increase of burst-firing after morphine, our results led us to hypothesize that, in addition to decreasing GABAergic inhibition of VTA DA neurons, morphine may increase glutamate-mediated excitatory inputs. Thus, it is tempting to propose that glutamatergic neurons innervating the VTA are involved, at least in part, in the activation of DA neurons during acute morphine administration. This idea is also consistent with recent findings that opiates (like other drugs of abuse) produce glutamate-mediated long-term potentiation in VTA DA neurons (Saal et al., 2003). Moreover, we recently demonstrated that synaptic plasticity in the VTA involving activation of glutamate receptors is necessary for both the acquisition and expression of morphine-conditioned place preference (Harris et al., 2004). Glutamate afferents to the VTA arise from three primary sources: the bed nucleus of the stria terminalis, the medial prefrontal cortex, and the pedunculo-pontine region (Fallon and Loughlin, 1995; Georges and Aston-Jones, 2002); this proposed elevated glutamate could originate in any of these inputs. It will be important in future studies to test this idea by examining the effect of morphine on VTA DA neuronal activity in the presence of glutamate antagonists or after specific inhibition of one of the three glutamatergic inputs to the VTA.

### Tolerance and hyperactivity in VTA DA neurons during morphine dependence

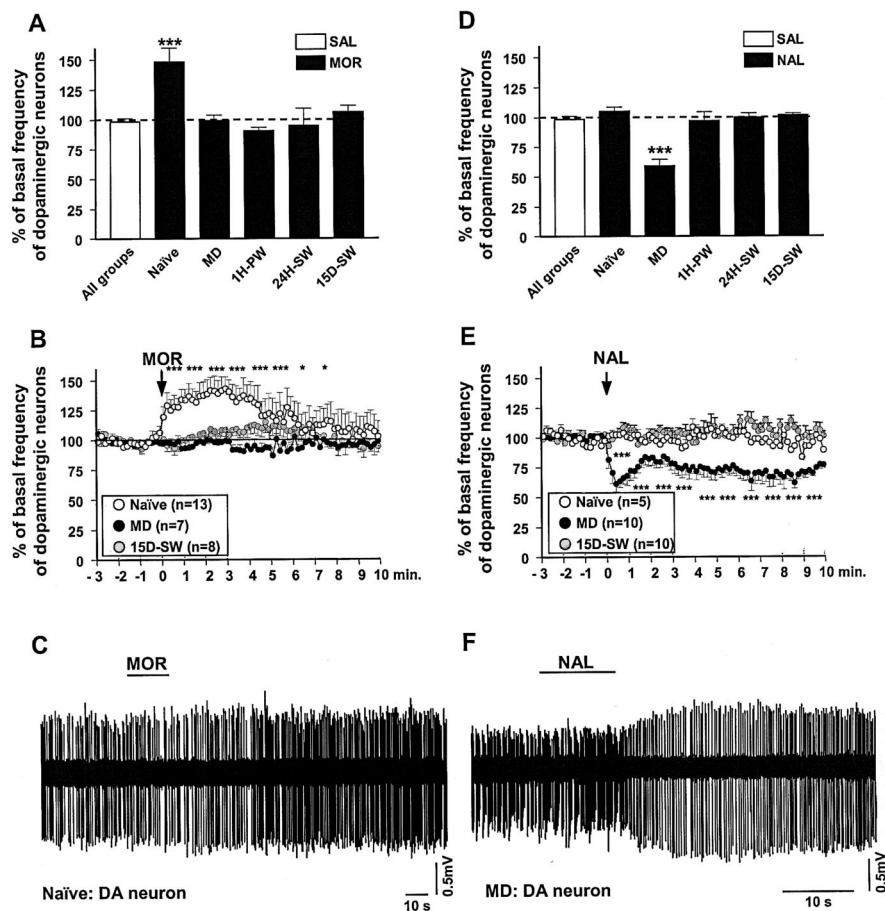
An important and somewhat unexpected finding from the present study was that VTA DA neurons exhibited elevated im-

pulse activity during morphine dependence. These data are consistent with a recent microdialysis study showing that continuous exposure to heroin elevates the basal level of DA in the nucleus accumbens (Leri et al., 2003). Notably, this change in tonic activity of VTA DA neurons during dependence coexisted with tolerance to the phasic excitatory effect of morphine. The observation of tolerance to the excitatory effects of morphine on VTA DA neurons during chronic morphine exposure is not surprising in light of a number of electrophysiological and biochemical findings indicating that morphine induces functional desensitization of opioid signaling (Sim et al., 1996; Williams et al., 2001) and that morphine-tolerant animals show adaptation in postreceptor signaling proteins (Nestler, 2001). Additional experiments are necessary to determine the mechanism by which VTA DA neuronal activity is elevated during chronic morphine treatment.

#### Lack of withdrawal and morphine-induced sensitization in VTA DA neurons

During acute withdrawal from morphine, we observed a drop in baseline activity of VTA DA neurons relative to their baseline activity measured during chronic morphine exposure; however, no differences were found among the baseline activities of VTA DA neurons from morphine-withdrawn compared with naive rats. These data are consistent with a recent microdialysis study showing that during acute withdrawal, basal extracellular DA levels in the nucleus accumbens were not different from those in vehicle-treated rats (Leri et al., 2003). Our data, however, contrast with the results of Diana et al. (1999), who found a lasting reduction in VTA DA activity after morphine withdrawal and an augmented magnitude of the response of these neurons to morphine after 14 d of withdrawal. These discrepancies with the present findings may result from differences in techniques used, such as the anesthetic procedures (halothane vs paralyzed, unanesthetized rat preparation), the routes of chronic morphine administration (pellet vs injection), the stress conditions, and/or the dose range of morphine studied (an acute challenge of morphine at 1 mg/kg vs cumulative doses up to 32 mg/kg). The reasons for the discrepancy between these results and our own are not entirely clear, however, and further work will be necessary to test the possible, above-mentioned explanations.

On the basis of the acute actions of morphine in the mesolimbic DA pathway, it has been suggested that this system is also involved in morphine-induced behavioral sensitization (Kalivas and Stewart, 1991; Robinson and Berridge, 2000). During acute spontaneous withdrawal (24H-SW) and protracted withdrawal (15D-SW), we observed a tolerance to the excitatory effect of morphine, as shown by the ineffectiveness of morphine to acti-



**Figure 4.** Overall effects of intravenous injections of morphine (MOR) (1 mg/kg) or NAL (0.1 mg/kg) on VTA DA neuronal impulse activity in naive rats (MOR:  $n = 13$  rats, 13 cells; NAL:  $n = 5$  rats, 5 cells), MD rats (MOR:  $n = 10$  rats, 10 cells; NAL:  $n = 7$  rats, 7 cells), and in both withdrawal conditions (precipitated: 1H-PW: MOR,  $n = 6$  rats, 6 cells; NAL,  $n = 5$  rats, 5 cells; spontaneous: 24H-SW: MOR,  $n = 6$  rats, 6 cells; NAL,  $n = 8$  rats, 8 cells; 15D-SW: MOR,  $n = 8$  rats, 8 cells; NAL,  $n = 10$  rats, 10 cells). Data are expressed as a percentage of mean basal levels and are the mean  $\pm$  SEM. **A, B**, Morphine in naive rats (1 mg/kg, i.v.) produced a marked increase in the firing of VTA DA neurons. Values are significantly different from baseline at time +1 to +8 min ( $n = 13$ ) ( $***p < 0.001$ ;  $*p < 0.05$ ; ANOVA followed by Dunnett's test); however, in MD and withdrawn rats, acute morphine failed to increase DA activity. **C**, Oscilloscope traces of impulses from a VTA DA neuron showing the typical increase of firing activity after MOR injection in a naive rat. The MOR injection is designated by the line above the trace. **D, E**, VTA DA activity in dependent rats is inhibited in response to precipitated withdrawal. Values are significantly different from baseline at time +1 to +10 min ( $n = 10$ ;  $***p < 0.001$ ; ANOVA followed by Dunnett's test). Note also that acute NAL had no effect on VTA DA activity in naive rats or in any withdrawal group. **F**, Oscilloscope traces of impulses from a VTA DA neuron showing the typical decrease of firing activity after NAL injection in an MD rat. The NAL injection is designated by the line above the trace.

vate VTA DA neurons (Acquas and Di Chiara, 1992; Spanagel et al., 1993). Our results obtained during acute spontaneous withdrawal are consistent with previous neurochemical findings indicating that challenge with morphine had no effect on DA outflow on the first day of withdrawal (Acquas and Di Chiara, 1992); however, our results are not consistent with microdialysis studies in the nucleus accumbens, indicating a sensitization to morphine-induced mesolimbic DA release maintained for 5 d (Acquas and Di Chiara, 1992) or 4 weeks (Spanagel et al., 1993). In contrast to our study, these previous reports used daily escalating doses of morphine. One possible explanation for the discrepancy between our electrophysiological results and those neurochemical findings is that a drug of abuse may produce sensitization or tolerance depending on the temporal pattern of drug delivery (Kreek, 1997; Samaha et al., 2002). An alternative possibility is that morphine dependence and withdrawal lead to adaptive changes in key elements controlling DA levels in the synapse, and these changes could produce alterations in the

**Table 1. Overall effects of acute injections of morphine or naltrexone on VTA DA neuronal activity**

Groups	200 s period preceding injection			Acute Injection (n)	200 s period following injection		
	Rate (Hz) Mean ± SEM	%SIB Mean ± SEM	VC Mean ± SEM		Rate (Hz) Mean ± SEM	%SIB Mean ± SEM	VC Mean ± SEM
Naïve	4.54 ± 0.54	16.63 ± 4.16	0.64 ± 0.05	MOR (13)	6.24 ± 0.63***	30.18 ± 4.88**	0.71 ± 0.06
MD	5.02 ± 0.69	7.62 ± 3.43	0.47 ± 0.06	MOR (7)	5.09 ± 0.81	8.12 ± 2.75	0.48 ± 0.07
1H-PW	5.51 ± 0.50	30.90 ± 5.79	0.78 ± 0.10	MOR (6)	4.98 ± 0.46**	26.41 ± 4.98**	0.76 ± 0.11
24H-SW	5.45 ± 0.35	24.20 ± 1.99	0.60 ± 0.03	MOR (6)	5.16 ± 0.86	23.14 ± 1.55	0.59 ± 0.02
15D-SW	4.68 ± 0.76	16.91 ± 3.52	0.55 ± 0.03	MOR (8)	4.98 ± 0.78	21.78 ± 4.20	0.64 ± 0.07
Naïve	3.61 ± 0.63	15.95 ± 5.50	0.68 ± 0.09	NAL (5)	3.74 ± 0.58	16.23 ± 6.50	0.66 ± 0.09
MD	7.07 ± 0.88	26.23 ± 7.84	0.61 ± 0.11	NAL (10)	4.20 ± 0.60***	11.68 ± 3.66**	0.49 ± 0.08
1H-PW	3.93 ± 0.69	14.29 ± 5.62	0.70 ± 0.09	NAL (5)	3.68 ± 0.44	13.62 ± 4.02	0.71 ± 0.09
24H-SW	4.10 ± 0.54	19.53 ± 3.09	0.68 ± 0.04	NAL (8)	4.08 ± 0.57	18.80 ± 3.94	0.66 ± 0.05
15D-SW	5.46 ± 1.06	17.28 ± 3.72	0.58 ± 0.05	NAL (10)	5.61 ± 1.17	19.12 ± 3.71	0.59 ± 0.04

Overall effects of intravenous injections of morphine (MOR) (1 mg/kg) or naltrexone (NAL) (0.1 mg/kg) on VTA DA neuronal firing rate, bursting activity (% spikes in bursts, SIB) or regularity of firing (coefficient of variation) in drug-naïve rats (Naïve: for MOR,  $n = 13$  rats, 13 cells; for NAL,  $n = 5$  rats, 5 cells), morphine-dependent (MD, for MOR,  $n = 10$  rats, 10 cells; for NAL,  $n = 7$  rats, 7 cells) rats, and in both withdrawal conditions (precipitated: 1H-PW: for MOR,  $n = 6$  rats, 6 cells; for NAL,  $n = 5$  rats, 5 cells; spontaneous: 24H-SW: for MOR,  $n = 6$  rats, 6 cells; for NAL,  $n = 8$  rats, 8 cells and 15D-SW: for MOR,  $n = 8$  rats, 8 cells; for NAL,  $n = 10$  rats, 10 cells). Note the lack of effect of acute morphine injection in rats during 24 h spontaneous withdrawal or during protracted withdrawal. \*\*\* $p < 0.001$ , \*\* $p < 0.01$ ,  $t$  test for paired observations (pre-drug vs post-drug for the three electrophysiological parameters).

amount of DA released without altering the pattern of VTA neuronal activity. Two possible adaptive changes of this kind are the dopamine transporter (DAT) and the  $D_2$  dopamine autoreceptor (Spielewoy et al., 2000; Rouge-Pont et al., 2002). The DAT plays a central role in determining the duration and amplitude of DA action by rapidly recapturing extracellular DA into presynaptic terminals after release (Benoit-Marand et al., 2000). Moreover, voltammetry studies have demonstrated that morphine in mice with a genetic disruption of the DAT produced a dramatic supernormal increase in the level of extracellular DA (Spielewoy et al., 2000). Thus, adaptive changes at the level of the DAT could produce the long-lasting changes in morphine-induced mesolimbic dopamine release observed (Spanagel et al., 1993) without altering the activity of VTA DA neurons (Budygin et al., 2004). This hypothesis is indirectly supported by studies showing that a history of opiate dependence can enhance the reinforcing effects of cocaine in a conditioned place preference (Lett, 1989; Leri et al., 2003).

The  $D_2$  dopamine autoreceptor is another potential target for neuroadaptive changes during morphine dependence that could produce elevated DA release without changing DA neural activity. Microdialysis studies have shown that the increase in extracellular DA induced by morphine was higher in mice lacking  $D_2$  dopamine receptors than in wild-type animals (Rouge-Pont et al., 2002). Thus, the development of DA-dependent behavioral sensitization (Kalivas and Stewart, 1991), together with our results and other neurochemical studies (Acquas and Di Chiara, 1992; Spanagel et al., 1993), suggests that alterations in the DAT and/or the  $D_2$  dopamine autoreceptor could play a role in the modulation of DA release in response to morphine reexposure after a period of abstinence without a change in the firing rate of DA neurons.

In summary, morphine increases VTA DA neuronal discharge and bursting activity in naive animals and triggers adaptive changes such as tolerance and hyperactivity in VTA DA neurons when administered chronically. Acute or protracted withdrawal causes the basal tonic activity of these DA neurons to return to the normal baseline of naive rats but also to become unresponsive to acutely administered morphine. Thus, long-term adaptations in VTA DA neurons induced by chronic opioid treatment are expressed in the absence of the drug and indicate long-lasting changes in the functioning of the VTA DA system.

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