Uniform Inhibition of Dopamine Neurons in the Ventral Tegmental Area by Aversive Stimuli

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Dopamine neurons play a key role in reward-related behaviors. Reward coding theories predict that dopamine neurons will be inhibited by or will not respond to aversive stimuli. Paradoxically, between 3 and 49% of presumed dopamine neurons are excited by aversive stimuli. We found that, in the ventral tegmental area of anesthetized rats, the proportion of presumed dopamine neurons that are excited by aversive stimuli is actually not dopaminergic. The identified dopamine neurons were inhibited by the aversive stimulus. These findings suggest that dopamine neurons are specifically excited by reward and that a population of nondopamine neurons is excited by aversive stimuli.

Dopamine neurons of the ventral tegmental area (VTA) and substantia nigra pars compacta exhibit rapid and brief bursts of activity in response to unexpected rewarding stimuli or conditioned stimuli associated with those rewards. When a reward becomes expected, dopamine neurons no longer fire; if the reward fails to occur, dopamine neurons are inhibited (1). It has thus been suggested that dopamine neurons encode a reward error-prediction rule (1). In addition, dopamine neurons might encode incentive salience for rewards (2, 3). The reward aspect of these hypotheses predicts that aversive stimuli should inhibit (or at least not excite) dopamine neurons. Most studies find that about 80% of presumed dopamine neurons are inhibited by or do not respond to aversive stimuli. However, between 3 and 49% of presumed dopamine neurons are excited by aversive stimuli (4–9). This finding clearly represents a major challenge to the reward hypotheses. In these studies, dopamine neurons have typically been identified as dopaminergic on the basis of their electrophysiological properties alone (10). Combined in vivo physiology and labeling experiments have confirmed that dopamine neurons do indeed possess these properties (11). However, it has been found in vitro that a population of nondopaminergic neurons exists in the VTA with similar electrophysiological characteristics to dopamine neurons but with slightly narrower action potentials (12). Presumed dopamine neurons that are excited by aversive stimuli often display a narrower action potential than those neurons that are inhibited (7). We therefore directly tested the possibility that the presumed dopamine neurons that are excited by aversive stimuli are not actually dopaminergic, and that, consistent with the reward hypothesis of their function, dopamine neurons are uniformly inhibited by aversive stimuli.

We recorded extracellular unit activity from single VTA neurons in anesthetized rats (13). Using standard electrophysiological criteria, we identified presumed dopamine neurons (10). We observed both excitatory and inhibitory responses to a standard aversive stimulus (foot pinch) (14). Neurons were subsequently labeled with the use of the juxta-cellular technique (15) and neurochemically characterized with immunofluorescence for tyrosine hydroxylase (TH), the essential rate-limiting enzyme for dopamine synthesis. Neurons that were immunopositive for TH, and therefore dopamine releasing (n = 12; Fig. 1, A and B), were typically inhibited by the aversive stimulus [10 of 12; Fig. 1C; mean change (±SEM) = −0.82 ± 0.23 Hz, P < 0.005]. In contrast, neurons that were immunonegative for TH (i.e., nondopamine; n = 6; Fig. 1, D and E) were typically excited by the aversive stimulus (4 of 6; Fig. 1F). These TH-negative neurons often exhibited burst firing (Fig. 1E) and an initial segment spike in the action potential, both of which are commonly considered to be characteristic of dopamine neurons (11). They were always in close proximity to TH-positive somata and within the dense TH-positive processes of the VTA (Fig. 1D).

Although the identified dopamine neurons had higher firing rates than the nondopamine neurons, the range of dopamine neuron firing rates fell entirely within the range of nondopamine neuron firing rates (dopamine: 4.6 ± 0.2 Hz, range 1.6 to 5.9, n = 12; nondopamine: 2.6 ± 1.1 Hz, range 0.3 to 7.7, n = 6; P < 0.05), and both groups had similar action potential amplitudes (dopamine: 0.75 ± 0.1 mV, range 0.32 to 1.32; nondopamine: 1.3 ± 0.4 mV, range 0.35 to 3.28; P > 0.05). All dopamine and nondopamine neurons had triphasic action potentials with widths that were greater than 2 ms but were broader in dopamine neurons than in nondopamine neurons (dopamine: 4.6 ± 0.2 msec, range 4.1 to 5.4; nondopamine: 3.8 ± 0.4 msec; range 2.4 to 4.9; P < 0.05). Because there was considerable overlap of the firing rates and triphasic action potential widths of the two

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Fig. 1. Identified dopamine neurons are inhibited by an aversive stimulus; excited neurons are not dopaminergic. (A) A neuron labeled with Neurobiotin (NB) is TH-positive and, therefore, dopaminergic. (B) Average extracellular waveform and baseline firing properties of the labeled dopamine neuron. (C) Firing of the dopamine neuron is decreased during the aversive stimulus. (D and E) Examples of baseline firing and average extracellular waveform of a neuron that is TH-negative and, therefore, nondopaminergic. (F) The nondopaminergic neuron was excited by the aversive stimulus. Data in (A) to (C) are from the same TH-positive neuron; data in (D) to (F) are from the same TH-negative neuron. Scale bars in (A) to (C) are the same as those in (D) to (F).
groups, we sought another electrophysiological measure that might allow us to distinguish more clearly between the two groups. The duration from the start of the action potential to the negative trough was significantly broader in dopamine neurons ($P < 0.001$; Fig. 2A). An analysis of all of the neurons we recorded (labeled and unlabeled; total $n = 70$) segregated the population into two groups on the basis of this criterion (Fig. 2B). Both the raw data and a predicted double Gaussian show that a duration of $\geq 1.1$ ms from the start of the action potential to the negative trough should exclude all nondopamine neurons and that, as a result, this may be a useful guide for the future identification of VTA neurons (Fig. 2C).

Because dopamine neurons can regulate their firing pattern and firing rate through independent mechanisms (16, 17), we next examined whether aversive stimuli also changed the firing pattern of dopamine neurons. In the absence of salient stimuli, (putative) dopamine neurons typically display slow irregular firing, with intermittent burst firing in vivo. At firing frequencies achieved during bursts (>12 Hz), dopamine release increases in a supralinear fashion because the dopamine transporter is overwhelmed (18). Therefore, decreased bursting could decrease dopamine release in addition to the effects of a reduction in firing rate. Because many dopamine neurons do not always fire in a bursting mode (19), we increased our sample size by conducting our analysis on identified (i.e., TH-positive) and putative dopamine neurons (identified with our electrophysiological criterion). In these neurons, we observed a reduction in the number of action potentials that occurred in a burst during the pinch.
Dopaminergic. Dopamine neurons were released at axon terminals (e.g., glutamate, on dopamine re-lease local actions of other neurotransmit-tors). The firing rates of dopamine neurons typically fired phasic spikes (i.e., each neuron fired one or two action potentials per burst), and these properties did not substantially vary across several species (4–9).

Our results show that aversive stimuli consistently reduce the phasic dopamine signal through a reduction in firing rate and bursting activity, and therefore strengthen the reward hypothesis of dopamine neuron function.

References and Notes
10. Properties used to identify dopamine neurons include a broad triphasic extracellular action potential of a width greater than 2 ms and a relatively slow firing rate (<10 Hz).
13. Materials and methods are available as supporting material on Science Online.
14. It is difficult to classify stimuli as aversive in the anesthetized preparation, because no behavioral measurements can be made. However, the foot pinch that we used would be aversive in the unanesthetized state, and because the responses of dopamine neurons to aversive stimuli are not strongly dependent on anesthesia, we believe that such a classification in this case is valid.
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