that the frequency ranges over which fictive locomotion could be entrained by applied movement to the rostral and to the caudal ends should be unequal and that the resting frequency should provide a limit for entrainment for one end but not the other. These nonintuitive predictions were confirmed by experiment [T. L. Williams, K. A. Sigvardt, N. Kopell, G. B. Ermentrout, M. P. Remler, *J. Neurophysiol.* **64**, 862 (1990)]. Furthermore, N. Kopell and G. B. Ermentrout [*SIAM J. Appl. Math.* **50**, 1014 (1990)] predicted a specific pattern of changes in phase lag when different portions of an in vitro preparation are bathed in different amino acid concentrations. These predictions were confirmed experimentally [(K. A. Sigvardt, N. Kopell, G. B. Ermentrout, M. P. Remler, *Soc. Neurosci. Abstr.* **17**, 122 (1991)]. Similar results were obtained by Matsushima and Grillner (*4*), but these authors placed a different interpretation on the data.

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11. Each cell in a simulation has an "activation" variable a_i, which represents its membrane potential trajectory. If a_i is above a threshold value th_i, it is taken as a measure of the firing rate f_i of the cell, with $f_i = 0$ when $a_i < th_i$ and $f_i = a_i - th_i$ when $a \geq th_i$. In each time step, the activation variable of cell i will change as a result of synaptic input or decay, as given by:

$$\Delta a_i = e_i(v_{ie} - a_i) + \tau_i(r_i - a_i) + \Sigma_j w_{ij} f_i(v_{ij} - a_i)$$

where e_i is the tonic excitatory drive to cell i, v_{ie} represents the "reversal potential" for the excitatory drive, r_i represents the resting potential, τ_i is a time constant for return to r_i, w_{ij} represents the weight of the synapse from cell j to cell i, and v_{ij} represents the "reversal potential" for the synapse from cell j to cell i.

Normalized values were used for all the parameters: $e_i = 0.1$ for C cells, 0.01 for L cells, and 0.025 for E cells; for all cells: $v_{ie} = 1.0$, $th_i = 0$, $\tau_i = 0.1$, and $r_i = 0$; $w_{ij} = 1.0$ for all synapses; $v_{ij} = 1.0$ for all excitatory synapses and -1.0 for all inhibitory synapses.

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Burst Firing in Dopamine Neurons Induced by *N*-Methyl-D-Aspartate: Role of Electrogenic Sodium Pump

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Dopamine-containing neurons of the mammalian midbrain are required for normal behavior and movements. In vivo they fire action potentials in bursts, but in vitro they discharge regularly spaced action potentials. Burst firing in vitro has now been shown to be robustly induced by the glutamate agonist *N*-methyl-D-aspartate (NMDA) although not by the non-NMDA agonists kainate or quisqualate. The hyperpolarization between bursts of action potentials results from electrogenic sodium ion extrusion by a ouabain-sensitive pump. This mechanism of burst generation in mammalian neurons may be important in the pathophysiology of schizophrenia and Parkinson's disease.

The dopamine-containing neurons of the ventral midbrain project predominantly to the prefrontal cortex, nucleus accumbens, and striatum and are involved in the control of affect, movement, and drug-seeking behavior (*1*). In freely moving rats, they discharge with single spikes or with bursts of spikes (*2*). The bursts are not seen in vitro (*3–8*), perhaps because of the loss of critical afferent inputs (*4*, *5*) that release excitatory amino acids (*9*, *10*). We tested this hypothesis by applying excitatory amino acids while recording from the cells in vitro (*6*, *7*, *11*).

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N-Methyl-D-aspartate (NMDA) (10 to 30 μM) changed the firing pattern from regularly spaced single spikes to one in which bursts of two to ten action potentials were separated by large hyperpolarizations (up to 40 mV) lasting 1 to 5 s (Fig. 1A). The action of NMDA continued throughout its application (up to 8 hours), reversed quickly on washing, and was antagonized by the NMDA receptor blocker D,L-2-amino-5-phosphonopentanoic acid (APV) although not by 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline (CNQX), which blocks non-NMDA receptors (*12*). Tetrodotoxin (TTX) blocked the bursts of action potentials but did not prevent the underlying oscillations of membrane potential (Fig. 1C); this result indicates that the potential oscillations underlying the bursts do not require Na^+ entry through TTX-sensitive channels and that burst firing probably does not involve local neuronal circuits within the tissue slice. Burst firing did not occur in Mg^{2+}-free solution, and it was not evoked by excitatory amino acids (kainate, quisqualate) that do not open Mg^{2+}-gated ion channels (*13*).

In other neurons, inward Ca^{2+} currents contribute to the onset of bursts of action potentials. Ca^{2+} current inactivation (*14*) or the development of an outward Ca^{2+}-dependent K^+ current (*15*) terminates the burst. However, burst firing of dopamine-containing cells persisted in Ca^{2+}-free solution ($n = 6$) (Fig. 1B), in apamin (1 μM), or with recording electrodes that contained the Ca^{2+} chelating agent 1,2-bis(2-aminophenoxy)ethane *N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA). BAPTA effectively buffered intracellular Ca^{2+} as demonstrated by blockade of the late component of the action potential after-hyperpolarization ($n = 5$) (*16*). Apamin, which blocks a Ca^{2+}-activated K^+ conductance in these (*17*) and other cells (*18*), actually increased the amplitude of the bursts, perhaps by blocking an opposing outward current during the depolarizing phase. Decreasing the K^+ concentration from 2.5 to 0.5 mM reversibly blocked burst firing ($n = 2$), and increasing the K^+ concentration to 10.5 mM increased the amplitude of the hyperpolarization between the bursts ($n = 2$). These findings are the opposite of those expected if the hyperpolarization had resulted from an increase in K^+ conductance.

Sodium was required for burst firing because, when its concentration was reduced (from 146 to 20 mM with choline or tris substitution), both the action potentials and the underlying membrane potential oscillations disappeared reversibly ($n = 3$) (Fig. 1D). One Na^+ current that has voltage and time dependence appropriate to burst firing is the hyperpolarization-activated current (I_h) (*19*). However, burst firing was not inhibited by Cs^+ (3 mM), which blocks I_h in dopamine-containing cells (*20*). We next hypothesized that Na^+ entry through NMDA-gated channels activated an electrogenic Na^+ pump. Both strophanthidin (2 to 10 μM, $n = 5$) and ouabain (2 to 10 μM) ($n = 6$) blocked burst firing in NMDA (Fig. 1E) but had little or no effect on the regular spike discharge observed in the absence of NMDA. Similar results were seen in the presence of dinitrophenol (30 to 100 μM) ($n = 5$) and in solutions without glucose ($n = 3$), both of which would be expected to inhibit active Na^+ extrusion.

Voltage-clamp experiments supported this hypothesis (*21*). The membrane was

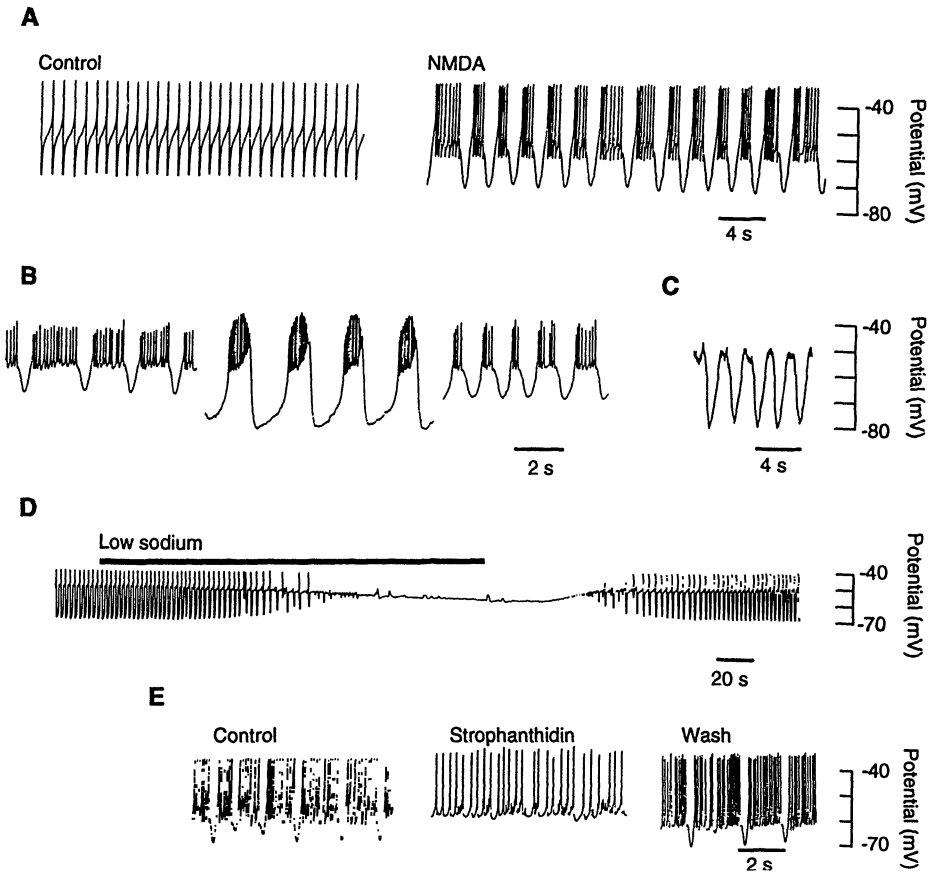

Fig. 1. NMDA induces burst firing. (**A**) Single spike firing (left), which is typical of dopamine neurons under control conditions, is converted to burst firing by NMDA (right). (**B**) Burst firing is not blocked by Ca^{2+}-free solution: (left) control, (middle) 15 min after changing to Ca^{2+}-free solution, and (right) wash. (**C**) Rhythmic hyperpolarizations persist in TTX (1 μM), although action potentials are blocked. (**D**) Rhythmic hyperpolarizations in TTX (1 μM) are reversibly blocked by Na^+-free solution. In all recordings, a steady hyperpolarizing current (150 to 300 pA) was applied in the presence of NMDA (20 μM) to maintain the spike threshold at the same potential (about −50 mV). All recordings were in apamin (100 nM). In (B) the solution also contained 3.2 mM Mg^{2+} and 3 mM Cs^+. (**E**) Bursting is blocked by strophanthidin (2 μM) (5-min application). Apamin (100 nM) was present. In strophanthidin, the holding current at −60 mV was increased from −200 to −260 pA.

depolarized from −60 to −40 mV for 1 s to simulate the depolarization that underlies the burst of action potentials; the action potentials themselves were blocked by TTX. In NMDA, although not in kainate or quisqualate, the depolarization was followed by an outward tail current that peaked quickly at 100 to 250 pA and then declined during a few seconds (τ = 1.3 ± 0.15 s, n = 10) (Fig. 2A). In 10.5 mM K^+ the current was larger and declined more rapidly (Fig. 2B). Most striking was the result that the current remained large and outward even at or beyond the K^+ equilibrium potential (6, 7) (Fig. 2, B and C). The tail current was also blocked by all the procedures that inhibited burst firing, including strophanthidin (3 to 10 μM, n = 2), ouabain (1 to 3 μM, n = 3) (Fig. 3A), low K^+ concentration (0.5 mM) (Fig. 3B), low Na^+ concentration (20 mM, n = 3) (Fig. 3C), dinitrophenol (30 to 100 μM, n = 2), and glucose-free solution (n = 3).

We conclude that Na^+ ions enter the neuron through NMDA-gated channels; this current is regenerative because of the negative slope conductance at these potentials (*13*). The rapid increase in intracellular Na^+ concentration activates a ouabain-sensitive pump. The outward current from the pump ends the burst by hyperpolarizing the neuron, which thereby leads to Mg^{2+} block of the NMDA current. As the intracellular Na^+ concentration falls, the pump current declines, and unopposed NMDA current initiates the next cycle of burst firing. Addition of strophanthidin (5 μM) in the presence of NMDA caused a net shift of holding current of 540 ± 87 pA (n = 5) at −60 mV; for a spherical neuron of 15-μm radius, this is within the range of electrogenic pump currents measured in other cells (*22*). Although these results clearly show that burst firing produced by NMDA in vitro is not Ca^{2+}-dependent, it is possible that other

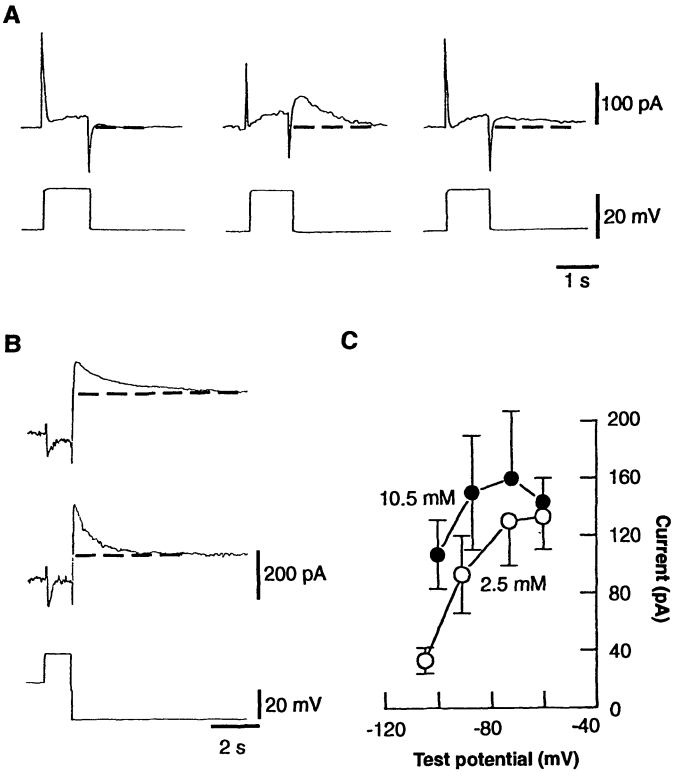

Fig. 2. NMDA-induced outward tail currents. (**A**) Depolarization from −60 to −40 mV for 1 s in control superfusate (TTX, 1 μM; apamin, 100 nM; Cs^+, 3 mM) induced no significant tail current (left). In NMDA (20 μM) (center), a large outward tail current is recorded after the voltage step. In NMDA and APV (50 μM) (right) the outward tail current is reduced. Holding currents at −60 mV were −130 pA (left), −650 pA (center), and −340 pA (right). (**B**) The outward tail current is increased at high extracellular K^+ concentration (10.5 mM) (lower trace) compared to normal (2.5 mM) (upper trace). Voltage steps were from −60 to −40 mV for 1 s and then back to −90 mV. (**C**) Outward tail currents at different potentials in 2.5 and 10.5 mM K^+. After a 1-s voltage step from −60 to −40 mV, the potential was returned to the test potential. Points are mean ± SEM (n = 3). Experiments in (B) and (C) were in TTX (1 μM), apamin (100 nM), Cs^+ (3 mM), tetraethylammonium (10 mM), and NMDA (20 μM).

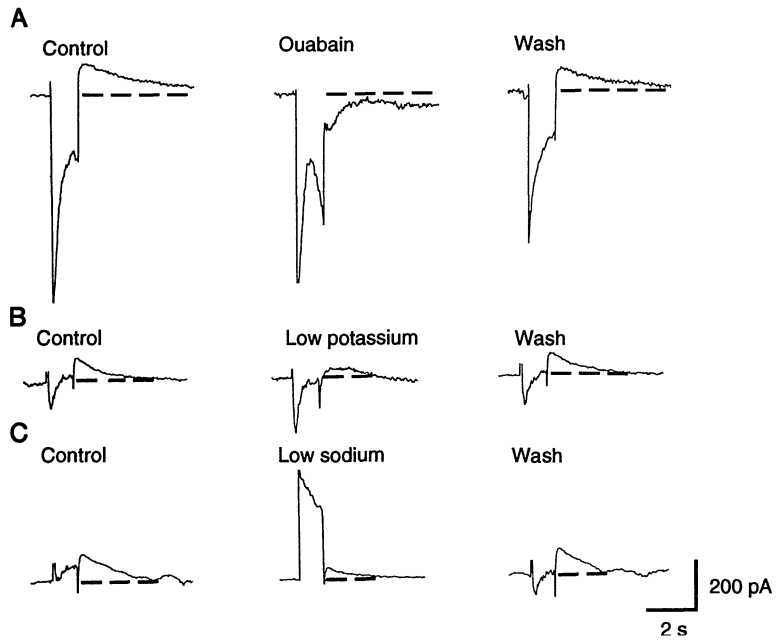

Fig. 3. Electrogenic pump current causes hyperpolarization between bursts. NMDA (20 μM) was present in all experiments illustrated. (**A**) The outward tail current in NMDA is blocked by ouabain (2 μM) (10-min application). In ouabain, the holding current at −60 mV was increased from −410 to −800 pA. Tetraethylammonium (10 mM), TTX (1 mM), and apamin (100 nM) were present. (**B**) Low K^+ (0.5 mM) concentration (5 min) reduced the NMDA-induced tail current. TTX (1 μM) and apamin (100 nM) were present. (**C**) Low Na^+ (20 mM) concentration reduced the NMDA-induced tail current (control was 146 mM). TTX (1 μM), apamin (100 nM), Cs^+ (3 mM), and high Mg^{2+} (3.2 mM) were present. Choline substituted for Na^+ in scopolamine (1 μM). The current during the depolarizing pulse turns strongly outward at low Na^+ concentration because the voltage-dependent K^+ current is not fully opposed by the inward NMDA-induced current.

mechanisms might also contribute in vivo. For example, Grace and Bunney (*3*) showed that intracellular loading of Ca^{2+} evoked bursts of action potentials in dopamine neurons in vivo.

A burst of action potentials releases more dopamine in projection areas than does the same number of evenly spaced action potentials (*23*). In mammals, including primates, single-spike firing in dopamine cells is thought to have a permissive role in initiating movement, whereas burst firing is correlated with behavioral arousal and motivation (*24*). We hypothesize that the frontal cortex, which sends a major excitatory amino acid–containing input to dopamine neurons (*10*), controls burst firing in dopamine cells in vivo (9). Indeed, loss of behavioral arousal and motivation are common symptoms of lesions in frontal lobes (*25*) and dopamine-containing pathways (*26*). In contrast, excessive burst firing might be expected to cause psychopathology, such as schizophrenia (*27*). There is evidence that abnormal cytoarchitecture in the cerebral cortex may be the primary defect in schizophrenia (*28*); this could lead to abnormal control of dopamine neuronal activity by cortical inputs.

About 40% of the metabolic activity of brain is devoted to supplying energy for the adenosine triphosphate (ATP)–dependent Na^+/K^+ ion pump (*29*). When intracellular Na^+ concentrations rise, which occurs during synaptic stimulation from inputs containing excitatory amino acids, this metabolic activity must increase. Failure to maintain intracellular Na^+ homeostasis is neurotoxic (*30*). In patients with Parkinson's disease, dopamine neurons may be deficient in mitochondrial enzymes necessary for the manufacture of ATP (*31*). Therefore, it is possible that excitatory amino acids are especially toxic to dopamine neurons in Parkinsonian patients (*32*) because these cells cannot keep pace with the energy requirements of the Na^+/K^+ ion pump.

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