Potent Regulation of Midbrain Dopamine Neurons by the Bed Nucleus of the Stria Terminalis

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Recent studies have revealed an important role of the ventrolateral (subcommissural) aspect of the bed nucleus of the stria terminalis (vBNST) in motivational aspects of drug abuse (Delfs et al., 2000). Dopaminergic (DA) neurons in the ventral tegmental area (VTA) have also long been linked to motivation and drug abuse (Koob and Le Moal, 2001). The present study tested whether activity in the vBNST influences discharge of midbrain DA neurons. Responses of DA neurons in the VTA to activation of the vBNST were characterized in anesthetized rats using extracellular recording techniques. Electrical or chemical [10–50 mM glutamate (Glu)] stimulation of the vBNST consistently activated DA cells (122% increase in activity with 50 mM Glu). However, stronger chemical stimulation of the vBNST (100 mM Glu) completely inactivated DA neurons. In addition, apomorphine restored the activity of DA neurons that were inactivated by 100 mM Glu stimulation of the vBNST, indicating possible depolarization blockade of DA cells by vBNST activity. These findings reveal that the vBNST exerts a strong excitatory influence on DA neurons. Also striking was the finding that chemical stimulation (50 mM Glu) of the vBNST yielded long-lasting oscillatory activity (>15 min) in VTA DA neurons. These results indicate that the vBNST can generate long-lasting alterations in the activity of DA neurons *in vivo*.

Key words: dopamine neurons; ventral tegmental area; bed nucleus of the stria terminalis; depolarization blockade; noradrenaline–dopamine interactions; extracellular recording techniques

The ventral tegmental area (VTA) is the source of dopaminergic (DA) neurons that project to structures in the ventral striatum and prefrontal cortex, known collectively as the mesocorticolimbic dopamine system. The firing of VTA DA neurons is thought to convey information about the rewarding or motivationally relevant properties of external stimuli (White, 1996). Excitatory synaptic inputs are a key component of the regulation of dopamine cell excitability and are known to play an important role in the actions of many drugs of abuse (Kalivas and Stewart, 1991).

The extended amygdala is also important in these reward circuits. The extended amygdala is composed of several basal forebrain regions that have similar morphology, immunoreactivity, and connectivity. It is essentially continuous rostrocaudally from the medial (shell) portion of the nucleus accumbens through the bed nucleus of the stria terminalis (BNST) (Alheid et al., 1998). The extended amygdala is connected with the VTA and several other brain structures hypothesized to be involved in the reinforcing effects of abused drugs (Koob and Le Moal, 2001).

The BNST is strongly interconnected to the mediocaudal shell portion of the nucleus accumbens (Brog et al., 1993; Georges and Aston-Jones, 2000) and the mediocentral amygdala (Alheid et al., 1998) and thus occupies a key position in the extended amygdala. We have focused on the ventrolateral (subcommissural) aspect of the BNST (vBNST) in recent studies of opiate abuse, because the vBNST receives one of the densest norepinephrine inputs in the brain (Delfs et al., 2000). In addition, we demonstrated recently a prominent direct projection from the vBNST to the VTA (Georges and Aston-Jones, 2000), consistent with previous studies (Phillipson, 1979). This connection and other results indicating a role of the vBNST in opiate abuse (Delfs et al., 2000; Walker et al., 2000) indicate that functional interactions may exist between the vBNST and VTA DA neurons; however, such interactions have not been reported previously. A major goal of this study was to examine the influence of vBNST activity on DA neurons in the VTA. Our findings provide physiological evidence that the vBNST is a powerful regulator of dopamine cell excitability.

MATERIALS AND METHODS

Surgery. A total of 31 Sprague Dawley rats (200–225 gm; Taconic, Germantown, NY) were used. Surgery was performed as described previously (Jodo and Aston-Jones, 1997). Two percent halothane was delivered through a tracheal cannula via spontaneous respiration. During recording experiments, the concentration of halothane was kept at 1.0–1.2%. Body temperature was maintained at 36–38°C. The skull was exposed, and a hole was drilled above the VTA (5.3 mm caudal to bregma; 0.3–0.5 mm lateral to the midline). Another hole was drilled

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above the BNST (0.3 mm caudal to bregma; 1.5 mm lateral to the midline).

Electrical stimulation of the ventral BNST. Bipolar electrical stimulation of the vBNST was conducted with a concentric electrode (100-μm-diameter inner electrode that extended 100 μm beyond the outer electrode; Frederick Haer & Co., Bowdoinham, ME). This electrode was inserted into the vBNST [coordinates relative to bregma (in mm): anteroposterior, 0.3; mediolateral, 1.5; dorsoventral, 7.2]. Electrical stimulation (1.0–5.0 mA, 0.5 Hz, 0.5-msec-duration pulses) was administered using a square pulse stimulator (Master-8; A.M.P.I, Jerusalem, Israel) and stimulus isolator (ISO-Flex; A.M.P.I.).

Chemical stimulation of the ventral BNST. An injection pipette (tip, <50 μm diameter) was filled with L-glutamate (Glu) [10, 50, or 100 mM in artificial CSF (aCSF)] and was lowered into the vBNST. Glu was microinjected into the vBNST using brief pulses of pneumatic pressure (Picospritzer; General Valve, Fairfield, NJ). In all experiments, a total volume of 60 nl was infused over 30 sec for each injection. Two injections at a single vBNST site were typically given at an interval >30 min.

Ventral tegmental area recordings. A glass micropipette (tip diameter, 2–3 μm; 4–6 MΩ) 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 (Grace and Bunney, 1983). Signals were amplified and filtered (0.1–5 kHz bandpass) using conventional electronics. Spikes of single neurons were discriminated, and digital pulses were led to a computer using a laboratory interface and software (CED 1401, SPIKE2; Cambridge Electronic Design, Cambridge, UK).

After isolating a single VTA neuron, preinjection spontaneous activity was recorded to establish baseline activity for at least 10 min. Subsequently in electrical stimulation experiments, single pulses were delivered to the BNST every 2 sec. At least 100 trials were administered per cell. For chemical stimulation experiments, Glu was injected at a rate of 120 nl/min for 30 sec.

Histology. At the end of each VTA recording penetration, the electrode placement was marked with an iontophoretic deposit of pontamine sky blue dye (−20 μA, continuous current during 12–15 min) (Fig. 1*A*). At the end of each experiment using chemical stimulation, the BNST injection placement was marked by inserting an injection pipette filled with dye at the location of the stimulating electrode and by similarly iontophoresing dye through the tip (Fig. 1*D*). To mark electrical stimulation sites, a lesion was performed by passing +10 μA through the stimulation electrode for 1 min (Fig. 1*C*). After the experimental procedures, the animals were deeply anesthetized with halothane (5%), and the brains were snap-frozen.

There are no sharp cytoarchitectural boundaries differentiating the BNST. However, the vBNST is well defined by a dense noradrenergic innervation (Delfs et al., 2000). Thus, the vBNST was identified by a classical immunohistochemical procedure for dopamine-β-hydroxylase (DBH) (rabbit anti-DBH primary antibody, 1:2000; Eugene Tech, Exeter, UK) to reveal noradrenergic fibers. Dopaminergic neurons and processes in the VTA were delineated by immunohistochemical staining for tyrosine hydroxylase (TH) (rabbit anti-TH primary antibody, 1:10,000; Institut Jacques Boy, Reims, France).

Data analysis. During electrical stimulation of the vBNST, cumulative peristimulus time histograms (PSTHs) (5 msec bin width) of VTA activity were generated for each neuron recorded. PSTHs were analyzed to determine excitatory and inhibitory epochs as described previously (Jodo and Aston-Jones, 1997). Response magnitude (R_{mag}) for excitation and inhibition was normalized for spontaneous firing (baseline) as described previously (Jodo and Aston-Jones, 1997). R_{mag} values essentially express the number of spikes during the response above or below that during baseline.

Results are expressed throughout as means ± SEM. These values were subjected to one-way ANOVAs followed by *post hoc* Newman–Keuls tests.

During chemical stimulation experiments, the discharge frequencies of dopaminergic neurons were pooled and averaged for each concentration of glutamate, and the time course for response to glutamate was determined. Statistical analysis was performed by ANOVA followed by *post hoc* Newman–Keuls tests.

RESULTS

Data are reported for 93 histologically verified VTA neurons that were identified as dopaminergic by their electrophysiological features (Fig. 1*A*). Spontaneously discharging nonburst- and burst-firing DA cells typically fired at an average rate of 4.2 ± 0.45 Hz. Action potentials of these cells had biphasic or triphasic waveforms with an average duration of 3.02 ± 0.05 msec. For the last DA neuron recorded in each experiment ($n = 7$), the D2 DA receptor agonist apomorphine (Apo) was administered systemically (0.1 mg/kg, i.v.). This drug consistently inhibited spontaneous impulse activity within 30 sec of injection (data not shown).

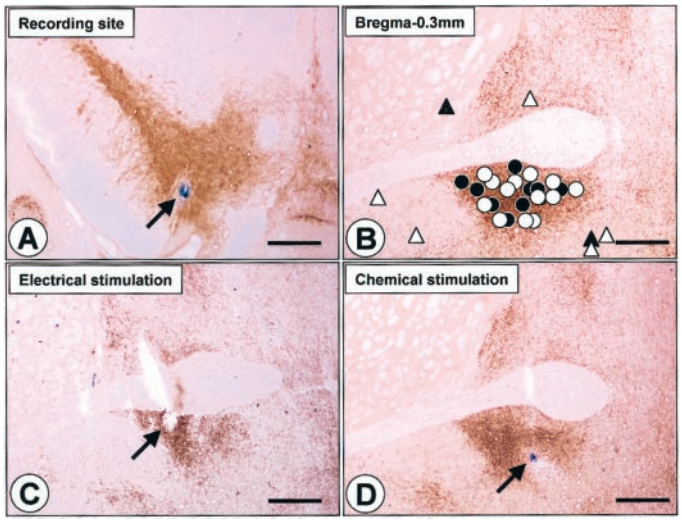

Figure 1. Stimulation and recording sites. *A*, Photomicrograph of a coronal section through the VTA. The section was counterstained by TH immunohistochemistry (in *brown*) to delineate DA neurons and processes. Iontophoretic ejection of pontamine sky blue (*spot* at *arrow*) marks the location of the last cell recorded. *B–D*, Photomicrographs of coronal sections through the BNST. The sections were counterstained by DBH immunohistochemistry (in *brown*) to delineate the region of dense noradrenergic innervation in the vBNST. *B*, Plots of effective sites of electrical (*filled circles*) or chemical (*open circles*) stimulation in the vBNST. *Triangles* show the locations of seven ineffective chemical (*open triangles*) and electrical (*filled triangles*) stimulation sites in the ventral pallidum, the caudate putamen, the LPO, or the dorsal BNST. *C*, An electrical stimulation site marked by passing positive current through the stimulation electrode (lesion at *arrow*). *D*, A chemical stimulation site marked by inserting an injection pipette at the location of the stimulating electrode and iontophoresing pontamine sky blue (*spot* at *arrow*). Scale bars: *A*, 1.0 mm; *B–D*, 0.8 mm.

VTA DA neurons were activated by electrical stimulation of the vBNST

Electrical stimulation of the vBNST synaptically activated 78% of VTA DA neurons (28 of the 36 tested; baseline firing rate, 4.1 ± 0.4 spikes/sec) (Fig. 2). Two characteristic responses were observed with single-pulse stimulation of the vBNST: activation with a short onset latency (<25 msec; 20 of 28 cells) (Fig. 2*A,C*) or activation with a long onset latency (>120 msec; 20 of 28 cells) (Fig. 2*B,D*). Short onset latencies ranged from 5 to 25 msec (mean onset latency, 16.1 ± 3.1 msec; response duration, 19.2 ± 1.9 msec; R_{mag}, +64.4 ± 12.8 spikes), whereas long onset latencies ranged from 185 to 270 msec (mean onset latency, 232.8 ± 15.6 msec; response duration, 58.5 ± 7.6 msec; R_{mag}, +64.1 ± 10.3 spikes). The mean threshold for both excitatory responses was ~500 μA. A sizeable fraction (28.6%) of the driven VTA DA neurons exhibited short latency activation followed by inhibition (8 of 28), and 28.6% of the neurons showed an inhibition followed by a long onset latency excitation (8 of 28). Inhibition onset latencies ranged from 5 to 170 msec (onset latency, 37.2 ± 6.1 msec; response duration, 155.5 ± 8.9 msec; R_{mag}, −64.9 ± 7.82

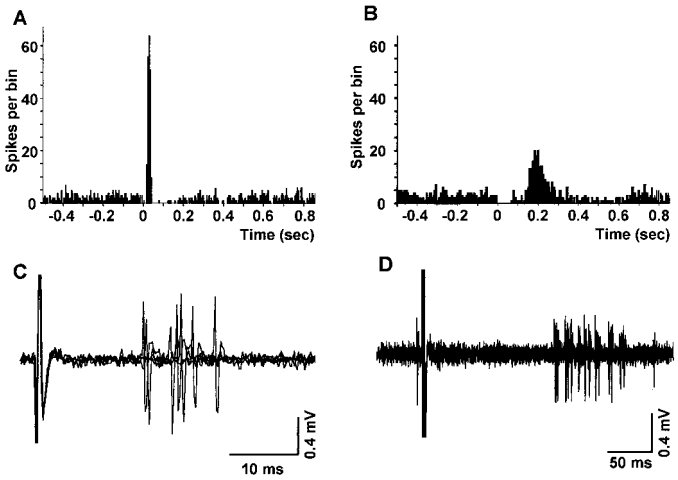

Figure 2. Responses of two typical VTA DA neurons to electrical stimulation of the vBNST. Two characteristic responses were observed after single-pulse stimulation of the vBNST: activation with short (*A*, *C*) or long (*B*, *D*) onset latency. *A*, *B*, PSTHs showing vBNST-evoked excitation of two typical VTA DA neurons with a short (*A*) or long (*B*) onset latency. *C*, Five superimposed traces showing spikes elicited by stimulation of the vBNST at a short latency (<25 msec). *D*, Five superimposed traces showing spikes elicited by stimulation of the vBNST at a long latency (>120 msec).

spikes). Notably, 42.8% of the VTA DA neurons activated by stimulation of the vBNST exhibited both short and long latency activations after single-pulse stimulation (12 of 28). No VTA neurons were driven antidromically by vBNST stimulation.

Stimulation through electrodes located in areas nearby but outside the vBNST induced inhibition or no activation of VTA DA neurons. For example, single-pulse stimulation of the caudate putamen had no effect on the cells tested (Fig. 1, one rat). Single-pulse stimulation of the lateral preoptic area (LPO) (Fig. 1, one rat) inhibited one of four DA neurons and had no effect on the other cell tested.

VTA DA neurons were activated by chemical stimulation of the vBNST

Microinjections of L-glutamate were used to chemically stimulate vBNST neurons without activation of passing fibers. Each concentration of Glu tested (10, 50, and 100 mM) produced a characteristic modulation of VTA DA neuronal activity (Fig. 3). As shown in Figure 3*C*, microinjection of 10 mM Glu into the vBNST transiently excited five of six DA neurons tested (also see typical responses in Fig. 3*A*,*D*). The onset of activation began 10 sec after initiation of Glu ejection. The maximal activation occurred 20 sec after the beginning of the L-glutamate injection and represented an increase of +43% above the basal frequency of the DA neurons (ANOVA; df = 30; $F = 2.85$; $p < 0.001$) (Fig. 3*C*). On average, this activation remained significant 40 sec after the L-glutamate injection.

Microinjection of 50 mM L-glutamate into the vBNST activated 85% of the DA neurons tested (12 of 14). This response was particularly long-lasting (several minutes) in 50% of the DA neurons tested (7 of 14) (typical responses in Fig. 3*B*,*E*). For all neurons considered together after stimulation of the vBNST with 50 mM L-glutamate ($n = 14$), significant activation began 10 sec after initiation of Glu ejection. The maximal activation occurred 70 sec after the beginning of the L-glutamate injection and represented an increase of +122% above basal discharge frequency (ANOVA; df = 30; $F = 3.08$; $p < 0.001$). The long-lasting responses ranged from 10 to 25 min and remained significantly elevated 15 min after the injection of L-glutamate in the vBNST (ANOVA; df = 30; $F = 3.08$; $p < 0.001$). Also, four DA neurons exhibited marked oscillatory activity after injection of 50 mM Glu into the vBNST (typical responses in Fig. 3*B*). The period of this oscillation was typically between 8 and 10 min. None of the DA neurons tested ($n = 20$) exhibited inhibitory responses with 10 or 50 mM L-glutamate microinjection.

As shown in Figure 3*C*, microinjection of 100 mM Glu into the vBNST produced a brief period of activation followed by strong inhibition of all DA neurons tested ($n = 12$; df = 30; $F = 44.74$; ANOVA; $p < 0.001$) (typical response in Fig. 3*F*). The maximal activation occurred 10 sec after the beginning of the Glu injection and was rapidly followed by a nearly total suppression of DA neuron activity. This inhibition did not recover for at least 15 min. The transient activation of DA neurons after injection of 100 mM Glu was accompanied by a progressive decrease in spike amplitude (Fig. 3*F*). Overall, this response to 100 mM Glu injection was consistent with changes reported to occur during the induction of DA cell depolarization block (Grace et al., 1997). Occurrence of depolarization block after microinjection of 100 mML-glutamate into the vBNST was confirmed in recordings from three rats (Fig. 4). In five of the seven VTA DA neurons tested, spontaneous firing was reinstated by intravenous administration of the DA autoreceptor agonist Apo (0.1 mg/kg) (typical response in Fig. 4*B*).

The ability of Glu to activate VTA DA neurons was critically dependent on injection placement. Glu microinjection in areas outside the vBNST induced short-lasting inhibition or no activation of VTA DA neurons. For example, microinjection of 10 or 100 mM Glu in the LPO (Fig. 1, two rats) inhibited three of nine DA neurons and had no effect on the other cell tested. Injection in the ventral pallidum (10 or 100 mM) (Fig. 1, two rats) had no effect on the five DA neurons tested. Glu injection in the dorsal BNST (100 mM) (Fig. 1, one rat) had no effect on the DA neuron tested. Administration of vehicle alone (60 nl of aCSF in the vBNST) had no effect on VTA DA neuronal activity ($n = 2$ rats, 4 neurons).

DISCUSSION

Previous studies have demonstrated that excitatory (glutamatergic) afferents in the VTA arise from three primary sources: the medial prefrontal cortex, the pedunculopontine tegmental nucleus (PPTg), and the subthalamic nucleus (Fallon and Loughlin, 1995). These inputs play an important role in regulating the activity of DA neurons in the VTA (Bonci and Malenka, 1999).

Our study provides functional evidence for strong and predominantly excitatory regulation of VTA DA neurons by the vBNST. Using tract-tracing methods, we have demonstrated recently a direct pathway linking the vBNST and the VTA (Georges and Aston-Jones, 2000), in accord with a previous study (Phillipson, 1979). In view of these anatomical data, the short latency excitations for many cells, and the distance between the vBNST and the VTA (~6 mm), we propose that at least some of these responses are mediated by a direct input to the VTA from the vBNST; however, additional experiments are needed to confirm this hypothesis. In addition, it is likely that the long latency responses were the result of multisynaptic connections and were conveyed indirectly to the VTA via one or several relays. Previous anatomical and electrophysiological studies indicate that the PPTg could be a relay between the BNST and the VTA: (1) neurons from the

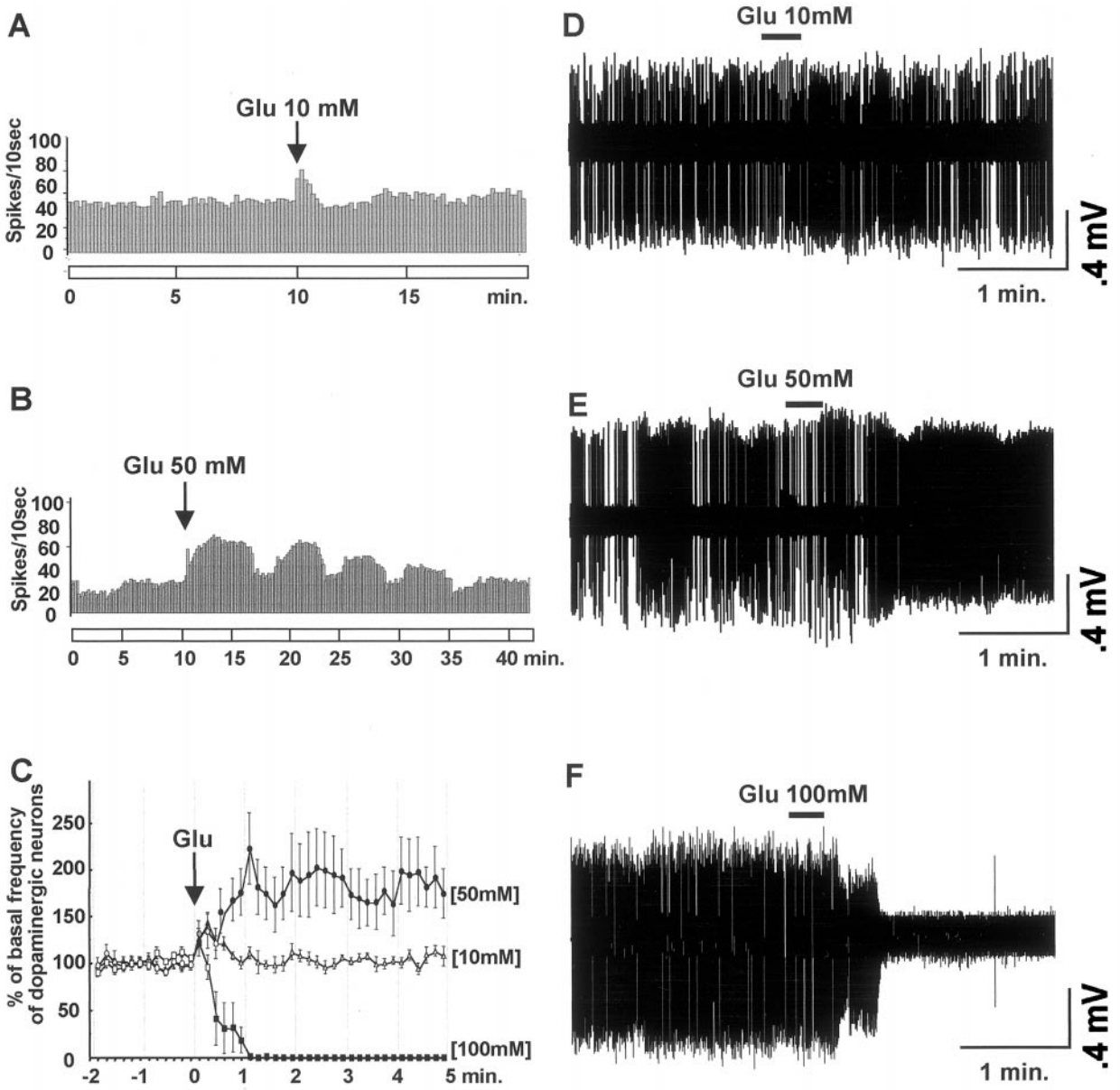

Figure 3. Effect of Glu microinjection (10, 50, or 100 mM) into the vBNST on VTA DA neuronal impulse activity. *A*, *B*, Firing activity of two typical DA VTA neurons before and after Glu injection (10 and 50 mM, as indicated) into the vBNST. A characteristic oscillatory pattern of VTA DA neuron firing activity is revealed after microinjection of 50 mM L-glutamate into the vBNST (*B*). *C*, Average activity of DA neurons after injection of different concentrations of Glu into the vBNST. Microinjection into the vBNST of 10 or 50 mM Glu produced a transient or long-lasting activation of DA neurons, respectively. Microinjection into the vBNST of 100 mM Glu produced a strong and long-lasting inhibition of DA neurons. $n = 6$, 14, and 12 cells for 10, 50, and 100 mM Glu, respectively. Significant points are indicated by *filled symbols*. *D–F*, Oscilloscope traces of three VTA DA neurons showing the typical firing activity before and after infusion of Glu at 10, 50, or 100 mM into the vBNST. Glu injection is designated by the *line* above each *trace*. Note the decrease in spike size with 100 mM Glu just before spiking stops. This is consistent with strong depolarization of these neurons and with our hypothesis that inactivation by 100 mM Glu results from a depolarization blockade (see Discussion for details). An ANOVA followed by a Newman–Keuls test for pairwise comparisons was performed for each concentration.

vBNST project directly to the PPTg (Semba and Fibiger, 1992); and (2) electrical stimulation of the PPTg produces an excitatory response of DA neurons (Di Loreto et al., 1992). In addition, the long latency excitation of VTA DA neurons may also be mediated by circuits involving the amygdala. Notably in this regard, there is a strong projection from the BNST to the amygdala (Swanson and Cowan, 1979) and the amygdala sends projections to the VTA. (Cassell et al., 1986). Additional experiments are needed to establish which if any of these structures act as relays between the BNST and the VTA. However, regardless of the pathway involved, our results show that the vBNST is a significant regulator of VTA DA neural activity.

The triphasic responses observed for some VTA neurons (short latency excitation, inhibition, long latency activation) after electrical stimulation of the vBNST indicate that effects on DA neurons may also involve a synaptic relay in the VTA. For example, vBNST inputs may induce a direct activation of DA neurons (short latency excitation), as well as activation of GABA cells in the VTA. These GABA cells provide a feedforward inhibition to the DA cells, which could give rise to the later

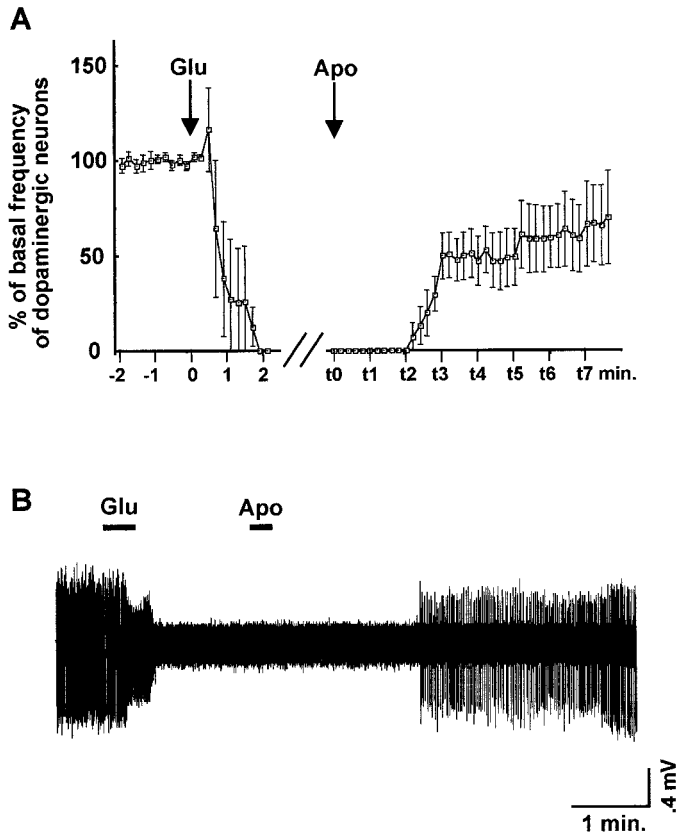

Figure 4. Effect of 100 mM Glu microinjection into the vBNST on VTA DA neuronal impulse activity. *A*, Microinjection into the vBNST of 100 mM Glu (60 nl) consistently caused VTA DA neurons to cease firing. Spontaneous firing was subsequently reinstated by intravenous administration of Apo (0.1 mg/kg). Injection time of Apo occurred between 2 and 3 min after Glu infusion in the vBNST and was normalized in this graph at time 0 (*t0*) for all neurons ($n = 5$). *B*, Filtered trace of a VTA DA neuron showing the typical firing activity during microinjection into the vBNST of 60 nl of Glu (100 mM) and reversal 2 min after intravenous administration of Apo (0.1 mg/kg). Drug injections are designated by the *lines* above the VTA spikes.

latency inhibitory responses observed. The functional significance of such an inhibitory circuit is uncertain but may be a regulatory step to limit the duration of firing of the DA cells.

A prominent finding here is that the chemical stimulation (50 mM glutamate) of the vBNST activated VTA DA neurons, often for many minutes (Fig. 3*B*). The long-lasting response of VTA cells could reflect a prolonged activation of BNST neurons by Glu. In a previous study, microiontophoresis of glutamate in the BNST produced a major but transient (lasting a few seconds) increase in firing rate in 90% of neurons tested (Casada and Dafny, 1993). However, that study did not examine Glu responses in the ventrolateral BNST specifically, and there may be a regionally specific response to Glu within this structure. It is also possible that the long-lasting response to Glu in the vBNST occurred as a result of our method of application (microinjection). However, previous studies using similar microinjection of Glu in other brain regions (e.g., cerebral cortex) found only a transient activation of neurons at the injection site (Jodo and Aston-Jones, 1997). In addition, high-affinity glutamate transporters are abundantly expressed in both neurons and glia throughout the brain and are important for maintaining the extracellular glutamate concentration at low levels (Kanai et al., 1995). Thus, it appears unlikely that the Glu applied here would remain active over a sufficient period of time to elicit the long-duration responses observed in the VTA. These uptake sites, and results from previous studies (Jodo and Aston-Jones, 1997), also make it appear unlikely that substantial Glu would diffuse from the BNST area to produce the responses observed. Moreover, ejection of Glu outside the BNST (Fig. 1) did not produce effects resembling those obtained when injections were within the BNST, demonstrating site specificity. The prolonged response of VTA neurons to 50 mM Glu injection in the vBNST may be produced by complex phenomena, such as activation of local circuits in the BNST, interactions with other neurotransmitters, or a prolonged release of transmitter from vBNST terminals in the VTA. Additional studies are needed to clarify this issue.

In contrast to electrical stimulation, chemical stimulation of the vBNST (50 mM glutamate) never produced purely inhibitory responses. However, inhibitory circuits activated during electrical stimulation may also be involved in the responses to chemical stimulation. Thus, the oscillatory activity in DA neuronal responses may be attributable to sustained excitatory input while inhibitory interneurons are also activated.

The data obtained after electrical or chemical stimulation of the vBNST (10 or 50 mM glutamate) indicate that neurons from the vBNST exert an excitatory influence on VTA DA neurons. However, injection of Glu at a concentration of 100 mM into the vBNST produced a rapid increase in firing rate and a progressive decrease in spike amplitude until electrophysiological activity terminated altogether. A similar complete loss of activity in DA neurons has been described *in vivo* after repeated treatment with antipsychotic drugs (Grace et al., 1997). In these cases, the inactivity was concluded to be attributable to hyperexcitation and inactivation by depolarization blockade. This mechanism may also be operative here, because the effect of 100 mM Glu in the vBNST was reversed by the DA autoreceptor agonist apomorphine (0.1 mg/kg, i.v.) (Fig. 4). This effect of apomorphine, which is normally strongly inhibitory on DA neurons, supports the possibility that DA neurons were in a state of depolarization block. Studies with intracellular recordings of VTA neurons are needed to confirm this hypothesis. Nonetheless, this result, and the fact that this is the first report for possible depolarization block of DA neurons by a synaptic input, indicates the strength of the excitatory influence of the vBNST on VTA DA neurons.

Given the role of the VTA DA system in normal reward processes and drug abuse (Koob and Le Moal, 2001), these findings for a novel and potent regulation of these cells by the vBNST have potentially important behavioral implications. Recent studies have defined a role for the vBNST in the aversive response to opiate withdrawal (Delfs et al., 2000). The current findings for regulation of VTA DA function by vBNST activity further expand functional considerations for the vBNST into possible roles in reward processing and motivation. It is noteworthy that these recent results and the findings here focus on the ventrolateral aspect of the BNST. It may be that this specific region has functional roles in addition to the autonomic and endocrine roles of other BNST regions explored previously. Additional work is needed to clarify the role of the vBNST in normal behavior and drug abuse.

In conclusion, the present results disclose a novel and potent excitatory influence on VTA DA neuronal function. Additional work is required to establish the neurotransmitters and the receptors involved. Our preliminary data suggest that activation of DA neurons by vBNST stimulation is mediated by non-NMDA

(AMPA–kainate) as well as by NMDA-type excitatory amino acid receptors (Georges and Aston-Jones, 2001). Also, it is intriguing that a transient stimulation of the vBNST can elicit a long-lasting activation of DA neuron activity. Thus, it is possible that phasic changes in vBNST activity could result in prolonged changes in VTA DA function.

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