Dorsal and Ventral Distribution of Excitable and Synaptic Properties of Neurons of the Bed Nucleus of the Stria Terminalis

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Egli, Regula E. and Danny G. Winder. Dorsal and ventral distribution of excitable and synaptic properties of neurons of the bed nucleus of the stria terminalis. J Neurophysiol 90: 405–414, 2003. First published March 20, 2003; 10.1152/jn.00228.2003. The bed nucleus of the stria terminalis (BNST) is a structure uniquely positioned to integrate stress information and regulate both stress and reward systems. Consistent with this arrangement, evidence suggests that the BNST, and in particular the noradrenergic input to this structure, is a key component of affective responses to drugs of abuse. We have utilized an in vitro slice preparation from adult mice to determine synaptic and membrane properties of these cells, focusing on the dorsal and ventral subdivisions of the anterolateral BNST (dBNST and vBNST) because of the differential noradrenergic input to these two regions. We find that while resting membrane potential and input resistance are comparable between these subdivisions, excitable properties, including a low-threshold spike (LTS) likely mediated by T-type calcium channels and an \( I_h \)-dependent potential, are differentially distributed. Inhibitory and excitatory postsynaptic potentials (IPSPs and EPSPs, respectively) are readily evoked in both dBNST and vBNST. The fast IPSP is predominantly GABA\(_\alpha\)-receptor mediated and is partially blocked by the AMPA/kainate-receptor antagonist CNQX. In the presence of the GABA\(_\alpha\)-receptor antagonist picotoxin, cells in dBNST but not vBNST are more depolarized and have a higher input resistance, suggesting tonic GABAergic inhibition of these cells. The EPSPs elicited in BNST are monosynaptic, exhibit paired pulse facilitation, and contain both an AMPA- and an N-methyl-D-aspartate (NMDA) receptor-mediated component. These data support the hypothesis that neurons of the dorsal and ventral BNST differently integrate synaptic input, which is likely of behavioral significance. The data also suggest mechanisms by which information may flow through stress and reward circuits.

INTRODUCTION

The bed nucleus of the stria terminalis (BNST) is considered an integral regulator of the hypothalamic-pituitary-adrenal stress axis (Cullinan et al. 1993; Dong et al. 2001a; Georges and Aston-Jones 2001, 2002; Herman and Cullinan 1997; Herman et al. 2002; McDonald et al. 1999; Moga et al. 1989; Saper and Loewy 1980; Weller and Smith 1982). The BNST receives “processive” stressor input from regions of the brain involved in cognitive information processing, such as the prefrontal cortex and the hippocampal formation. This information only becomes a stressor once it has been processed and compared with prior events in one’s experience. The BNST also receives “systemic” stressor information, i.e., hypotension or hemorrhage. This input comes directly from visceral efferent pathways and elicits a stress response without any need of higher order processing. Evidence suggests that the BNST acts as a key intermediary by receiving these inputs and then sending its own projections to the paraventricular nucleus of the hypothalamus (PVN), the key central regulator of peripheral glucocorticoid levels.

In addition to being interconnected with the stress axis, the BNST is a key anatomical bridge in an assemblage of brain regions referred to as the extended amygdala, which also includes the central nucleus of the amygdala and the shell of the nucleus accumbens (NAc) (Alheid and Heimer 1988). Furthermore, the BNST projects to and regulates the firing of dopaminergic cells within the ventral tegmental area (VTA) (Georges and Aston-Jones 2001, 2002). Thus the BNST is uniquely positioned to receive stress axis information and integrate it into reward/motivation circuitry. Consistent with these anatomical interconnections, recent data demonstrate that the BNST plays a key role in mediating stress-induced relapse to cocaine-seeking behavior (Erb et al. 1996; Shaham et al. 2000; Sinha et al. 1999), as well as in stress-induced maintenance and reinstatement of morphine-conditioned place preference (Wang et al. 2001). Furthermore, DeLisi et al. (2000) showed that the BNST plays a key role in morphine withdrawal–induced conditioned place aversion.

The systemic stress input to the BNST consists primarily of input from the central nucleus of the amygdala and noradrenergic input from the A1 and A2 cell groups of the caudal medulla. Indeed, the BNST is one of the heaviest sites of noradrenergic innervation in the CNS. This input is regionally compartmentalized within the BNST: NE input is much more dense to the ventral BNST (vBNST) than to the dorsal BNST (dBNST) in the rat and primate brain (Freedman and Shi 2001; Georges and Aston-Jones 2001; Woulfe et al. 1990). Interestingly, NE levels increase in the BNST as a result of restraint stress (Pacak et al. 1995), and inhibiting \( \alpha_1 \) and/or \( \beta_1 \)-adrenergic receptors directly in the BNST can reduce anxiety-like behaviors in rats (Cecchi et al. 2002), as well as the cocaine- and morphine-related behaviors described above, as does lesioning the ventral noradrenergic bundle (DeLisi et al. 2000; Sinha et al. 1999).
Wang et al. 2001). Moreover, cocaine self-administration in subhuman primates decreases metabolic activity in BNST, as well as upregulating norepinephrine transporter levels in this region (Macey et al. 2003).

Currently, little is known of the properties of BNST neurons. The BNST consists primarily of neurons that stain positively for glutamic acid decarboxylase (GAD), the enzyme responsible for the conversion of glutamate to GABA (Sun and Cassell 1993), and also expresses a variety of neuropeptides (Ju et al. 1989). Despite a growing literature on anatomical and neurochemical properties of the BNST, little is known of the electrophysiological properties of neurons within this structure. In a recent initial study, Rainnie (1999) recorded from rat dorso-medial and dorsolateral BNST cells via whole cell patch methods, demonstrating that these cells can be recorded from in vitro and that they are regulated by serotonin.

Here, we have confirmed and extended the findings of this initial report by electrophysiologically characterizing neurons of the anterolateral BNST in vitro, comparing dorsal versus ventral anterolateral BNST. We characterized several properties of excitability in BNST, including a low-threshold spike, an \( I_h \)-dependent depolarization, and inward rectification. These properties of excitability are differentially distributed across BNST neurons along a dorsal-ventral gradient, suggesting that common inputs to these two areas may evoke distinct outputs that differentially regulate behavior. Moreover, local fiber stimulation evokes both an AMPA- and \( N\)-methyl-\( D\)-aspartate (NMDA) receptor-mediated excitatory postsynaptic potential (EPSP) and a \( GABA_A \)-receptor mediated inhibitory postsynaptic potential (IPSP). These data allow us to suggest mechanisms by which information flows through stress and drug reward pathways.

METHODS

Animal care

All animals were housed in the Vanderbilt Animal Care Facilities in groups of 2–5. Food and water were available ad libitum. All procedures were approved by the Animal Care and Use Committee at Vanderbilt University.

Brain slice preparation

Male C57Bl/6J mice (5–10 wk old, Jackson Laboratories) were decapitated under anesthesia (Isoflurane). The brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) (in mM) 124 NaCl, 4.4 KCl, 2 CaCl\(_2\), 1.2 MgSO\(_4\), 1 NaH\(_2\)PO\(_4\), 10.0 glucose, and 26.0 NaHCO\(_3\). Slices 350 \( \mu \)m in thickness were prepared using a vibratome (Pelco). Rostral slices containing anterior portions of BNST (Bregma 0.26–0.02 mm) (Franklin and Paxinos 1997) were identified using the internal capsule, anterior commissure, fornix, and stria terminalis as landmarks (Fig. 1, A and B). Slices were then transferred to an interface recording chamber where they were perfused with heated (approximately 28° C), oxygenated (95% O\(_2\)-5% CO\(_2\)) ACSF at a rate of about 1–1.5 ml/min. Slices were allowed to equilibrate in normal ACSF for 1 h before experiments began. Increased divalent cation ACSF consisted of (in mM) 116 NaCl, 2.2 KCl, 7.0 CaCl\(_2\), 7.0 MgSO\(_4\), 1.0 NaH\(_2\)PO\(_4\), 10.0 glucose, and 26.0 NaHCO\(_3\).

FIG. 1. Representative slice and electrode placement and T-type calcium channel dependence of the low-threshold spike. A: schematic from Franklin and Paxinos rat brain atlas. Dorsal bed nucleus of the stria terminalis (dBNST) is highlighted in blue, ventral BNST (vBNST) is highlighted in purple. Landmarks used to identify slices containing BNST are labeled, anterior commissure (AC), internal capsule (ic), and the stria terminalis (St). B: corresponding slice from adult male C57Bl/6J mice. Stimulus electrode and recording electrode are shown as they would be placed for recording from the vBNST with local stimulation. C: vBNST cell resting at −60 mV does not display the low-threshold spike on injection of a depolarizing step (solid trace) but does display the spike on repolarization after a hyperpolarizing step current injection (dotted trace). D: loss of low-threshold spike after application of 200 \( \mu \)M NiCl\(_2\). The same cell as in C held via DC current injection at −80 mV before and during application of 200 \( \mu \)M NiCl\(_2\). Representative current injection traces shown below voltage traces are the same both pre- and post-NiCl\(_2\) (range −0.25 to +0.15 nA, increments of 0.1 nA). Scale bars: 50 ms, 20 mV.
Intracellular recording

Recordings from a total of 121 neurons in the BNST were utilized in this study. Recording electrodes were pulled on a Flaming-Brown Micropipette Puller (Sutter Instruments) using thick-walled filament-containing borosilicate glass capillaries. Electrodes were filled with 2 M potassium acetate and were of approximately 120–170 MΩ resistance. A current/voltage relationship from each cell was obtained by injecting a range of current via the recording electrode. A bipolar nichrome wire stimulating electrode was placed locally within the BNST approximately 500 μm from the recording electrode. An input/output curve of synaptic responses was generated by stimulating with a range of input voltages with stimulus durations from 50 to 100 μs, starting at 3 V and increasing the stimulus in 2-V increments until the cell fired an action potential (AP). If the cell did not fire, the maximum stimulus intensity administered was 35 V. Input resistance (IR) was calculated by measuring the slope of the line created by plotting current versus voltage from a series of current injections (–0.25 to +0.25 nA). IR was monitored throughout the duration of the experiment by injecting a current pulse at the end of each stimulus test pulse. AP amplitude was calculated by measuring the difference between the membrane potential at which the AP fired in an all-or-none manner and the membrane potential at which the AP reversed. AP amplitude was measured at the beginning and end of each experiment. If APs did not consistently overshoot zero, the experiment was excluded from final analysis. AP duration was calculated by measuring the width of the AP at the membrane potential corresponding to one-third of total AP amplitude. The membrane time constant (τ) was calculated using the standard exponential curve fitting function provided in pClamp9 (Axon Instruments, Union City, CA). Synaptic transmission experiments were analyzed by measuring the peak amplitude of the synaptic response which was normalized and averaged across experiments for each manipulation using Excel.

Pharmacology

All drugs were bath applied. Picrotoxin, CNQX, d-2-amino-5-phosphono-valeric acid (d-AP5), and nickel chloride hexahydrate were purchased from Sigma (St. Louis, MO). 4-Ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidium chloride (ZD7288) and [R-(R*,S*)]-5-[(6,8-dihydro-8-oxofuro[3,4-e]-1,3-benzodioxol-6-yl)-(1,2-dimethyl-6-methylaminopyrimidium chloride (ZD7288) and [R-(R*,S*)]-5-[(6,8-dihydro-8-oxofuro[3,4-e]-1,3-benzodioxol-6-yl)]-5,6,7,8-tetrahydro-6,6-dimethyl-1,3-dioxolo[4,5-g]isoquinolinium bromide (bicuculline) were purchased from Tocris (Ellisville, MO). Dimethyl sulfoxide (DMSO) (0.02% vol/vol) was the carrier for picrotoxin and CNQX.

Data analysis

Electrophysiological data were collected using an Axoclamp 2B amplifier and digitized and analyzed using pClamp 8.2 and 9.0 software. Appropriate statistical analyses (indicated within figure legends) were performed using Prizm and InStat software (Graphpad).

RESULTS

Basic membrane properties

Neurons were sampled within the dorsal and ventral lateral portions of the BNST in an anterior section (Fig. 1). We found that the resting membrane potential (RMP) recorded in normal ACSF was comparable between dorsal and ventral BNST, as was the apparent IR (Table 1). AP amplitudes, durations, and thresholds were also comparable between dBNST and vBNST. However, we found that the τ of cells in vBNST was on average considerably faster than the τ of cells in dBNST. Table 1 compares these properties between dBNST, vBNST, and a neighboring brain region, the NAc core, since these regions receive overlapping excitatory input. We recorded from 15 NAc core cells for analysis of basic properties. We found that the RMP of NAc neurons was significantly more hyperpolarized than the RMP of either dBNST or vBNST neurons, and the IR was significantly lower. AP amplitude was significantly lower in NAc neurons than in dBNST and vBNST neurons. AP duration and threshold were comparable between BNST and NAc neurons, but the τ for NAc neurons was significantly lower than for either dBNST or vBNST neurons.

Depolarization evokes low-threshold spiking in BNST neurons

A substantial proportion of neurons in the BNST displayed a low-threshold spike (LTS) in response to depolarizing current injections (Fig. 1, C and D). This LTS was observed as a relatively rapidly activating and inactivating envelope lasting 63.5 ± 5.8 ms on which were frequently superimposed one to five action potentials. The first action potential on the LTS was similar in amplitude and duration to APs elicited in the absence of a LTS (Table 2). Subsequent APs on the LTS became progressively significantly smaller in amplitude and longer in duration than the first AP on the LTS (Table 2), in contrast to groups of multiple APs observed on non-LTS–inducing depolarizing steps. The LTS was not observable when the cell was held at −60 mV via constant current injection prior to the depolarizing step (Fig. 1C, solid trace). However, on recovery from a hyperpolarizing pulse, the LTS was observable (Fig. 1C, dotted trace). The LTS seen on depolarization from a

![Graph](https://via.placeholder.com/150)

**TABLE 1. Comparison of basic properties of cells in dBNST, vBNST, and the NAc core**

<table>
<thead>
<tr>
<th></th>
<th>dBNST</th>
<th>vBNST</th>
<th>NAc Core</th>
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<tbody>
<tr>
<td>RMP (mV)</td>
<td>−69.1 ± 2.0</td>
<td>−68.6 ± 1.7</td>
<td>−89.6 ± 1.7</td>
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<tr>
<td></td>
<td>(−50 to −90)</td>
<td>(−52 to −82)</td>
<td>(−75 to −91)*</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>115.2 ± 10.8</td>
<td>127.9 ± 10.9</td>
<td>35.1 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>(26 to 167)</td>
<td>(42 to 250)</td>
<td>(12 to 67)*</td>
</tr>
<tr>
<td>Action potential amplitude, mV (peak-threshold)</td>
<td>61.5 ± 1.9</td>
<td>62.1 ± 1.5</td>
<td>54.7 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>(52 to 71)</td>
<td>(50 to 70)</td>
<td>(44 to 64)*</td>
</tr>
<tr>
<td>Action potential width, ms (at one-third total amplitude)</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>(1.0 to 2.7)</td>
<td>(0.8 to 1.5)</td>
<td>(1.0 to 1.6)</td>
</tr>
<tr>
<td>Action potential threshold, mV</td>
<td>−50.7 ± 1.1</td>
<td>−51.8 ± 1.3</td>
<td>−47.8 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>(−42 to −56)</td>
<td>(−41 to −58)</td>
<td>(−27 to −59)</td>
</tr>
<tr>
<td>Tau (ms)</td>
<td>17.4 ± 3.4</td>
<td>9.7 ± 1.0</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>(6.6 to 41.5)</td>
<td>(4.7 to 18.5)</td>
<td>(2.1 to 6.6)*</td>
</tr>
<tr>
<td></td>
<td>*P &lt; 0.01</td>
<td>†P &lt; 0.05</td>
<td>†P &lt; 0.05</td>
</tr>
<tr>
<td>n</td>
<td>22</td>
<td>27</td>
<td>15</td>
</tr>
</tbody>
</table>

Values are ± SE with range in parentheses. One-way ANOVA followed by Bonferroni’s multiple comparison test: *Statistically significant difference with dBNST and vBNST; † statistically significant difference with dBNST alone.
hyperpolarized state could be reduced by bath application of 200 μM Ni²⁺ (n = 3; Fig. 1D) at a concentration known to selectively inhibit T- and R-type calcium channels (Su et al. 2002). Application of NiCl₂ had no apparent effect on the other basic properties of these cells, i.e., membrane potential and input resistance remained constant during nickel application. The LTS we observed had an activation threshold of approximately −60 mV, which is consistent with being driven by T-type Ca²⁺ channels rather than R-type Ca²⁺ channels, which have an activation threshold of −40 to −25 mV (Randall and Tsien 1997). The resting state dependence and activation threshold of the LTS coupled with its Ni²⁺ sensitivity suggest that it is mediated by activation of T-type Ca²⁺ channels. Interestingly, the LTS also occurred on EPSPs (data not shown), suggesting that cells demonstrating the LTS may be more prone to synaptically-induced burst firing behavior.

BNST neurons display a depolarizing sag in response to hyperpolarization as well as inward rectification reminiscent of medium spiny neurons

Another property we observed in BNST neurons was a depolarizing sag in response to a hyperpolarizing current injection (Fig. 2A). This sag was somewhat slowly developed, with the time at which the depolarization began ranging from 13.8 to 120.8 ms, and it had an amplitude ranging from 1 to 15 mV (measured from the peak hyperpolarization after initiation of the current step to the final steady-state hyperpolarization reached before current injection was turned off). Because this type of potential is often mediated by Iᵦ, we tested this possibility via bath application of the Iᵦ channel blocker ZD7288 (n = 3, 100 μM; Fig. 2B). While this drug had no consistent effect on RMP, ZD7288 abolished the depolarizing sag. Inspection of the I-V relationships in the absence and presence of ZD7288 suggests that this conductance contributes to lowering the apparent input resistance in these cells (Fig. 2B, inset).

Finally, a number of cells displayed moderate inward rectification in response to current injection (Fig. 2, C and D). While this property is reminiscent of medium spiny neurons of the dorsal striatum and NAc, these BNST cells had substantially higher input resistances (113.3 ± 7.4 MΩ) and more depolarized resting membrane potentials (−66.3 ± 1.8 mV) than NAc core medium spiny neurons.

Electrophysiological properties of BNST neurons are differentially distributed within dorsal and ventral BNSTs

Interestingly, many of the basic properties we observed in the BNST were not homogeneously expressed within the BNST, but rather were regionally differentiated. The τ of cells in vBNST was on average considerably faster than in dBNST neurons (Table 1). The percentage of neurons displaying a LTS

<table>
<thead>
<tr>
<th>AP Induced by Depolarizing Step</th>
<th>First AP on LTS</th>
<th>Second AP on LTS</th>
<th>Third AP on LTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude (mV)</td>
<td>62.8 ± 0.8 (50–73)</td>
<td>64.4 ± 0.9 (55–75)</td>
<td>48.6 ± 2.2 (16–61)†</td>
</tr>
<tr>
<td>Duration (ms)</td>
<td>1.3 ± 0.04 (0.8–2.7)</td>
<td>1.1 ± 0.03 (0.8–1.4)</td>
<td>1.4 ± 0.05 (0.8–2.1)‡</td>
</tr>
</tbody>
</table>

Values are ± SE with range in parentheses. One-way ANOVA followed by Tukey’s multiple comparison test: * statistically significant difference vs. 1st AP on the LTS (P < 0.01); † statistically significant difference vs. AP induced by depolarizing step (P < 0.001); ‡ statistically significant difference vs. 2nd AP on LTS (P < 0.05).
was significantly greater in vBNST than in dBNST—while a LTS could be evoked in 74.5% of cells in vBNST, it could only be evoked in 22.9% of cells in dBNST ($P < 0.0001$, Fig. 3A). As with the LTS, the $I_h$-dependent depolarizing sag was differentially distributed between dorsal and ventral BNST; however, rather than being found predominantly in vBNST, this property was significantly more frequently observed in dBNST (15.7 and $48.6\%$, respectively, $P = 0.0002$, Fig. 3A). Cells displaying inward rectification were found only slightly more abundantly in dBNST (Fig. 3A). Interestingly, multiple cells from vBNST and dBNST showed two of these three characteristics, and two cells we recorded from, one in vBNST and one in dBNST, exhibited all three characteristics (Fig. 3B).

**Neurons in the dorsal BNST are tonically inhibited by GABA**

The BNST receives strong GABAergic input from both intrinsic and extrinsic sources. To begin to explore the role of GABAergic transmission in BNST physiology, we examined the properties of dBNST and vBNST neurons in the presence and absence of the GABA$_A$-receptor antagonist picrotoxin. RMP and IR of cells in vBNST appeared unaffected by the inhibition of fast GABAergic transmission. In contrast, the RMP of cells in dBNST depolarized significantly, from $-69.1 \pm 2.0$ mV in normal ACSF to $-64.1 \pm 1.3$ mV in picrotoxin ($P = 0.0289$, Fig. 4A), suggesting tonic inhibition of these cells by GABA. Accordingly, the IR in dBNST cells also increased significantly in the presence of picrotoxin, from 115.2 ± 10.8 M$\Omega$ to 143.2 ± 6.2 M$\Omega$ ($P = 0.0197$, Fig. 4B). It should be noted that the $I_h$ current was still active in the presence of picrotoxin; therefore the increase in the apparent IR of dBNST neurons seen in the presence of picrotoxin is not likely due to an inhibition of $I_h$ current activation.

**Inhibitory synaptic transmission**

Local fiber stimulation in the BNST results in an EPSP and an IPSP in both dBNST and vBNST. Depending on the membrane potential, the EPSP, the IPSP, or both are observable (Fig. 4C). The IPSP occurs with a latency of 2.39 ± 0.04 ms (measured in the presence of 10 μM CNQX, an AMPA/kainate-receptor antagonist). The major component of the IPSP is mediated by the GABA$_A$-receptor because it can be blocked by application of 25 μM picrotoxin (Fig. 4D) or another GABA$_A$-receptor antagonist, 20 μM bicuculline (Fig. 5E, trace 2). A small late hyperpolarization was occasionally observed after the initial IPSP that could be a GABA$_B$-mediated IPSP and/or a glycinegic IPSP (Fig. 4D), but this was inconsistently seen and would need to be analyzed pharmacologically to definitively identify its origins. To begin to determine the source of the GABA$_A$-mediated IPSP, we recorded under conditions of increased concentrations of Mg$^{2+}$ and Ca$^{2+}$ (7 mM each) to increase AP firing threshold. An increase in AP threshold would be predicted to reduce the probability of multisynaptic events relative to monosynaptic events. Thus these conditions allow one to begin to discriminate a monosynaptic response (which should be unaffected under these conditions) from a polysynaptic response (which should be reduced). As predicted, this manipulation substantially shifted the threshold for somatic AP firing to the right, with APs only elicited on greater amounts of depolarization, i.e., APs fired at a membrane potential of about −40 mV instead of −55 mV (Fig. 4E, insets). Under these conditions of high divalent cation concentrations, the IPSP was elicited with no failures (Fig. 4E), suggesting that at least a component of the IPSP is monosynaptic GABAergic transmission. Curiously though, this IPSP was partially reduced by application of CNQX (10 μM, Fig. 4F), suggesting that the IPSP may have both monoo- and polysynaptic components that may be due to both direct GABAergic input stimulation as well as either indirect low-threshold GABAergic inputs stimulated by AMPA/kainate-receptor mediated circuits, or conceivably, GABAergic terminals presynaptically regulated by AMPA/kainate receptors (Braga et al. 2003).
Excitatory synaptic transmission

In addition to an IPSP, synaptic stimulation evoked a substantial EPSP, which by raising stimulus strength, was routinely of sufficient size to generate APs (Fig. 5A). This EPSP appeared to be monosynaptic in origin. First, the response was elicited with a very short latency with very little variability (2.56 ± 0.04 ms, measured in the presence of 25 μM picrotoxin). Second, in ACSF containing increased divalent ion concentrations, the EPSP was still reliably elicited and comparable in amplitude to EPSPs recorded in normal ACSF. In addition, there was no increase in failure rate during low frequency stimulation (Fig. 5B). The EPSP recorded in 25 μM picrotoxin ACSF was consistently elicited during relatively high-frequency (400 ms of 25 Hz) stimulation (Fig. 5C) and could be almost entirely blocked by 10 μM CNQX (Fig. 5D).

An NMDA-receptor-mediated EPSP could be revealed after blocking an IPSP recorded in the presence of 10 μM CNQX (Fig. 5E, trace 1) with 20 μM bicuculline (Fig. 5E, trace 2). This remaining component of the EPSP could be blocked by the NMDA-receptor antagonist, d-AP5 (100 μM; Fig. 5E, trace 3). In the presence of picrotoxin, EPSPs in cells in both vBNST and dBNST showed paired pulse facilitation (Fig. 5, F and G) across a range of interpulse intervals, with the most robust facilitation at the shorter time points. The presence of paired pulse facilitation indicates that these cells can undergo at least short-term forms of plasticity.

DISCUSSION

The BNST plays a key role in integrating information from stress input pathways and in turn regulating stress output and reward pathways. In this study, we have performed an initial characterization of the electrophysiological properties of neurons within this brain region in the adult male C57Bl/6J mouse and have demonstrated that while several basic membrane properties are conserved between cells of dorsal and ventral BNST, many properties of excitability are differentially distributed within these two regions in a manner that parallels their differential catecholaminergic innervation. These data provide us with information with which we can begin to make hypotheses about the way the BNST participates in processing information in stress and reward circuits.
Excitable properties

From a circuit perspective, it is useful to compare the properties of BNST neurons to a population of cells receiving heavily overlapping afferent input. When compared with medium spiny neurons in the core of the NAc, a neighboring population of neurons that receives many of the same excitatory inputs, we find that neurons of the BNST are much more excitable, having more depolarized resting membrane potentials, much higher input resistances, longer membrane time constants, and slightly larger AP amplitudes. In addition, we report three voltage-dependent properties present in a large percentage of BNST neurons. First, we found that these cells express a Ni²⁺-sensitive low-threshold spike. The LTS is most likely mediated by T-type calcium channels, as its activation properties were consistent with those of a T-type calcium channel (Randall and Tsien 1997), and it could be blocked by the T- and R-type calcium channel blocker Ni²⁺. Second, we found that many cells exhibited a depolarizing sag in response to hyperpolarizing current injection that was sensitive to the I_h blocker ZD7288. The excitable properties we report here in mouse brain slices with sharp microelectrodes are very similar to those reported by Rainnie in rat brain slices (Rainnie 1999). We have here further extended his results by pharmacologically probing these properties. Third, we found that many cells exhibited inward rectification in response to increasing current injection steps that was reminiscent of that observed in medium spiny neurons of the dorsal striatum and the NAc (Nisenbaum and Wilson 1995; O’Donnell and Grace 1993).

Inhibitory transmission

We have found that on synaptic stimulation, both a GABA_A receptor-mediated IPSP and an AMPA/kainate- and NMDA-receptor-mediated EPSP could be elicited in BNST neurons. The IPSPs elicited in these cells via modest distal stimulation (approximately 500 µm away from the recording electrode) appeared to be at least partially monosynaptic, because they could be elicited in the presence of high divalent cation concentrations and had a very short, relatively invariant latency. These IPSPs likely originated from both a large GABAergic projection from the central nucleus of the amygdala as well as from intra-BNST projections of GABAergic BNST neurons. However, even in the presence of high divalent cation concentrations we found that the IPSP could be partially reduced by application of 10 µM CNQX. These IPSPs are likely due to highly excitable local circuit interneurons, although it is possible that GABAergic transmission in this brain region may be
modulated by either axonal or presynaptic terminal localized ionotropic glutamate receptors (Braga et al. 2003). In future studies, analysis of spontaneous transmission will be needed to resolve this issue.

In addition to activity-dependent fast GABAergic inhibition, we found that cells in dBNST were tonically inhibited by GABAergic transmission. In the presence of 25 μM picrotoxin, the RMP of cells in dBNST was significantly depolarized relative to dBNST cells in normal ACSF. Consistent with relief of tonic inhibition, the IR of dBNST cells also increased significantly when picrotoxin was present. This change in IR does not appear to have been driven by an alteration in $I_h$ activation because $I_h$ persisted in the presence of picrotoxin. Tonic inhibition by GABA has been demonstrated in hippocampus (Yeung et al. 2003) and is correlated with the presence of specific subunits of the GABA$_A$-receptor. The δ subunit of the GABA$_A$-receptor has been clearly demonstrated to have an extrasynaptic localization, and heteromeric assemblies of this subunit produce inactivating and nondesensitizing GABA$_A$-receptors (Bianchi et al. 2002). The δ subunit is present in the BNST, particularly in neuronal processes, although it is not clear whether there is a dorsal versus ventral distribution (Pirker et al. 2000). It is also possible that this basal inhibition of cells in dBNST may be the result of a steady stream of spontaneous inhibitory transmission, as opposed to tonic presence of GABA. However, we have no evidence that there is an exceptionally high level of spontaneous neurotransmitter release (either GABA or glutamate) that could contribute to tonic regulation of membrane potential or input resistance, although our experiments have not been performed under conditions which would optimize the detection of these events.

In total, our data suggest at least three different forms of fast GABAergic inhibition in the BNST: 1) glutamatergic transmission-independent forms of GABAergic transmission, possibly arising from the central nucleus of the amygdala and/or local GABAergic transmission, 2) glutamatergic transmission-dependent GABAergic transmission which may reflect local circuit interactions, and/or presynaptic actions of glutamate on GABAergic terminals, and 3) tonic GABAergic tone in dBNST.

Excitatory transmission

In addition to an IPSP, on local stimulation in the BNST, an EPSP could also be elicited. The ventral subiculum and cortical areas provide the predominant known glutamatergic input into the BNST (Herman et al. 2002), and since the vast majority of cells intrinsic to the BNST are GABAergic, are likely responsible for the EPSPs recorded here. The EPSP was largely mediated by non-NMDA ionotropic glutamate receptors, but also had a clear NMDA-receptor dependent component. The EPSP was most likely monosynaptic, because it could be elicited in high divalent cation concentrations, followed relatively high-frequency stimulation with no failures, and had a very short and invariant latency. EPSPs recorded from cells in dBNST and vBNST exhibited paired pulse facilitation, a form of short-term plasticity. The amount of facilitation at longer interpulse intervals was not pronounced, suggesting that the release probability at these synapses under the present conditions may be relatively high.

It is also worth noting that many of the glutamatergic inputs to the BNST also project to the NAc core, a neighboring population of neurons also implicated in the drug-reward pathway. Based on our comparison of properties of these cells, it would be predicted that neurons of the BNST would be much more responsive to these inputs than NAc neurons, suggesting that BNST neurons may be activated by glutamatergic stimuli from these regions that would not activate NAc neurons, which may be of functional significance when the stress and drug reward pathways are recruited.

Dorsal and ventral BNST neurons display divergent properties

The differing distribution of excitable properties between dBNST and vBNST may confer different characteristics of synaptic processing on these two regions. The low-threshold spike that predominates in the vBNST promotes burst firing, which may alter transmitter release from these cells. For example, in dopaminergic cells burst firing results in a more efficient release of DA from terminals (Suad-Chagny et al. 1992), and in many systems, is correlated with release of peptidergic transmitters from dense core vesicles. Efferents from the BNST contain many different types of neuropeptides, including corticotrophin releasing factor, neuropeptide Y, galanin, substance P, and cholecystokinin (Ju et al. 1989), which may be released in a similar manner from BNST burst firing cells. Moreover, cells in vBNST also tended to have relatively shorter $\tau_r$ compared with cells in dBNST. The combination of the shorter $\tau_r$ with the LTS could serve to increase signal-to-noise ratios by dampening temporal summation of weak inputs but rewarding larger inputs with burst firing evoked by the LTS.

In contrast to cells of the vBNST, neurons within the dBNST tended not to express a LTS, were under tonic GABAergic inhibition, and had $I_h$ activity. The lack of the LTS and the presence of tonic GABAergic inhibition suggest that these cells may be more quiescent in the native state and may require greater input in order for effenter connections to be activated. However, it is interesting to note that the $\tau_r$'s for these neurons were significantly longer than for cells in the vBNST, despite the presence of $I_h$ activity. This suggests that either these cells are significantly larger in size than cells of the vBNST, that compensatory effects with other channel types are employed to compensate for $I_h$ activity, or a combination of these two.

Potential functional significance

It has been suggested that the BNST acts as an intermediary for regions of the brain involved in perceiving stress and regions of the brain producing responses to stress (Herman and Cullinan 1997). The ventral subiculum and cortical areas that transmit “processive” stressor information and the noradrenergic projections from the nucleus of the solitary tract that transmit “systemic” stressor information project to the PVN via a relay in the BNST, as well as via direct projections to the hypothalamus. Interestingly, the BNST also projects to brain systems involved in reward responses, including the VTA and...
the NAc. The interactions between the BNST, PVN, and VTA are complex, but one prediction would be that activation of the BNST by processive stressors (i.e., ventral subiculum and cortical areas) would result in increased output from BNST to the NAc, VTA, PVN and/or the hypothalamic area surrounding the PVN (PVN surround). However, it is as yet unclear whether this would have a net excitatory or inhibitory effect on these regions. For example, while the majority of the neurons of the BNST appear to be GABAergic, strong electrophysiological evidence suggests an excitatory projection between the BNST and the VTA (Georges and Aston-Jones 2002) that could result in increased dopamine release in the NAc as a result of BNST activation.

It is important to note that, although there are similarities in the organization of dBNST and vBNST, these regions do have anatomically distinct inputs and outputs. For example, while the dBNST may send a stronger projection to the NAc (Dong et al. 2001b), vBNST acts as a GABAergic relay for glutamatergic ventral subicular inputs to the PVN (Cullinan et al. 1993) and has also been shown to project directly to the VTA via an excitatory projection (Georges and Aston-Jones 2002). There also appears to be differential expression of various neuropeptides in dBNST and vBNST (Ju et al. 1989; Moga et al. 1989), which may also be of functional consequence in integrating their respective inputs and outputs. Additionally, Rainnie (1999) described differences in input resistance and r between cells in dorsomedial and dorsolateral BNST, providing more evidence in support of functional differences between subregions of the BNST.

In addition to the differences described above, a major distinction between these regions is the differential noradrenergic innervation of BNST, with the ventral portions receiving substantially more noradrenergic innervation by the caudal medulla than the dorsal regions (Woulfe et al. 1990). This NE input to vBNST is of key functional relevance given the dependence on adrenergic receptor activity for many of the roles the BNST plays in drug related behaviors (Delfs et al. 2000; Wang et al. 2001). Indeed, one possibility to be investigated in future studies is whether the differential expression of excitabile properties we find reflects differential states of noradrenergic neuromodulation within BNST. Thus while at present it is difficult to predict the net effects of activation of BNST cells on target brain regions, these studies coupled with more detailed understanding of the connectivity of these regions will greatly aid in our understanding of the integration of the stress and reward axes.

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