Role of the bed nucleus of the stria terminalis in the control of ventral tegmental area dopamine neurons

Marion Jalabert [a,b], Gary Aston-Jones [c], Etienne Herzog [d,e,f], Olivier Manzoni [a,b], François Georges [a,b,*]

[a] INSERM, U862, Neurocentre Magendie, Pathophysiology of synaptic plasticity group, Bordeaux, F-33000, France
[b] Université de Bordeaux, Bordeaux, F-33000, France
[c] Department of Neurosciences, Medical University of South Carolina, 173 Ashley Ave., Suite 403 BSB, MSC 510, Charleston, SC 29425-5100, USA
[d] INSERM U952, 9 Quai St Bernard, 75005, Paris, France
[e] CNRS UMR 7224, 9 Quai St Bernard, 75005, Paris, France
[f] Université Pierre et Marie Curie (UPMC), Paris 06, Paris, France

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ABSTRACT

Projections from neurons of the bed nucleus of the stria terminalis (BST) to the ventral tegmental area (VTA) are crucial to behaviors related to reward and motivation. Over the past few years, we have undertaken a series of studies to understand: 1) how excitatory inputs regulate *in vivo* excitable properties of BST neurons, and 2) how BST inputs in turn modulate neuronal activity of dopamine neurons in VTA. Using *in vivo* extracellular recording techniques in anesthetized rats and tract-tracing approaches, we have demonstrated that inputs from the infralimbic cortex and the ventral subiculum exert a strong excitatory influence on BST neurons projecting to the VTA. Thus, the BST is uniquely positioned to receive emotional and learning-associated informations and to integrate these into the reward/motivation circuitry. We will discuss how changes in the activity of BST neurons projecting to the VTA could participate in the development or exacerbation of psychiatric conditions such as drug addiction.

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1. Introduction

The bed nucleus of the stria terminalis (BST), a component of the extended amygdala (ExtA), has been implicated both in rodents and primates as a key mediator of stress and reward interactions (Walker et al., 2003; Burow et al., 2005; Choi et al., 2007; Harris and Aston-Jones, 2007). The mesolimbic dopamine (DA) system is necessary for the processing of naturally rewarding stimuli, as well as for self-administration of drugs of abuse (Grace et al., 2007). Numerous electrophysiological data (Murase et al., 1993; Georges and Aston-Jones, 2002; Floresco et al., 2003; Lodge and Grace, 2006) indicate that

potent excitatory afferents of the ventral tegmental area (VTA) arise in the medial prefrontal cortex (mPFC), BST, and laterodorsal and pedunculopontine tegmental nuclei and are essential for burst firing of VTA DA neurons. We previously reported that the BST projects to and exerts a strong excitatory influence on the firing of DA neurons within the VTA (Georges and Aston-Jones, 2001, 2002). This particular excitatory projection has been implicated in physiological and pathological reward-directed behaviors, such as food and cocaine self-administration (Dumont et al., 2005; Grueter et al., 2006) and drug seeking (Aston-Jones and Harris, 2004). Excitatory synaptic inputs are a key component of the regulation of BST cell excitability and are important in the central control of the autonomic system, the actions of many drugs of abuse and the mediation of stress responses (Wilkinson and Pittman, 1995; Dumont and Williams, 2004; Egli et al., 2005; Kash et al., 2007). The BST integrates information from stress input pathways, and subsequently regulates both stress output and reward pathways (Herman and Cullinan, 1997; Georges and Aston-Jones, 2002). The endocannabinoid system participates in these functions, since we have recently reported that the infralimbic cortex (ILCx) glutamatergic projections control mesolimbic DA neurons through the CB1 receptor located in the BST (Massi et al., 2008). Moreover, inhibition of fast excitatory transmission in the BST by injection of an AMPA receptor antagonist blunts anxiety responses (Walker and Davis, 1997), indicating that regulation of glutamatergic transmission in this region is an important target for anxiolytic and

anxiogenic stimuli. We will focus here on the function of BST in brain reward circuitry, particularly in terms of its connectivity with the mesolimbic DA system, and the major role it plays in modulating DA neuronal activity. We refer also to an excellent review for studies on the role of BST in the pathophysiology of stress-related psychiatric disorders (Forray and Gysling, 2004).

The anterior part of the BST receives excitatory inputs primarily from the ventral subiculum of the hippocampus (vSUB) and limbic cortical regions (Cullinan et al., 1993; McDonald et al., 1999; Dong et al., 2001). The mPFC and the vSUB play an important role in regulating adaptive responses to emotional stress (Spencer et al., 2005; Herman and Mueller, 2006) and reward-directed behaviors (Morgan and LeDoux, 1995; Seamans et al., 1995; Tzschentke and Schmidt, 1999; Vertes, 2006; Grace et al., 2007).

The mPFC is a major component of the motivation network, as it regulates general motivational significance of stimuli and determines the intensity of associated behavioral responses (Goldstein and Volkow, 2002). There is an increasing recognition of the important functional distinctions within discrete subdivisions of the mPFC, namely ILCx, prelimbic (PL) and anterior cingulate (Cg) cortices (Morgan and LeDoux, 1995; Seamans et al., 1995; Tzschentke and Schmidt, 1999; Vertes, 2006; Grace et al., 2007). The ILCx may also have an important role in regulating adaptive responses to emotional stress (Spencer et al., 2005; Radley et al., 2006) as well as in diverse functions including attention, rewarding-directed behavior and working memory, in accordance to its projection to the ExtA (for review: Uylings et al., 2003). The PL/Cg sends 90% of the mPFC excitatory projections to the VTA. These synapses primarily connect to GABAergic interneurons, but also to mesocortical DA neurons (Carr and Sesack, 2000; Geisler et al., 2007). In contrast, the ILCx sends only about 10% of the prefrontal excitatory projections to the VTA (Geisler et al., 2007), but little is known regarding a potential implication of the ILCx in the control of the DA neurons activity. In our recent research, we combined tract tracing approaches and *in vivo* electrophysiological recordings to demonstrate that the BST actively relays the excitatory drive from the ILCx to VTA DA neurons (Massi et al., 2008).

The vSUB plays an important role in the relapse to cocaine seeking behavior (Rogers and See, 2007) and interacts with key limbic structures through its output connections with the PFC, BST, nucleus accumbens (NAc) and the amygdala (Ishikawa and Nakamura, 2006). A series of seminal studies by Grace and colleagues defined the vSUB as an interface between the hippocampal formation and structures in the brain reward circuitry. Direct stimulation of the vSUB increases the population of active DA neurons in the VTA *via* polysynaptic pathways relayed through the NAc (Grace et al., 2007). Although the vSUB does not project directly to the VTA DA neurons, it can indirectly activate DA neuronal impulse activity and increase DA levels in the terminal regions (Legault and Wise, 1999; Legault et al., 2000). Pyramidal neurons in the vSUB exhibit transitions between two modes of action potential outputs: bursting and single spiking. The transitions between these two modes are thought to be important in integrating spatial location and context information where reward occurs (Tabuchi et al., 2000).

Some of our experiments demonstrating the prominent role of the BST in the control of DA neurons excitability are summarized below (see also: Georges and Aston-Jones, 2001, 2002; Massi et al., 2008). These studies were designed to evaluate the influence of the projection of the ILCx and the vSUB to the BST and to further elucidate the interactions between prefrontal and hippocampal regions to control the activity of VTA DA neurons.

2. BST as a hub connecting hippocampus, prefrontal cortex and dopamine system

The anterolateral group of the BST plays a critical role in anxiety and reward related behaviors (Forray and Gysling, 2004; Dumont et al., 2005). It is a complex structure that can be divided into anterolateral, subcommissural (anteroventral), oval, juxta-capsular,

fusiform and rhomboid nuclei (Dong et al., 2001). This region of the BST has a large number of GABAergic neurons, mostly classified as medium-sized spiny neurons (McDonald, 1983; Cullinan et al., 1993; Sun and Cassell, 1993). Only recently, physiological properties of neurons from these regions have been studied. It has been reported by
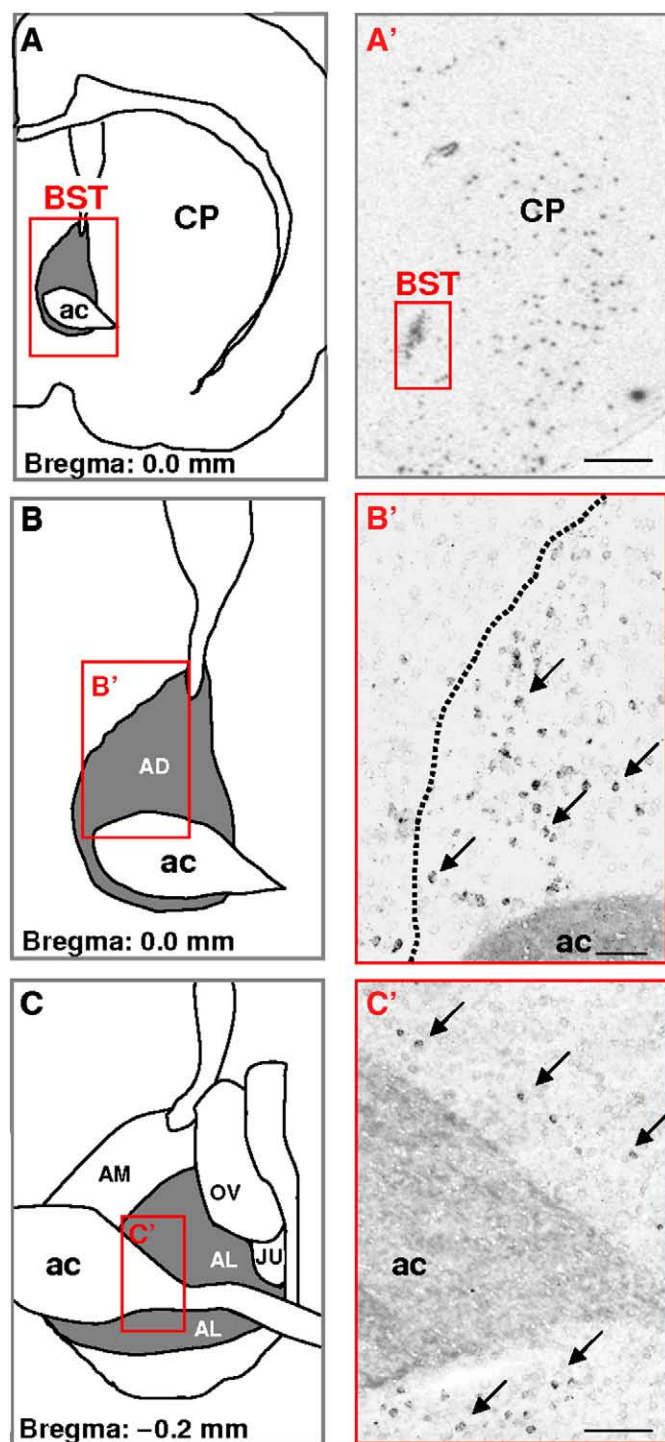
Fig. 1. *In situ* hybridization analysis of VGLUT3 transcript distribution in the rat BST. A–C: Schematics illustrating the anterior region of the BST at two levels (A–B: AP = 0.0 mm from bregma; C: AP = − 0.2 mm from bregma. A′–C′: Coronal brain sections were hybridized with antisense 35S-labeled oligonucleotides (A′) or DIC-UTP-labeled cRNA probes (B′ and C′). Neurons expressing VGLUT3 mRNA (arrows) are distributed in the anterior lateral group of the BST. *ac*, anterior commissure; *AD*, anterodorsal BST; *AL*, anterolateral BST; *AM*, anteromedial BST; *OV*, oval nucleus; *JU*, juxtacapsular nucleus. Scale bars: A′ = 1.5 mm; B′ and C′ = 0.2 mm. These illustrations are issued from experiments published in Gras et al. (2002) and Herzog et al. (2004).

Table 1
Summary of basic electrophysiological properties, onset latencies and durations of excitatory responses of BST and VTA neurons to ILCx and vSUB stimulations.

BST neurons projecting to the VTA	ILCx stimulation	vSUB stimulation
Number of neurons	$n = 32$	$n = 12$
Spontaneous firing rate (Hz)	10.5 ± 2.6	0.73 ± 0.47
Action potential width (ms)	0.74 ± 0.04	0.72 ± 0.02
Neurons responsive to tait pinch (%)	21.8	ND
Excitatory response to stimulation (% of neurons)	81.25	83.05
Response magnitude of excitation (spikes)	64.3 ± 12.9	96.20 ± 6.2
Onset of excitation (ms)	9.4 ± 0.7	12.8 ± 0.6
Duration of excitation (ms)	10.5 ± 1.0	11.0 ± 1.6
Onset of inhibition (ms)	10.9 ± 3.3	15.0 ± 2.2
Duration of inhibition (ms)	85.0 ± 29.7	132.5 ± 11.4

All values are means $\pm$ sem. BST neurons projecting to the VTA are those antidromically driven by the VTA stimulation. Adapted from Massi et al. (2008).

our group using *in vivo* preparations (Georges and Aston-Jones, 2002; Massi et al., 2008) and by others using *ex vivo* preparations (Rainnie, 1999; Dumont and Williams, 2004; Dumont et al., 2005, 2008; Hammack et al., 2007; Kash et al., 2007) that there are electrophysiologically distinct populations of neurons in the BST. Specifically, the anterolateral and anteroventral regions of the BST send a monosynaptic excitatory output to DA neurons in the VTA (Georges and Aston-Jones, 2002; Dumont and Williams, 2004; Kash et al., 2007). Our studies demonstrate that a subgroup of BST neurons

project to the VTA and potently activate neuronal activity of DA neurons (Georges and Aston-Jones, 2002). However, these data do not allow to conclude formally that the BST contains glutamatergic neurons. But, two converging lines of evidences support the existence of a distinct population of glutamatergic neurons in the anterolateral BST: 1) Using radioactive (Fig. 1A′) or colorimetric (Fig. 1B′ and C′) *in situ* hybridization techniques, we detect prominent labeled neurons for the glutamate transporter 3 (VGLUT3) mRNA in BST (Fig. 1; (Gras et al., 2002; Schafer et al., 2002; Herzog et al., 2004; Riedel et al., 2008); and 2) Using [H³] D-aspartate retrograde tracing technique, numerous radiolabelled cell bodies were detected in the anterolateral and anteroventral BST (Csaki et al., 2000; Kocsis et al., 2003). This data taken together with our retrograde and anterograde labelings, and antidromic activation of BST neurons by VTA stimulation (Georges and Aston-Jones, 2002), give anatomical and physiological support for a glutamatergic projection from the BST to the VTA.

Glutamatergic synapses in the ExtA are involved in behaviors related to stress and the effects of addictive drugs (for review: (Koob, 2003)). Plasticity at excitatory synapses onto the subgroup of VTA-projecting BST neurons correlates with operant learning, suggesting a role of the BST in goal-directed behaviors towards natural and drug associated rewards (Dumont et al., 2005). The vSUB and cortical areas provide the prominent known glutamatergic input into the BST (McDonald et al., 1999; Dong et al., 2001; Herman et al., 2002) and these inputs are topographically organized (McDonald, 1998). Support for this anatomical organization is provided by data from our
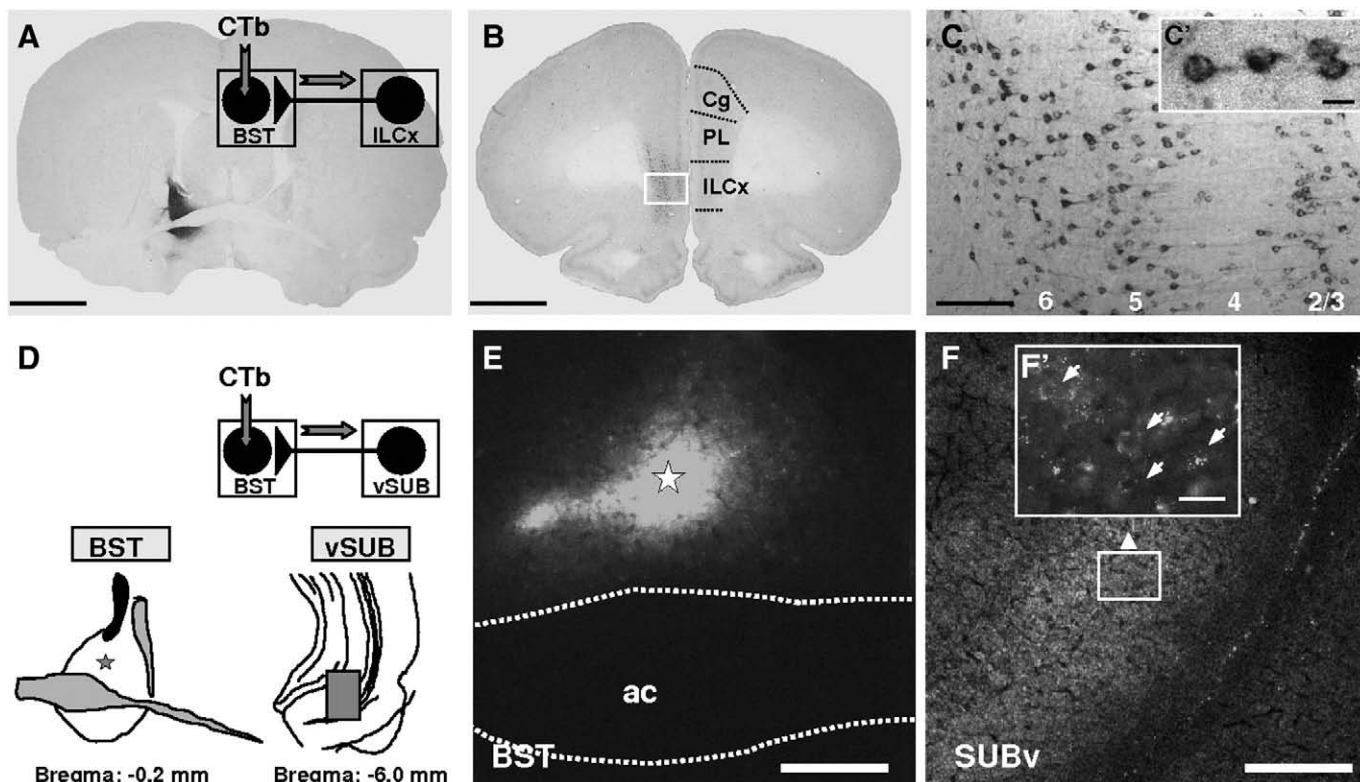

Fig. 2. ILCx and vSUB connect directly to BST neurons. A–C: Projections from ILCx to BST revealed by retrograde labeling in ILCx after injection of the subunit b of the cholera toxin (CTb) within the BST. A: Representative photomicrograph of a BST CTb injection (dark labeling). Inset: Diagram of the injection protocol used in this experiment B, C: Bright-field photomicrographs of retrograde labeling in ILCx after BST CTb injection. Injections of CTb into the BST revealed that cortical projections to the BST originate exclusively from the ILCx. In each of the four animals injected with CTb, we observed numerous retrogradely labeled neurons in the ILCx. No anterograde labeling was detected in the ILCx. In sections processed for CTb (dark labeling), cell bodies are observed in ILCx layers 2/3, 5 and 6. Cell bodies retrogradely labeled in the ILCx are shown at higher magnification in C and C′. Scale bar: A and B, 2.0 mm; C, 0.1 mm; C′, 10 μm. D–F: Projection from vSUB to BST revealed by retrograde labeling in vSUB after BST CTb injection. D: Diagram of the injection protocol used in this experiment. BST was injected with Alexa Fluor® 488 conjugated to CTb (Invitrogen; 60 nl). Five rats were sacrificed 7 days after receiving CTb into the BST. Below, Anatomical localization on a cartography of the injection site and retrograde labeling represented in E and F. E: Representative photomicrograph of a BST CTb injection (bright labeling show the CTb fluorescence). F: Fluorescent photomicrographs of retrograde labeling in vSUB after BST CTb injection. In each of the five animals injected with CTb, we observed numerous retrogradely labeled neurons in the vSUB. Cell bodies retrogradely labeled in the vSUB are shown at higher magnification in F′. Scale bar: C and D, 0.1 mm; C′, 10 μm; E; F, 0.5 mm and F′, 20 μm. ac, anterior commissure; BST, bed nucleus of the stria terminalis; CTb, Cholera toxin-B subunit; ILCx, infralimbic cortex; PL, prelimbic cortex; Cg, cingular cortex; vSUB, ventral subiculum. Adapted from Massi et al. (2008).

laboratory (Massi et al., 2008), reporting the first functional evidence of a strong excitatory regulation by ILCx and vSUB of the majority of BST neurons including those projecting to VTA. Considering the short latency excitations for most cells (<15 ms; Table 1), and the distance between the ILCx and the BST (approximately 5 mm) or the vSUB and the BST (approximately 6 mm), we propose that the excitatory responses of BST neurons to ILCx or vSUB stimulations are mediated by direct and monosynaptic glutamatergic inputs. Thus, that study provides the first anatomical and functional evidence (Figs. 2–4 and Table 1) that inputs from the ILCx and vSUB exert a strong excitatory influence on BST neurons projecting to the VTA. We demonstrate that blocking GABA$_A$ receptors within the BST by local picrotoxin, enhanced the probability of spike discharge evoked in BST neurons by ILCx stimulation (Massi et al., 2008). These data raise questions about the potential role of feedforward inhibitory circuits, including interneurons within the BST, and/or projections from GABAergic regions like NAc and central nucleus of the amygdala (Le Gal LaSalle et al., 1978; Sun and Cassell, 1993), in the regulation of BST neurons excitability. The BST sends a monosynaptic excitatory output to DA

neurons in the VTA (Georges and Aston-Jones, 2002; Dumont and Williams, 2004). However, the majority of BST neurons are GABAergic (70–90%; (Le Gal LaSalle et al., 1978; Sun and Cassell, 1993). Moreover, a particular feature characterizing the BST is its large density of NMDA receptor (subunit: NMDAR1) immunoreactive terminals, forming symmetric synapses and displaying GABA immunoreactivity (Paquet and Smith, 2000). Thus, this population of presynaptic NMDARs, if activated by extrasynaptic glutamate diffusing from neighboring synapses (Kullmann and Asztely, 1998), may modulate the release of GABA. Our data with GABA$_A$ antagonist provide further support for the hypothesis that glutamatergic and GABAergic afferent pathways regulate BST neuron population activity (Massi et al., 2008).

Our data show that the ILCx and the vSUB exert their powerful phasic control over the activity of BST neurons via ionotropic glutamate receptors (NMDA and AMPA/kainate; Fig. 4). NMDA and AMPA/kainate receptor antagonists dramatically decreased basal-steady state activity suggesting a stimulatory tonic influence of glutamatergic synapses within the BST. Other neurotransmitters may also participate in the ILCx-BST or vSUB-BST pathway. For instance,
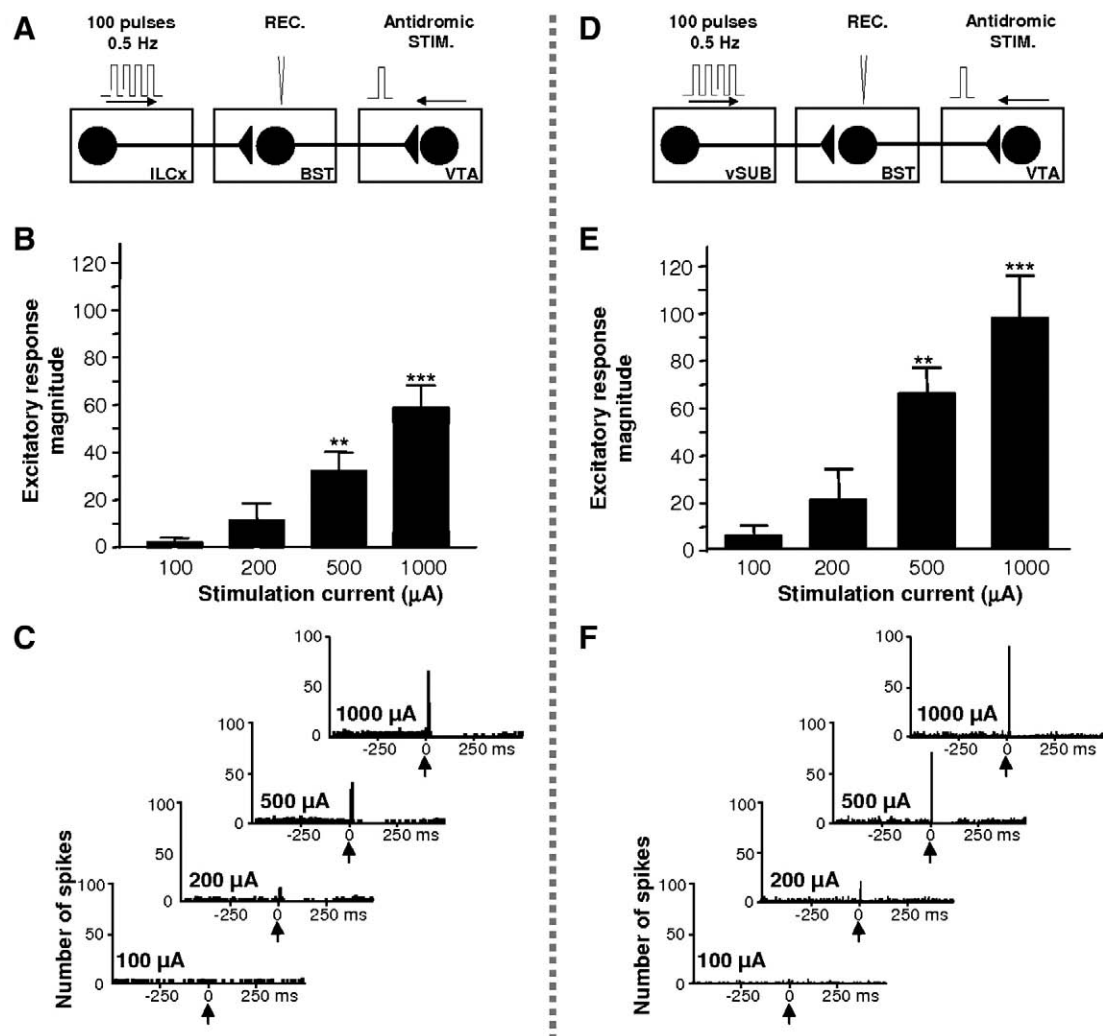

Fig. 3. Results of input/output tests on BST neurons projecting to the VTA after stimulation of ILCx or the vSUB. A, Diagram of the stimulation protocol used in this experiment. ILCx was stimulated with a 100-pulse train. B, Increasing intensity of stimulation current in ILCx evoked higher response magnitude in VTA-projecting BST neurons. C, Typical post-stimulus time histograms (PSTHs) illustrate activity of VTA-projecting BST neurons in response to ILCx stimulation at increasing intensities (100, 200, 500 and 1000 μA). D, Diagram of the stimulation protocol used in this experiment. Here, the vSUB was stimulated with a 100-pulse train. E, Increasing intensities of vSUB stimulation evoked higher response magnitudes in VTA-projecting BST neurons. C, Typical PSTH illustrate activity of a VTA-projecting BST neuron in response to ILCx stimulation at increasing intensities (100, 200, 500 and 1000 μA). BST neurons projecting to the VTA are those antidromicaly driven by VTA stimulation. The slope of the relationship between injected current and evoked firing rate is similar in VTA-projecting BST neurons after stimulation of the ILCx or vSUB. BST, bed nucleus of the stria terminalis; ILCx, infralimbic cortex; vSUB, ventral subiculum; VTA, ventral tegmental area. Adapted from Massi et al. (2008).
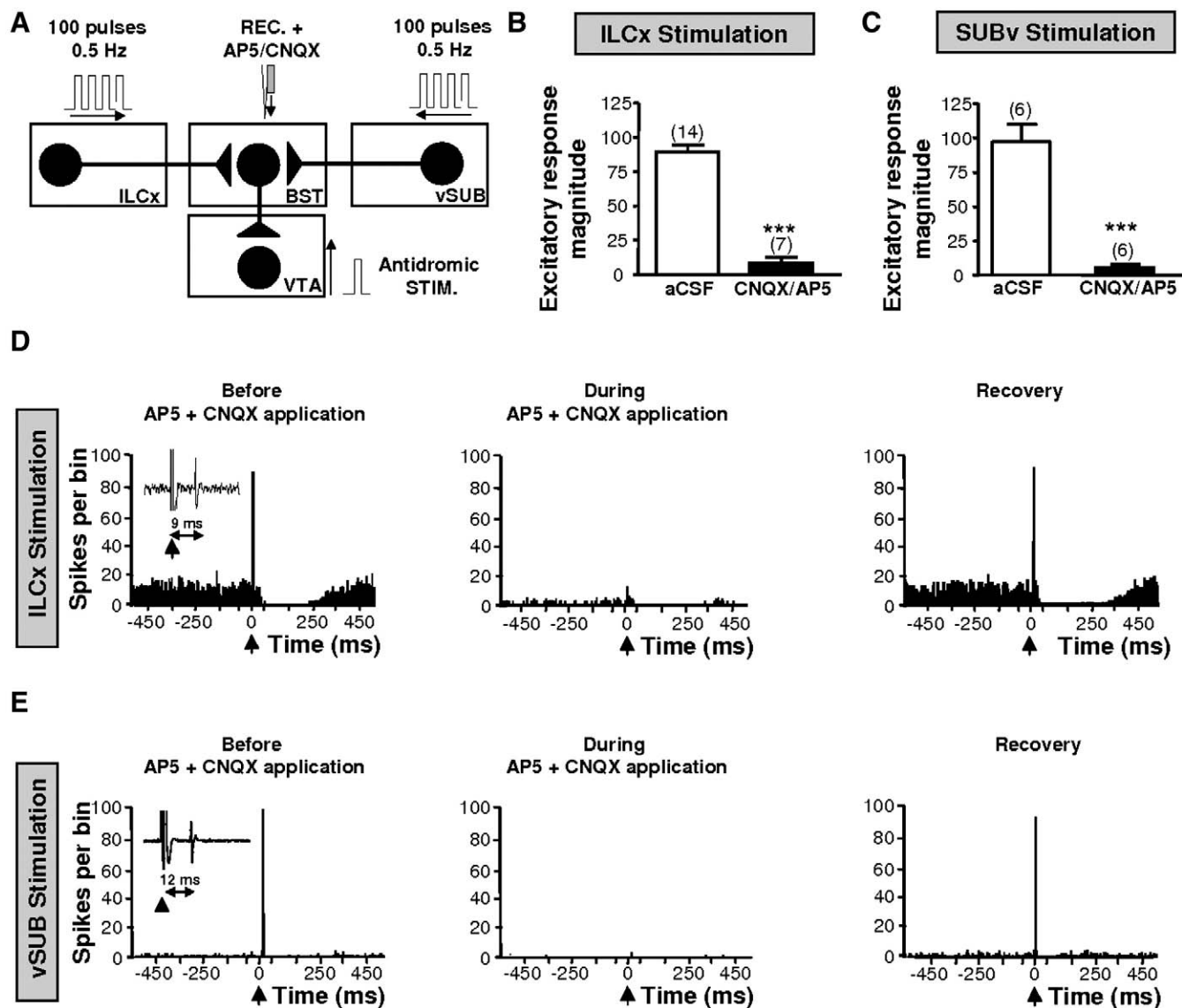
Fig. 4. Infusion of glutamatergic receptor antagonists within the BST blocks the excitatory drive from the ILCx or the vSUB on BST neurons that project to the VTA. A, Diagram of the stimulation protocol: ILCx or vSUB was stimulated with a 100-pulse train. A mixture of 100 µM amino-5-phosphonopentanoic acid (AP5) and 50 µM 6-cyano-7nitroquinoxaline-2,3-dione (CNQX) are microinfused through a pipette adjacent to the recording electrode. VTA-projecting BST neurons were identified after antidromic stimulation of the VTA. B and C: Graphs illustrating the effects of ionotropic glutamatergic (black bars) antagonists on excitation of BST neurons projecting to the VTA, after stimulation of the ILCx (B) or the vSUB (C). Scores are percentage ± SEM of baseline response magnitudes for VTA projecting BST neuronal responses evoked by ILCx or vSUB electrical stimulation during microinjection into the BST of aCSF (white bars), the mixture of 50 µM CNQX plus 100 µM AP5 (black bars). Numbers of neurons recorded in each group are mentioned in brackets above each histogram bar. A Student test for pairwise comparisons was performed for excitation. ***$p < 0.001$. D, Effect of the CNQX+AP5 cocktail on a characteristic BST neuron projecting to the VTA during ILCx electrical stimulation. Typical peristimulus time histograms (PSTHs) show ILCx-evoked responses before, during and after (recovery) CNQX+AP5 injection into the BST for the same BST neuron identified as projecting to the VTA. Inset in D shows orthodromic spikes evoked by stimulation of ILCx. Microinjection of CNQX+AP5 prevented the short latency activation of BST neurons evoked by ILCx stimulation, and decreased basal activity but had no effect on the inhibition. E, Effect of the CNQX+AP5 cocktail on a characteristic BST neuron projecting to the VTA during vSUB electrical stimulation. Typical PSTH show vSUB evoked responses before, during and after (recovery) CNQX+AP5 injection into the BST, for the same BST neuron identified as projecting to the VTA. Inset in E shows orthodromic spikes evoked by stimulation of vSUB. Microinjection of CNQX+AP5 prevented the short latency activation of BST neurons evoked by ILCx or vSUB stimulation, and decreased the basal activity but had no effect on the inhibition. BST, bed nucleus of the stria terminalis; ILCx, infralimbic cortex; REC, recording electrode; vSUB, ventral subiculum; VTA, ventral tegmental area; ac, anterior commissure; STIM, stimulating electrode. Adapted from Massi et al. (2008).

inhibitory responses (Table 1) may be mediated by co-release of an inhibitory transmitter from the cortical pyramidal neurons projecting from the ILCx to the BST or from cells nearby that are activated at an early latency. The prodynorphin immunoreactivity of pyramidal cells (Evans et al., 2007), together with the presence of kappa opioid receptor on BST neurons, support the role of the endogeneous opioid system in the inhibitory control of BST neurons (Mansour et al., 1996). The ILCx and the vSUB may also send glutamatergic afferents to GABA terminals that make synapses onto BST neurons. Accordingly, anatomical and pharmacological studies suggest that NMDA and non-NMDA receptors may act pre-synaptically to modulate release of GABA (Young and Bradford, 1993; Glitsch and Marty, 1999; Paquet and

Smith, 2000). However, such interactions between glutamatergic and GABAergic systems within the BST appears unlikely, as the evoked inhibition of BST neurons by ILCx stimulation is not affected neither by intra-BST infusion of GABA$_A$ or NMDA/AMPA/kainate antagonists (Massi et al., 2008). It remains unknown whether external GABAergic projections targeting the BST, GABA$_B$ receptors or other neurotransmitter systems like the endocannabionoid system could participate in the inhibitory response evoked by ILCx stimulation. Additional studies are needed to address this issue.

Excitatory afferents to the VTA are of diverse origins (Geisler et al., 2007) and they differentially target subpopulations of VTA DA neurons on the basis of their projections. The mesolimbic DA system

represents the vast majority of the VTA DA neurons and projects to limbic structures, including the NAc, the basolateral amygdala (BLA) and the BST (Marinelli et al., 2006). DA projections to these target areas are involved in the rewarding properties of nearly all drugs of abuse (Carboni et al., 2000; Koob and Le Moal, 2008; Wise, 2008). Carr and Sesack revealed that: 1) the majority of VTA DA neurons project to the NAc (65%–85%) and 2) the mPFC send excitatory glutamatergic projection to DA neurons in the VTA, which, in turn are connected in a reciprocal manner to the mPFC (Carr and Sesack, 2000). This reciprocal mesocortical pathway represents 30–40% of VTA DA neurons. The mPFC powerfully activates the VTA DA neurons by evoking bursting activity (Tong et al., 1996a,b). Although the mPFC has been shown to send an excitatory projection to the VTA, which synapses directly with DA neurons (Sesack and Pickel, 1992), it is not necessarily the case that the mPFC monosynapticaly activates the majority of VTA DA neurons (Tong et al., 1996b). Given the long latency of cortically induced bursts (122 ms), it seems likely that these events were produced by indirect excitatory inputs to the DA neurons (Tong et al., 1996b). We have tested the BST as a glutamatergic relay terminating in the VTA in a recent series of experiments. Our anatomical and *in vivo* electrophysiological evidence demonstrate that glutamatergic projections arising exclusively from the ILCx converge on BST neurons, which in turn project to and excite DA neurons (Figs. 2 and 3). We demonstrated that BST is necessary for the efficient relay of cortical excitation to DA neurons of the VTA (Massi et al., 2008). Thus, our finding that more than 80% of the VTA DA cells respond to ILCx stimulation with an increased spiking probability (Massi et al., 2008), strongly suggests that the BST processes, amplifies and actively distributes the excitatory drive from the ILCx to the majority of DA neurons. However, recent evidence with electrical stimulation of the PrL/ILCx region demonstrated a stronger short-latency (<25 sec) excitatory effect on VTA DA neurons than previously reported, indicating that direct effects of mPFC on VTA activity may also exist (Moorman and Aston-Jones, 2007).

The vSUB (Blaha et al., 1997; Legault and Wise, 1999; Legault et al., 2000; Taepavarapruk et al., 2000; Floresco et al., 2001) powerfully activates the DA system. Although the circuitry used by the vSUB to activate the DA system remains to be fully elucidated, the mechanisms proposed to account for this effect involve: 1) an increase of the population of active DA neurons in the VTA through a multisynaptic inhibition of GABAergic inputs to the VTA, and 2) a presynaptic modulation of DA release by the action of glutamatergic inputs arising from the vSUB. Conversely, in a related series of microdialysis and electrophysiological experiments, Wise and colleagues have shown that NMDA injections into the vSUB have a net excitatory effect on VTA dopaminergic transmission, increasing DA neurons firing and increasing both somatodendritic and terminal DA release (Legault and Wise, 1999). Additionally, ionotropic glutamate receptor activation in the VTA is necessary for vSUB stimulation to increase DA efflux in the NAc (Legault et al., 2000). Finally, intra-VTA perfusion of glutamatergic antagonists completely abolished vSUB-evoked DA release in the NAc (Legault et al., 2000). These experiments clearly demonstrated that the vSUB activates VTA DA neurons through a glutamatergic link terminating in the VTA. In light of these data, we propose a possible circuit involving projections from the vSUB to the BST. The BST, in turn, provides well-characterized excitatory inputs to the VTA (Georges and Aston-Jones, 2001, 2002; Massi et al., 2008).

3. Potent regulation of midbrain dopamine neurons by the BST

Midbrain DA neurons are importantly involved in most reward-directed behaviors, motivation and learning (Hyman and Malenka, 2001; Jones and Bonci, 2005; Chen et al., 2008). Furthermore, accumulating evidence indicates that increasing the glutamatergic drive enhances DA neuronal activity and produces the characteristic bursting pattern expressed *in vivo* (for review: (Marinelli et al., 2006).

This increase in bursting activity of VTA DA neurons has been shown to be important in mediating a number of motivated behaviors (Fields et al., 2007). Anatomically, BST neurons are in a good position to control reward-related behaviors. For instance, they innervate the DA-rich VTA (Phillipson, 1979; Georges and Aston-Jones, 2002; Dong and Swanson, 2006) and the NAc (Groenewegen and Russchen, 1984; Dong and Swanson, 2006), structures that drive behaviors motivated by natural or drug rewards. We (Georges and Aston-Jones, 2001, 2002) and others (Rainnie, 1999; Dumont and Williams, 2004; Dumont et al., 2005, 2008; Hammack et al., 2007; Kash et al., 2007) reported that a sub-population of BST neurons project to the VTA. We used antidromic activation of BST neurons following stimulation of the VTA and retrograde labeling between the BST and VTA (Fig. 5) to demonstrate the monosynaptic input from the BST to the VTA. A recent paper by Dumont et al. (2005) provides direct and specific evidence that increased glutamate receptor activity on the sub-population of BST neurons projecting to the VTA might be a key component of the limbic circuit underlying operant behaviors in relation to reward. Thus, while at present it remains difficult to predict the net functional and behavioral effects of activation of BST on target brain regions, our recent series of experiments (summarized in Fig. 6) illustrates potential mechanisms by which excitatory input from the BST enhances activity of VTA DA neurons (Georges and Aston-Jones, 2001, 2002).

The short latency excitations for many cells following BST electrical stimulation (<20 ms), and the distance between the BST and the VTA (approximately 6 mm), indicate that at least some of these responses are probably mediated by a direct input to the VTA from the BST (Fig. 6). Our anatomical data demonstrate a direct projection from the BST to the VTA, supporting this hypothesis (Fig. 5). Our results describing a similar activation of VTA DA neurons after microinjections of glutamate in the BST (Fig. 6), indicate that the activation of VTA DA neurons following electrical stimulation of the BST are mediated by activation of BST neurons and not by « en passant » fibers. Our electrophysiological and anatomical results (Figs. 5 and 6) revealed that neurons of the BST project directly to the VTA and exert a strong excitatory influence on DA neurons mediated by NMDA and AMPA glutamatergic receptors (Georges and Aston-Jones, 2001, 2002).

Furthermore, we investigated potential influence of the BST on burst activity of VTA DA neurons. Microinfusion of glutamate into the BST increased burst activity of DA neurons (Fig. 6), whereas microinfusion of GABA into the BST decreased burst activity in DA cells (Georges and Aston-Jones, 2002). These results reveal that the BST can tonically regulate the amount of bursting of VTA DA neurons. Striatal DA efflux has been shown to correlate strongly with DA neuron activity, and is highly dependent on the pattern of discharge (Marinelli et al., 2006). In fact, it has been shown that bursts of action potentials are twice as potent as the same number of regularly spaced spikes to trigger DA release (Suaud-Chagny et al., 1992). Thus, the tonic modulation of burst firing by the BST could be an important element in the regulation of the VTA DA system.

4. Role of BST in reward-related behaviors

4.1. Acute opiate withdrawal

The ventral BST (vBST) has a remarkably dense innervation by norepinephrine (NE) fibers (Phelix et al., 1992), which largely originate in the medullary A1 and A2 cell groups (Delfs et al., 2000). This indicated to us that the vBST is a possible site for the ability of NE drugs to alleviate opiate withdrawal. Indeed, we found that systemic administration of the beta NE antagonist propranolol decreased the strong Fos induction in vBST associated with acute morphine withdrawal (Aston-Jones et al., 1999). In addition, microinjections of selective beta NE antagonists, or the alpha2 NE agonist clonidine, into

vBST markedly attenuated the aversiveness of acute opiate withdrawal in rats (Delfs et al., 2000). Previous work established a role for BST in anxiety (Walker and Davis, 1997), and specifically for NE innervation in vBST with stress-induced anxiety (Cecchi et al., 2002). This led us to hypothesize that the aversiveness of opiate withdrawal involved, at least in part, anxiety generated by elevated NE inputs (Delfs et al., 2000; Smith and Aston-Jones, 2008). Mechanistically, NE acting in the BST may drive dysphoria by decreasing excitatory projections from the extended amygdala to its efferent targets,

including VTA. This effect may be achieved via NE actions to increase GABA and decrease glutamate inputs onto excitatory VTA-projecting neurons in BST. NE application to neurons in ventrolateral BST (with a physiological profile like that of VTA-projecting neurons) was found to cause increased GABA(A)-IPSC frequency during acute opiate withdrawal (Dumont and Williams, 2004). This effect was mediated through alpha-1 and beta adrenoceptor mechanisms, whereas NE effects were modulated only through alpha-1 mechanisms in naive animals (Dumont and Williams, 2004). These findings are consistent
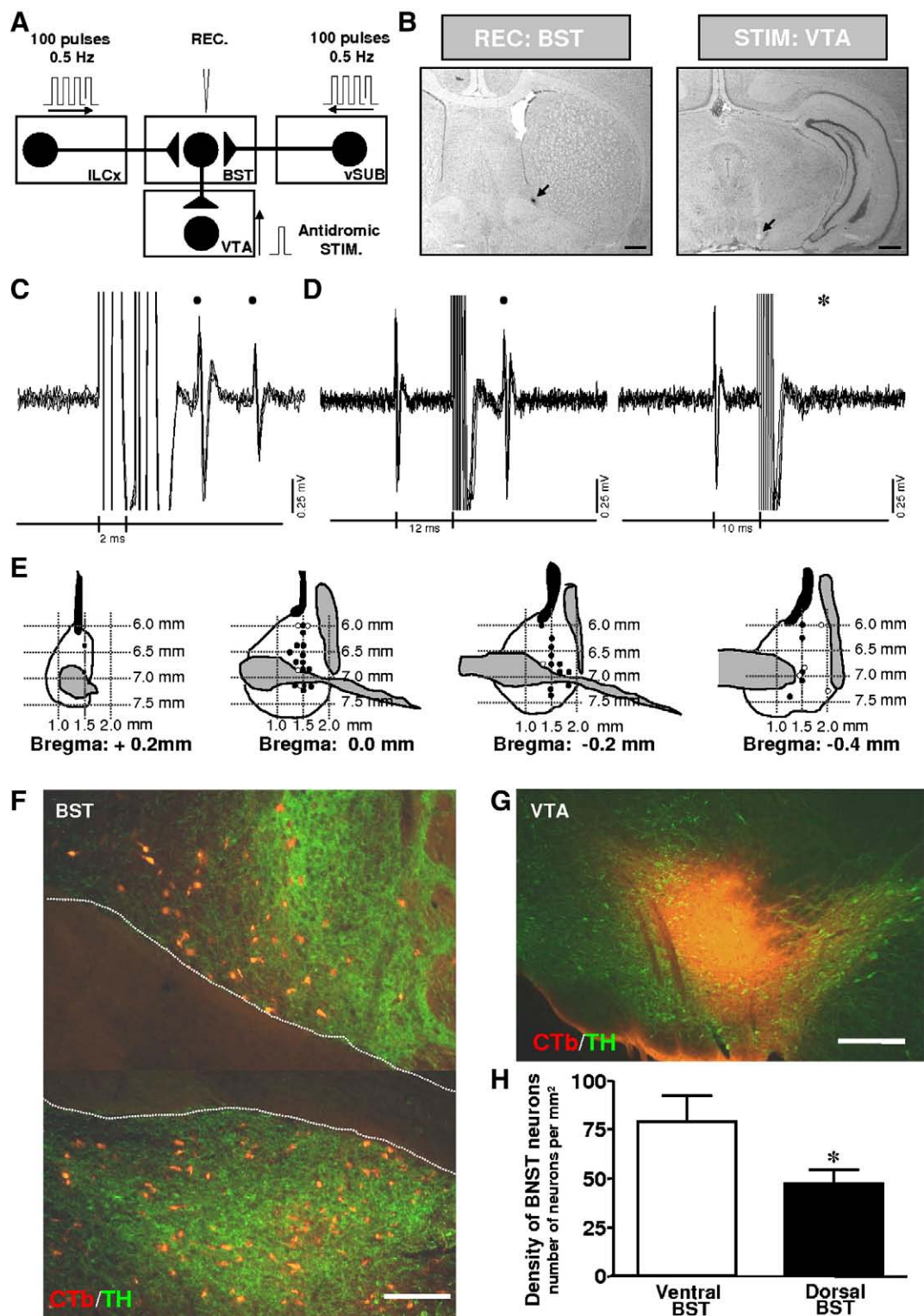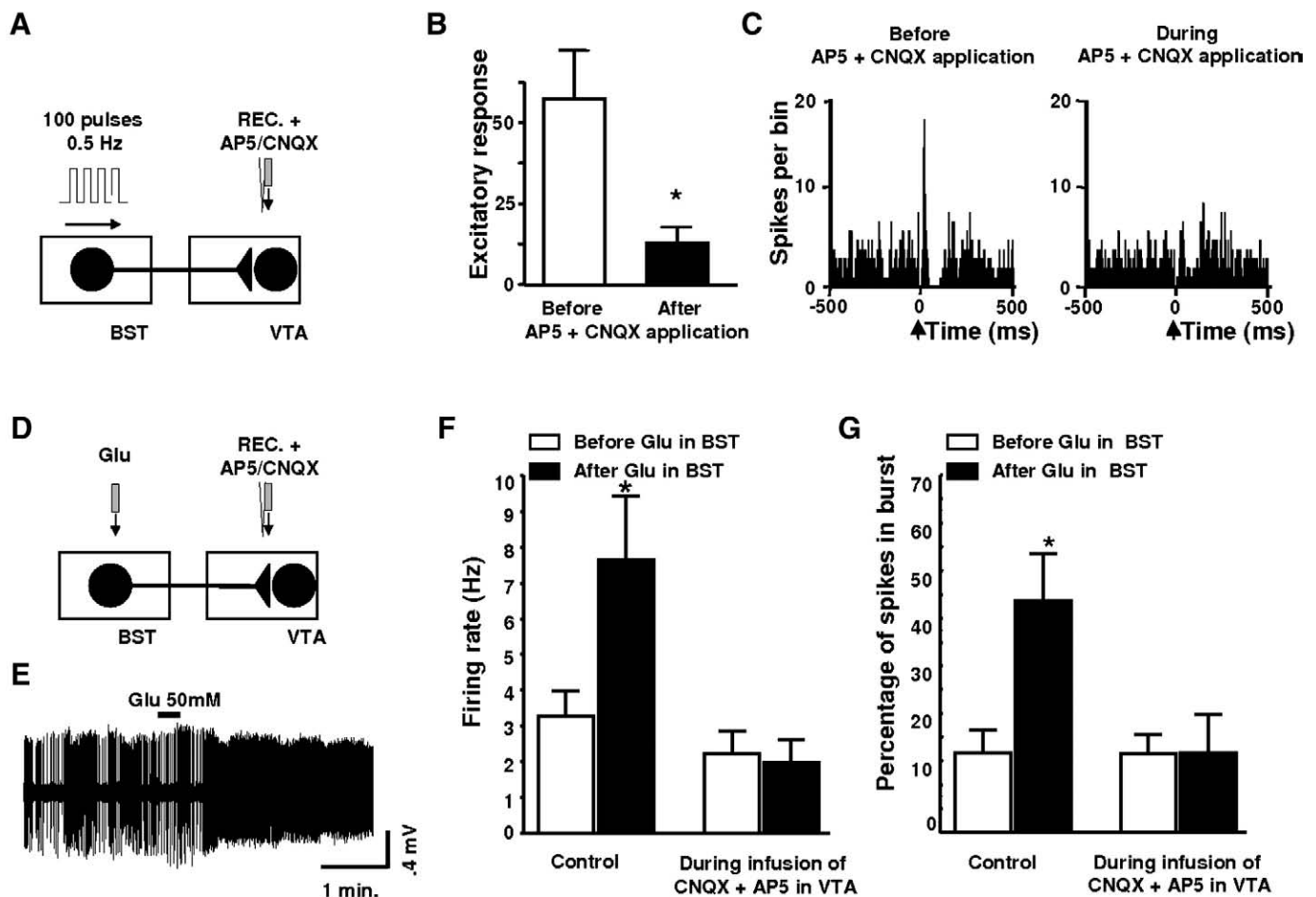
Fig. 6. Effects of AP5+CNQX on VTA DA neuronal responses evoked by BST electrical stimulation A, Diagram of the stimulation protocol used in this experiment. Here, the BST stimulated with a 100-pulse train. DA neurons were recorded within the VTA and the CNQX+AP5 cocktail was microinfused through a pipette adjacent to the recording electrode. B, Mean (±SEM) response magnitudes (Rmags) of VTA DA neuronal responses evoked by BST stimulation before (black bars) and during (white bars) microinjection of AP5 (100 μM) + CNQX (50 μM) into the VTA. Microinjection of AP5+CNQX prevented the short latency activation of DA VTA neurons evoked by BST stimulation. The same cells were used before and after drug application. A Student t-test for pairwise comparisons was performed. *$p<0.05$. C, PSTHs showing VTA evoked responses before and during drug injection into the VTA for a typical DA neurons. Single pulse stimuli (0.5 ms, 0.5/s) were delivered at time zero. D, Diagram of the stimulation protocol used in this experiment. Here, the BST stimulated with local infusion of glutamate (Glu, 50 mM, 60 nl). DA neurons were recorded within the VTA and the CNQX+AP5 cocktail was microinfused through a pipette adjacent to the recording electrode. E, Oscilloscope traces of a VTA DA neuron showing the typical firing activity before and after infusion of Glu at 50 mM into the BST. Glu injection is designated by the line above each trace. F and G, Effects of AP5+CNQX on VTA DA neuronal responses evoked by BST stimulation by Glu microinjection : Graphs comparing firing rate (F) and bursts (G) of VTA DA neurons before (white bars) and during local microinfusion of 50 mM CNQX + 100 mM AP5 into the VTA. Note that the CNQX/AP5 mixture blocked both the increase in bursting as well as the increased in firing rate of VTA DA neurons evoked by chemical stimulation of the BST. BST, bed nucleus of the stria terminalis; Glu, glutamate; REC, recording electrode; VTA, ventral tegmental area; STIM, stimulating electrode. Data were analyzed by two-way ANOVA. *$p<0.005$. Adapted from Georges and Aston-Jones (2001, 2002).

with those of Delfs et al. (2000) indicating that the aversiveness of opiate withdrawal is due to NE acting at beta adrenoceptors in BST. VTA may be influenced by the BST during acute withdrawal, as indicated by the finding that acute opiate withdrawal inhibited VTA

DA neuron firing and decreased extracellular DA in NAc, and that pretreatment with clonidine prevented these effects (Pothos et al., 1991; Spanagel et al., 1994; Diana et al., 1995; Georges and Aston-Jones, 2003). This inhibition of VTA DA output may contribute to withdrawal

Fig. 5. Projection from the BST to the VTA revealed by antidromic stimulation from the VTA and retrograde labeling in the BST after injection of CTb into the VTA. A, Diagram of the stimulation protocol used in this experiment. Here, the ILCx or vSUB are stimulated with a 100-pulse train, and BST neurons projecting to the VTA were identified by antidromic activation from the VTA. B, Left, Recording location for a BST neuron projecting to the VTA (dark spot, black arrow). Right, electrical stimulation site in the VTA (lesioned area, black arrow). Scale bars: 0.5 mm. C and D: Five superimposed traces illustrating high-frequency activation and collision test for a BST cell driven from the VTA. C, Driven spikes (black circles) elicited by each of paired stimuli (vertical lines, 2 ms interpulse interval), indicating frequency following for this cell at 500 Hz. D, Left, Stimulation of the VTA 12 ms after spontaneous spikes (left side of traces) elicit driven spikes (black circle) at 9.5 ms latency. Right, Driven spikes are occluded for similar stimuli delivered 10 ms after spontaneous impulses indicating collision between spontaneous and driven spikes. The asterisk indicates when driven spikes would have occurred in the absence of collision. E, Schematic presentation of neuronal recording sites in the BST of neurons projecting to the VTA. Black circles: locations of synaptically driven BST neurons by single-pulse ILCx electrical stimulation. White circles: locations of synaptically driven BST neurons by single-pulse vSUB electrical stimulation. Numbers refer to stereotaxic coordinates. Note that all the BST neurons projecting to the VTA tested were driven by ILCx or vSUB stimulations. F–H, Projection from the BST to the VTA revealed by retrograde labeling in the BST after injection of CTb into the VTA. F, Epifluorescence photomicrographs illustrating retrograde labeling in the dorsal and ventral parts of the BST after CTb injection into the VTA. G, Photomicrograph illustrating a representative CTb injection site in the VTA. The sections have been processed dually for CTb (red) and tyrosine hydroxylase (TH; green). Scale bars: F, 100 μm; G, 200 μm. H, Bar histograms comparing the density of CTb-immunoreactive neurons in the dorsal and ventral BST after an injection of CTb in the VTA ($n = 15$ rats perfused 7 days after receiving CTb into the VTA). t-test was used to establish statistical differences between the dorsal and the ventral part of the BST. BST, bed nucleus of the stria terminalis; ILCx, infralimbic cortex; REC, recording electrode; vSUB, ventral subiculum; VTA, ventral tegmental area; ac, anterior commissure; STIM, stimulating electrode. Adapted from Massi et al. (2008). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

effects, as indicated by the finding that D2 agonist injection into NAc decreased opiate withdrawal behaviors (Harris and Aston-Jones, 1994).

4.2. Enhanced drug preference during protracted withdrawal

Prolonged exposure to drugs causes neuronal and behavioral changes that exist long after symptoms of acute withdrawal have dissipated. These alterations cause a variety of maladaptive effects, including anxiety, vulnerability to stress-induced relapse, and enhanced drug-seeking. A series of experiments from our laboratory have shown that post-dependent animals process reward-related stimuli in an aberrant manner, such that conditioned place preference (CPP) is increased for stimuli associated with morphine or cocaine, whereas CPP for natural rewards such as food or novel objects is less than normal (Harris et al., 2001; Harris and Aston-Jones, 2003b, 2007).

To determine whether the extended amygdala plays a role in the enhanced preference observed in CPP tests during protracted morphine abstinence, neuronal activity was investigated using immunohistochemistry for the immediate early gene protein Fos. For these experiments, animals were sacrificed 2 h following the drug-free CPP test session, so that Fos expression reflects re-exposure to, and conditioned response to, the drug-paired environment. Elevated Fos was observed in anterior cingulate, NAc core and shell, ventral BST, BLA and central nucleus of the amygdala (CeA), lateral hypothalamus and nucleus of the tractus solitatius (NTS) in post-dependent rats after five weeks of protracted abstinence, as compared to non-dependent and non-conditioned rats (Harris and Aston-Jones, 2003b, 2007; Aston-Jones and Harris, 2004). Fos in anterior cingulate and BLA correlated with CPP in all conditioned animals; however, only Fos in BST also correlated with CPP in post-dependent rats (Harris and Aston-Jones, 2003a). This indicates that activation in BST is uniquely associated with the elevated drug preference found during protracted abstinence. These data, in view of the strong NE innervation and effects in BST, led our lab to hypothesize that drug-conditioned stimuli

can activate A1 and A2 NE neurons that release NE into BST. This was proposed to cause increased anxiety and produce negative reinforcement for drug rewards given during conditioning sessions. These negatively reinforcing effects were proposed to summate with positively reinforcing effects to increase motivation to seek out drugs (Aston-Jones and Harris, 2004; Smith and Aston-Jones, 2008).

Animals also exhibited decreased CPP for food or novel objects during protracted morphine abstinence. Food CPP in previously morphine-dependent rats was negatively correlated with Fos expression in CeA, ventrolateral BST and NTS (Harris and Aston-Jones, 2007). This, in view of the increased drug preference (described above), indicates that Fos activation in BST and CeA in the extended amygdala, and their major NE afferent in NTS, is associated with enhanced drug preference and decreased food preference. This indicates that increased activity in these stress systems may decrease the rewarding properties of natural rewards, while increasing the reward associated with drug rewards. These findings add to others reviewed above indicating that activation in extended amygdala areas, including the BST, is strongly associated with altered reward processing during protracted abstinence.

5. Potential functional significance

The BST plays critical roles in responses to both stress/anxiety and reward through mechanisms that involve excitatory glutamatergic transmission (Winder et al., 2002; Kash and Winder, 2006). Dumont and collaborators highlighted the role of increased excitatory synaptic transmission in the BST in both cocaine and food self-administration (Dumont et al., 2005). The BST plays an important role in addictive behaviors such as drug-seeking (Harris and Aston-Jones, 2007) and stress-induced relapse to cocaine (Erb et al., 2001). Activation of the BST neurons projecting to the VTA seems to be necessary for learning to associate drug rewards with specific environmental cues (Dumont et al., 2005). Although the DA system is classically associated with reward (Schultz, 2007), goal-directed behaviors can be altered by
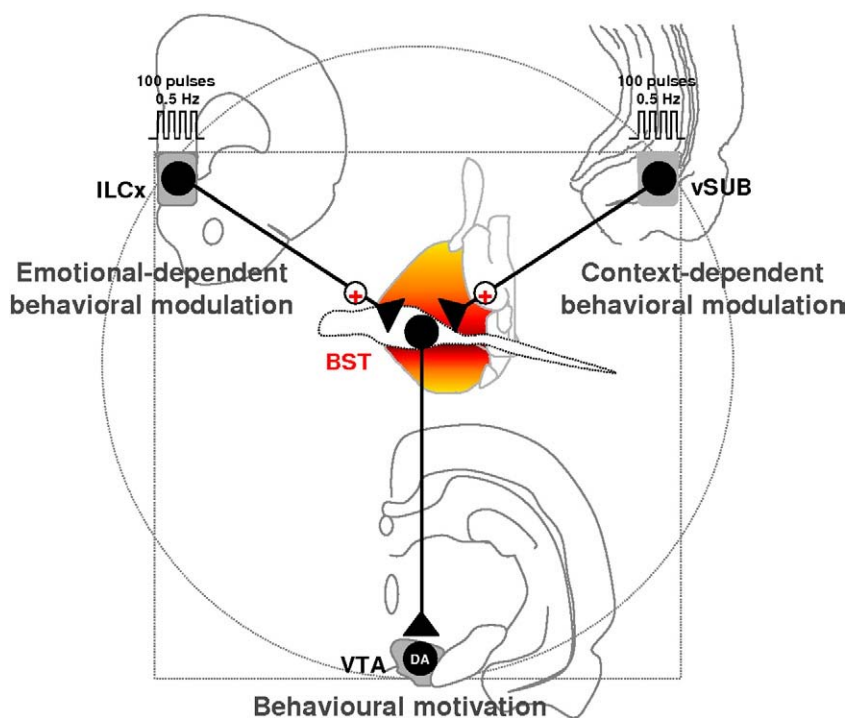

Fig. 7. We propose a model to describe the neural circuitries by which the ILCx and vSUB modulate the BST neurons projecting to the VTA. Electrical stimulation of the ILCx and vSUB activate excitatory afferents to the BST which in turn activates BST neurons projecting to the VTA. As a result, we recently demonstrated that activation of the ILCx produces phasic activation of midbrain DA neurons. In this model we propose that the ILCx-BST and vSUB-BST projections provide sufficient excitatory influence to activate the sub-population of BST neurons projecting to the VTA. BST, bed nucleus of the stria terminalis; ILCx, infralimbic cortex; VTA, ventral tegmental area; DA, dopaminergic neurons.

adding a stressful component (Ghiglieri et al., 1997; Phillips and Barr, 1997; Bowers et al., 1999; Calvo-Torrent et al., 1999). This indicates that final behavioral outcomes result from interactions between stressful and rewarding stimuli. The BST, driven by the ILCx and the vSUB may be involved in processing both emotional- and context-dependent stimuli, which in turn influence motivated-behaviors through the DA system (Fig. 7). Peters and colleagues recently reviewed convincing evidences showing that the ILCx regulates the expression of both fear and drug memories after extinction, through divergent projections to the amygdala and NAc respectively (Peters et al., 2009). We propose that the BST could be part of parallel circuit adjusting the impact of events coming from the ILCx or the vSUB in order to filter and to amplify the excitatory drive on DA neurons. The BST, with its roles in both stress activation and reward based learning and memory, could provide an important target for future pharma-cotherapies designed to prevent pathologies associated with reward/motivation circuitry.

Acknowledgements

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