Published in final edited form as:

Neuroscience. 2008 April 22; 153(1): 232–239. doi:10.1016/j.neuroscience.2008.01.039.

MORPHINE PRODUCES CIRCUIT-SPECIFIC NEUROPLASTICITY IN THE BED NUCLEUS OF THE STRIA TERMINALIS

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Abstract

The bed nucleus of the stria terminalis (BST) is a brain structure located at the interface of the cortex and the cerebrospinal trunk. The BST is a cluster of nuclei organized in a complex intrinsic network that receives inputs from cortical and subcortical sources, and that sends a widespread top-down projection. There is growing evidence that the BST is a key component in the neurobiological basis of substance abuse. In the present study, the regulation of excitatory inputs onto identified neurons in the BST was examined in rats treated chronically with morphine. Neurons projecting to the ventral tegmental area (VTA) were identified by retrograde transport of fluorescent microspheres and recorded in the whole-cell voltage clamp configuration in brain slices. Selective excitatory inputs to these neurons were electrically evoked with electrodes placed in the medial and lateral aspects of the dorsal BST. The chronic morphine treatment selectively increased AMPA-dependent excitatory postsynaptic currents in a subset of inputs activated by dorso-lateral stimulation in the BST. Inputs activated by medial stimulation were not affected by morphine. Likewise, the inputs to neurons that did not project to the VTA were not changed by morphine. Altogether, these results extend the understanding of neuronal circuits intrinsically sensitive to drugs of abuse within the BST.

Keywords

bed nucleus of the stria terminalis; BST; electrophysiology; morphine; AMPA; NMDA

The bed nucleus of the stria terminalis (BST) is a cluster of 11 nuclei surrounding the caudal part of the anterior commissure. An increasing number of anatomical and behavioral observations suggest that the BST is part of a system that coordinates autonomic, neuroendocrine, and behavioral functions, particularly with regards to energy balance, defense, and reproduction (Dong et al., 2000, 2001a,b; Dong and Swanson, 2003, 2004a,b, 2006a,b,c). The projection pattern of the BST is widespread but primarily directed toward the cerebrospinal trunk with more limited projections to cortical, striatal and pallidal targets. The BST projects to the nucleus accumbens and the ventral tegmental area (VTA), suggesting a role in goal-directed behaviors. In fact, plasticity at excitatory synapses within the BST correlates with operant learning, supporting a role of the BST in goal-directed behaviors toward natural rewards (Dumont et al., 2005). Likewise the BST may be part of the neurocircuitry underlying addiction since it contributes to the reinforcing properties of

several drugs of abuse (Walker et al., 2000; Fudge and Haber, 2001; Georges and Aston-Jones, 2002; Dumont et al., 2005). In addition, the BST is a key contributor in negative reinforcement, where it is thought to mediate aversive aspects of drug withdrawal resulting in drug seeking in dependent animals (Delfs et al., 2000; Aston-Jones and Harris, 2004; Georges et al., 2005).

The lateral region of the anterior bed nucleus of the stria terminalis (alBST) is an important component of the brain motivation pathways. The alBST sends a monosynaptic excitatory output to dopamine neurons in the VTA (Georges and Aston-Jones, 2002; Dumont and Williams, 2004); excitatory synapses onto this subgroup of VTA-projecting neurons show an increase in strength in rats trained to self-administer cocaine (Dumont et al., 2005). This suggests that the regulation of synaptic inputs onto alBST projection neurons can influence the reward pathways, perhaps by synaptic activation of dopamine neurons.

The BST receives bottom-up and top-down information respectively from the cerebrospinal trunk and the cortex. Cortical inputs to the BST are particularly intriguing since they are topographically organized (McDonald, 1998). Specifically, afferents from the insula and the amygdalopiriform area enter the laterodorsal aspect of the BST whereas inputs from the ventral entorhinal, ventral subiculum, and infralimbic regions reach the BST medially (McDonald, 1998). Given that cortical regions, such as the insula and infralimbic cortex, are circuits that mediate components of addiction, the BST could serve as an interface between the cortex and lower regions of the brain (Bechara and Damasio, 2002; Kalivas, 2004; Contreras et al., 2007).

The objective of the present study was to determine if excitatory inputs to the BST were altered in morphine-dependent rats. Whole-cell patch-clamp recordings were made from alBST neurons in brain slices and excitatory post-synaptic currents (EPSCs) were evoked by stimulation of distinct afferent inputs. Morphine selectively strengthened a population of excitatory fibers entering the dorso-lateral BST and making synapses on a subpopulation of alBST neurons projecting to the VTA. This circuit-specific effect of morphine in the BST extends our understanding of brain pathways involved in the addictive properties of drugs of abuse such as opioids.

EXPERIMENTAL PROCEDURES

Identification of BST neurons projecting to the VTA

The alBST is a complex region of the brain with at least three distinct subpopulations of neurons (Dumont and Williams, 2004; Hammack et al., 2007). In order to study BST neurons intrinsic to the neurocircuitry of goal-directed behaviors, fluorescent microspheres were stereotactically microinjected (10 nl, 0.04 μ m, 488/560 EX/EM, www.invitrogen.com) into the VTA (−5.3 mm from bregma, 0.8 mm lateral, vertical 7.8 mm vertical (Swanson, 2005)) of 20 rats (Sprague–Dawley, 38–51 days, 126–150 g, www.criver.com). During the procedure, the rats were deeply anesthetized with isoflurane (5% isoflurane V/V with O₂, 100 ml/min, www.bensonmedical.ca). Pain and inflammation were minimized with Anafen™ (1 mg/kg, s.c., www.cdmv.com) pre-operatively and twice daily for 2 days following surgery. In addition, topical Xylocaine-jelly™ (lidocaine-HCl 2%, www.cdmv.com) was applied on the surgical incision immediately post-operatively. Animal protocols were approved by the Queen's University Animal Care Committee in accordance with the guidelines set by the Canadian Council on Animal Care. All efforts were made to ensure that the number of animals used and suffering was kept to a minimum.

Morphine treatment

Thirty rats (including those with intra-cranial micro-injections) were randomly assigned to placebo or morphine groups. The rats were anesthetized with isoflurane (5% isoflurane V/V with O₂, 100 ml/min) and received placebo- or morphine-containing s.c. pellet (75 mg morphine, National Institute on Drug Abuse) implants. The morphine treatment consisted of one pellet on day 1 and two pellets on days 3 and 5; experiments were done on days 6 or 7.

Brain slices preparation

Twenty-four hours after the last treatment, the rats were deeply anesthetized (isoflurane) and killed for brain slice preparation. Coronal slices (250 μ m) containing the alBST were prepared in a physiological solution containing (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 1.2 NaH₂PO₄, 25 NaHCO₃, 11 D-glucose, and morphine sulfate 0.001 at 15 °C. The slices were incubated at 34 °C for 30 min and transferred to a chamber constantly irrigated (1.5 ml/min) with the physiological solution maintained at 34 °C and equilibrated with 95% O₂/5% CO₂. Whole-cell voltage-clamp recordings were made in the ventral part of the alBST (Bregma -0.26 mm, Fig. 1A), using glass microelectrodes (2–5 M Ω) filled with a solution containing (in mM) 130 Cs⁺MeSO₃⁻, 4 NaCl, 1 MgCl₂, 1 EGTA, 10 Hepes, 2 MgATP, and 0.3 GTP. The recordings were done under visual guidance using an upright transmitted light microscope (BX-51W1, www.olympus.com) equipped with Dodt-Gradient-Contrast-System (www.Luigs-Neumann.com). The microscope was also equipped with an X-Cite 120 fluorescence illumination system (EXFO Life Sciences & Industrial Division, www.exfo-lifesciences.com) to visualize neurons labeled with fluorescent beads. Recordings were made using a Multiclamp 700B amplifier and a Digidata 1440A (www.mds.com). Data were acquired and analyzed with Axograph X (www.axographx.com) running on an Apple computer (www.apple.com).

Evoked EPSCs

EPSCs were evoked by local stimulation (two pulses at 40 Hz, 0.1ms duration, 0.1 Hz) using bipolar tungsten electrodes in the presence of picrotoxin (100 μ M) while neurons were voltage-clamped at -60 mV. Stimulation intensity was adjusted to obtain approximately 80% of the maximum response. Neurons were then voltage-clamped at +40 mV to release Mg²⁺-induced blockade of NMDA receptors. After a stabilization period of 5–15 min, the NMDA receptor antagonist D-AP5 (5 min, 50 μ M) was bath-applied to isolate AMPA currents. Isolated AMPA currents were digitally subtracted offline from the total EPSC to obtain the NMDA currents and compute AMPA to NMDA ratios. The addition of naloxone (1 μ M) during the recordings precipitated morphine withdrawal. At the end of each experiment, NBQX (5 μ M) and D-AP5 (50 μ M) were added to completely abolish all EPSCs.

Isolation of multiple excitatory inputs to the alBST

To stimulate multiple excitatory inputs to the alBST, multiple bipolar stimulating electrodes were placed in the dorso-medial ($\pm$ 1.5 mm from recording), dorso-lateral ($\pm$ 1.5 mm from recording), and ventro-lateral ($\pm$ 0.5 mm from recording) aspects of the anterior BST. With this configuration, two electrical pulses could be evoked and paired-pulse ratios (PPR) calculated from a single or from two different stimulation sites during recordings from a single neuron. We hypothesized that fiber stimulation in the immediate vicinity of the recorded neuron recruited a mixed population of excitatory inputs. Stimulation from remote sites in the dorsal alBST produced EPSCs with longer latencies. Short-term plasticity of excitatory synapses was measured using a paired-pulse protocol (two stimuli applied at 40 Hz) in an attempt to isolate various excitatory inputs to the alBST. Two stimuli from a single stimulation site always produced facilitation (PPR >1 when stimulus two was divided by

stimulus 1). The absence of cross short-term plasticity between two stimuli (40 Hz) from two different stimulation sites reflected the recruitment of independent excitatory inputs to the alBST.

Drugs

Stock solution of NBQX (50 mM) was prepared in DMSO (100%) and further diluted in the physiological solution at the desired concentration. DMSO concentration never exceeded 0.001%. Stock solutions of morphine (10 mM), naloxone (10 mM), picrotoxin (100 mM), and AP-5 (10 mM) were prepared in water and dissolved in the physiological solution at the desired concentration. All drugs were obtained from Sigma-Aldrich (www.sigmaaldrich.com).

RESULTS

Retrograde tracer from the VTA labeled a population of anterior BST neurons

Consistent with previously published observations (Dumont and Williams, 2004), microinjections of fluorescent microspheres into the VTA produced retrograde labeling in the alBST (Fig. 1B). Eighteen out of 20 microinjections were restricted to the VTA and in all cases produced retrograde labeling in the BST (Fig. 1B). In the remaining two cases, the microinjections were above the VTA and did not produce labeling in the BST. As previously reported, BST neurons projecting to the VTA shared common electrophysiological properties when submitted to a series of voltage-steps in the whole-cell voltage clamp configuration, including a time-independent and barium-sensitive inwardly rectifying conductance at hyperpolarized membrane potentials (Fig. 1C, left). These neurons were similar to type III alBST neurons of Hammack et al. (2007). As previously reported, we also recorded from a subpopulation of alBST neurons that were never labeled with the tracer. These neurons displayed a time-dependent sag (I_h) in the inward direction at hyperpolarized membrane voltages (Fig. 1C, right) and likely correspond to type I and/or type II of Hammack et al. (2007).

Chronic morphine increases AMPA currents in VTA-projecting alBST neurons

Local stimulation in the alBST evoked fast EPSCs with both AMPA and NMDA glutamate ionotropic receptor-mediated components in both subpopulations of neurons (Fig. 2B). In VTA-projecting neurons, chronic morphine doubled AMPA EPSCs (81 ± 8 pA vs. 39 ± 3 pA, $P < 0.05$, $n = 10$ neurons per group) whereas NMDA EPSCs remained unchanged (53 ± 8 pA vs. 51 ± 3 pA, $P > 0.05$, $n = 10$ neurons per group). As a result, the ratio of AMPA to NMDA currents increased significantly after the chronic morphine treatment (1.57 ± 0.1 vs. 0.76 ± 0.04 , $P < 0.05$, $n = 10$ neurons per group, Fig. 2B, C). The PPR was not affected by chronic morphine suggesting that the increase in AMPA EPSCs resulted from a postsynaptic mechanism (Fig. 2C1).

In contrast with VTA-projecting neurons, morphine did not alter AMPA or NMDA EPSCs in the second subpopulation of alBST neurons (Fig. 2C). There was no change in the kinetics of AMPA or NMDA-dependent EPSCs. Rise-times of AMPA (2.44 ± 0.4 and 2.55 ± 0.4 ms, placebo and morphine-treated, respectively) and NMDA (16.6 ± 0.9 and 12 ± 1.1 ms, placebo and morphine-treated, $n = 10$ neurons per group) EPSCs were statistically comparable. Similarly, the decay to 90% of the peak was not different between the placebo and morphine-treated groups for both AMPA (8.1 ± 1.0 and 7.5 ± 1.1 ms, placebo and morphine-treated, $n = 10$ neurons per group) and NMDA EPSCs (108.5 ± 21 and 89 ± 17 ms, placebo vs. morphine-treated, $n = 10$ neurons per group). Finally, the morphine-induced increase in AMPA EPSC measured in VTA-projecting neurons was not changed when acute

morphine withdrawal was precipitated by bath-application of naloxone (AMPA/NMDA=1.51±0.1, $P>0.05$, $n=10$ neuron per group, Fig. 1C).

Excitatory inputs onto projection neurons were distinguished

Placing stimulating electrodes in various regions of the BST allowed isolation of independent excitatory inputs to alBST VTA-projecting neurons (Fig. 3). Consistent EPSCs could be evoked through local stimulation in the ventral aspect of the alBST, close to the recorded neurons, as well as by stimulation in the lateral or medial regions of the dorsal BST (Fig. 3). Paired-pulse facilitation was observed with stimulation at all three stimulation sites (two pulses at 40 Hz to the same site, Fig. 3, left traces). However, pairing two pulses (at 40 Hz) from two different sites revealed evidence of independence between excitatory inputs entering the BST in its lateral and medial dorsal aspects. Pairing a lateral with a medial stimulation (or vice versa) did not produce short-term plasticity (facilitation or depression) suggesting independent populations of synapses (Fig. 3B, right traces). In contrast, paired-pulse facilitation was measured when a stimulus from the lateral BST was paired with a local stimulation in the ventral alBST (Fig. 3C, right traces). This suggests that stimulation of local fibers in the alBST recruited a mix of excitatory inputs to the BST, including those entering the lateral part of the dorsal BST.

Chronic morphine increased excitatory inputs activated by dorso-lateral stimulation

With the ability to distinguish separate inputs to projection neurons in the BST, the regulation of these inputs was examined after the chronic morphine treatment. EPSCs evoked from both dorso-lateral and dorso-medial stimulations were sequentially evoked (1.6 Hz) while neurons were voltage-clamped at +40 mV. In slices from morphine-treated rats, dorso-lateral stimulation resulted in an increase in the AMPA to NMDA ratio (1.5±0.06 vs. 0.81±0.05, $P<0.05$, morphine and placebo, respectively, $n=8$ neurons per group, Fig. 4). This change resulted from an increase in AMPA currents (80±11 pA vs. 51±5, $P<0.05$, morphine vs. placebo, respectively, $n=8$ neurons per group) and not from a decrease in NMDA currents (50±10 pA vs. 53±11, morphine vs. placebo, $n=8$ neurons per group). There was no change in the kinetics of the AMPA or NMDA EPSCs (AMPA rise: placebo 2.18±0.9, morphine 2.21±0.6 ms, decay to 90%: placebo 10.1±0.7, morphine 8.5±0.9 ms; NMDA rise: placebo 9.85±1.2, morphine 10.9±1.3 ms; decay to 90%: placebo 101.1±22, morphine 92±14 ms).

Stimulation from the dorso-medial position evoked EPSCs that were not different in slices from placebo- and morphine-treated rats. The AMPA to NMDA ratios were the same in slices from placebo and morphine-treated rats (morphine 0.9±0.1, placebo 1.0±0.1, $n=8$ neurons per group, Fig. 4). AMPA and NMDA currents were comparable (Fig. 4D). The kinetics of AMPA and NMDA EPSCs were also not modified by the morphine treatment.

DISCUSSION

In the present study, we observed that a five-day morphine treatment produced plasticity of alBST excitatory synapses. This neuroplasticity was both input- and output-specific: only a subset of excitatory inputs entering the latero-dorsal BST (making synapses onto a subpopulation of VTA-projecting alBST neurons) was strengthened by the morphine treatment.

Plasticity at excitatory synapses is a primary mechanism for memory formation in many areas of the brain, including the motivation and reward pathways. Natural rewards and drugs of abuse can both produce plasticity of excitatory synapses in multiple sites within the brain reward pathways (Wolf, 2002; Gerdeman et al., 2003; Kauer, 2004; Kelley, 2004; Robinson

and Kolb, 2004; Jones and Bonci, 2005; Hyman et al., 2006). Drug-induced plasticity is, however, a pathological form of memory formation that may contribute to the addictive properties of these drugs. Psychostimulants (Ungless et al., 2001; Dumont et al., 2005), opioids (Saal et al., 2003), cannabinoids (Mato et al., 2004), and alcohol (Melis et al., 2002; Weitlauf et al., 2004) all produce plasticity at excitatory synapses in various structures of the brain reward pathways. The VTA (Ungless et al., 2001; Melis et al., 2002), the nucleus accumbens (Thomas et al., 2001; Mato et al., 2004; Kourrich et al., 2007), and the BST (Weitlauf et al., 2004; Dumont et al., 2005) are all structures of brain reward pathways where drugs of abuse produce plasticity at excitatory synapses. The BST is a key brain structure within the reward pathways since it contributes to both the rewarding and the negative affective states related to substance abuse (Delfs et al., 2000; Walker et al., 2000; Erb et al., 2001; Leri et al., 2002; Harris and Aston-Jones, 2003; Dumont and Williams, 2004; Dumont et al., 2005). Chronic treatments with psychostimulants or alcohol produce plasticity at excitatory synapses in the BST, further suggesting that this structure is part of the circuitry involved in drug-associated neuroadaptations (Weitlauf et al., 2004; Dumont et al., 2005). The BST may also be involved in incentive learning since there is a clear increase in AMPA EPSCs in the BST of rats that self-administer rewards (cocaine or palatable food) but not in those receiving rewards passively (yoked controls) (Dumont et al., 2005). In the present study, a chronic morphine treatment also strengthened excitatory synapses in the BST although the morphine was administered passively. This disparity between the effects of opioids and psychostimulants is currently unknown but may be secondary to different mechanisms of action, the various routes of administration of the drugs, or a combination of both factors. Nonetheless, the morphine-induced BST neuroplasticity herein measured supports a role for long-term associative memory in the pathology of addiction. The morphine-induced increase in the peak amplitude of AMPA EPSCs was a postsynaptic effect since NMDA-dependent EPSCs were not affected. The exact mechanism of drug-induced changes in AMPA currents is not known although there were no changes in the kinetics of AMPA currents, which is consistent with other observations (Ungless et al., 2001; Borgland et al., 2004; Kourrich et al., 2007). This suggests the recruitment of more AMPA receptors to the membrane rather than intrinsic changes in the activity of AMPA channels, although this necessitates further investigation.

A key finding of the present study was the specificity of the effects of morphine on excitatory transmission in the BST. The effect of chronic morphine treatment was specific to excitatory synapses innervating a subpopulation of alBST neurons that project to dopamine-rich regions in the VTA. In contrast, excitatory synapses innervating a separate group of anatomically and physiologically identifiable neurons in the alBST were insensitive to the morphine treatment. Evidence suggests that alBST neurons projecting to the VTA are glutamatergic, sending an excitatory signal to dopamine neurons (Georges and Aston-Jones, 2002). Thus, the alBST neurons projecting to the VTA would be an intrinsic part of an excitatory circuit contributing to morphine-induced activation of dopamine neurons (Nowycky et al., 1978; Johnson and North, 1992; Grant and Sonti, 1994).

In addition, the effect of chronic morphine on excitatory transmission in the BST was input-specific. The BST receives multiple excitatory inputs from various brain regions (e.g. cortex, thalamus, amygdala) (McDonald, 1998; Dong et al., 2001a; Li and Kirouac, 2008). Furthermore, excitatory inputs are topographically organized in the BST and in some cases, clearly delineated in the medio-lateral axes in the anterior BST (McDonald, 1998; Dong et al., 2001a). This topographical organization of excitatory inputs was exploited by placing stimulating electrodes in both the medial and lateral regions of the dorsal BST in order to study independent excitatory inputs. Medial and lateral excitatory inputs were independent because no cross-short-term plasticity was detected. In contrast, pairing either of the dorsal stimulation sites (medial or lateral) with local stimulation in the ventral alBST produced

paired-pulse facilitation. This suggests that inputs entering the lateral and the medial BST ultimately converge on alBST neurons. Examples of excitatory inputs specific to the lateral part of the BST include cortical afferents from the insula, a cortical region critically involved in addiction (McDonald, 1998; Contreras et al., 2007; Naqvi et al., 2007). Our results showed a functional dissociation between excitatory fibers entering the lateral and medial aspects of the BST that correlates anatomical observations. However, further experimentation is required to unequivocally identify the origin of these inputs. For example, sagittal or horizontal preparation might allow stimulating directly within cortical areas and record excitatory transmission in the BST.

The mechanism(s) underlying the selective effect of morphine on the latero-dorsal inputs to alBST projection neurons is currently unknown. However, the dorso-lateral BST receives a very dense dopamine projection as demonstrated by intense TH-positive fibers (Deutch et al., 1988; Freedman and Cassell, 1994; Hasue and Shammah-Lagnado, 2002). This suggests a potential feed-forward excitatory loop between the BST and the VTA that could result in activity-dependent increased strength of excitatory synapses in the presence of morphine. Furthermore, D1 dopamine receptors are present in the dorso-lateral BST and it is well documented that these receptors contribute to plasticity at excitatory synapses (Kerr and Wickens, 2001; Wolf et al., 2003; Sun et al., 2005).

CONCLUSION

In conclusion, our results show that chronic morphine treatment facilitated an input–output specific excitatory transmission in the BST. This confirms previous observations that drugs of abuse selectively target a neurocircuit that includes a subpopulation of presumably glutamatergic alBST neurons that project to the VTA. Selective stimulation of afferent excitatory inputs entering the BST revealed that a dorso-lateral pathway was selectively facilitated after chronic morphine. These results extend the understanding of neuronal circuits sensitive to drugs of abuse and further strengthen the suggestion that the BST plays a role in addiction.

Acknowledgments

Work supported by the Canadian Institutes of Health Research (MOP-79277 to ECD) and NIH-NIDA (DA08163 to J.T.W.). E.C.D. was a fellow of the Canadian Institutes of Health Research (MFE-49878).

Abbreviations

alBST	lateral region of the anterior bed nucleus of the stria terminalis
BST	bed nucleus of the stria terminalis
EPSC	excitatory post-synaptic current
PPR	paired-pulse ratio
VTA	ventral tegmental area

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Fig. 1.

Identification and characterization of alBST neurons projecting to the VTA. (A) Schematics representing the anterior region of the BST where neurons labeled with a retrograde tracer were observed following microinjection of fluorescent microspheres in the VTA. (B) Photomicrographs showing alBST filled with fluorescent microspheres. (C) Superimposed current responses (middle traces) to graded series of voltage pulses (top) in alBST neurons filled (left) or not filled with fluorescent microspheres (right). Graphs at the bottom represent the current responses as a function of membrane voltage in alBST neurons filled (left) or not filled (right) with fluorescent microspheres. Vm: membrane voltage; ic: internal capsule; ac: anterior commissure; st: stria terminalis; fx: fornix; s: soma; p: processes.

Fig. 2. Effect of a chronic morphine treatment on excitatory synaptic transmission in the alBST. (A) Schematic representing the placement of recording and stimulating electrodes for whole-cell voltage clamp recordings of evoked excitatory postsynaptic currents in the alBST. (B) Application of the NMDA receptors antagonist AP-5 (50 μ M, 5 min) revealed both AMPA- and NMDA-mediated excitatory synaptic transmission when neurons were voltage-clamped at +40 mV, in brain slices prepared from non-treated/placebo (upper traces) and morphine-treated rats (lower traces). Each trace is the average of 5 to 10 evoked EPSCs. (C) The bar chart displays the ratio of AMPA to NMDA components of the evoked EPSCs in alBST neurons projecting (left bars) or not projecting (right bars) to the VTA in placebo (open

bars), morphine-treated (closed bars) or after acute withdrawal produced by a 10-min bath-application of naloxone (1 μ M). (C1) Bar chart representing the PPRs of two sequentially applied stimuli at 40 Hz in VTA-projecting neurons in placebo (open bars), morphine-treated (closed bars) or after acute withdrawal produced by a 10-min bath-application of naloxone (1 μ M). * $P < 0.05$, $n = 10$ neurons per group. s: Stimulation site; st: stria terminalis; ic: internal capsule; fx: fornix; ac: anterior commissure.

Fig. 3.

The alBST receives multiple independent excitatory inputs. (A) Schematic representing the placement of multiple bipolar stimulating electrodes in (a) the latero-dorsal BST, (b) the medio-dorsal BST, or (c) the ventral alBST, to evoke EPSCs in the ventral alBST of non-treated rats. (B) Pairing two stimuli (40 Hz) produced short-term plasticity (paired-pulse facilitation) in alBST EPSCs evoked in both the latero-dorsal (top left traces) and medio-dorsal (bottom left traces) BST. No paired-pulse facilitation was observed when stimuli were paired between both dorsal BST stimulation sites (top and bottom right traces). Each trace is the average of 5 to 10 evoked EPSCs. Numbers (1, 2, or 3) next to site symbols indicate a single stimulus from a particular site. (C) Pairing two stimuli also produced

paired-pulse facilitation in the alBST when EPSCs were evoked by local fiber stimulation (left traces). Pairing local alBST stimulation with an evoked EPSC from a remote site in the latero-dorsal BST also produced paired-pulse facilitation (right traces). Insets demonstrate that stimulation in remote sites produced longer latencies than local stimulation in the alBST. ic: Internal capsule; ac: anterior commissure; fx: fornix.

Fig. 4. Chronic morphine selectively increased the aBST excitatory inputs entering the latero-dorsal BST. (A) Schematic representing the placement of multiple bipolar stimulating electrodes in the lateral (a) and medial dorsal BST (b) to evoke EPSCs in the ventral aBST. (B) Bar chart representing AMPA to NMDA ratios measured in VTA-projecting aBST neurons after stimulation in the dorso-lateral (left) and dorso-medial BST (right), in both placebo (open bars) and morphine-treated (closed bars) conditions. (C) AMPA and NMDA EPSCs recorded in VTA-projecting aBST neurons after stimulation in the latero-dorsal (left traces) and medio-dorsal (right traces) BST, in placebo (top traces) and morphine-treated (bottom traces) conditions. The interval between both stimuli was increased to 800 ms to

avoid short-term plasticity. Each trace is the average of five evoked EPSCs. (D) Bar chart summarizing the amplitudes of AMPA (left) and NMDA (right) EPSCs measured in VTA-projecting alBST neurons in the dorso-lateral (site a) and dorso-medial BST (site b), in placebo (open bars) or morphine-treated (closed bars) conditions. * $P < 0.05$, $n = 8$ neurons per group. ic: Internal capsule; st: stria terminalis; ac: anterior commissure; fx: fornix.