Double-dissociation of the catecholaminergic modulation of synaptic transmission in the oval bed nucleus of the stria terminalis

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Abstract

The bed nucleus of the stria terminalis (BST) is a cluster of nuclei within the extended
amygdala, a forebrain macrostructure with extensive projection to motor nuclei of the hindbrain.
The subnuclei of the BST coordinate autonomic, neuroendocrine and somato-motor functions
and receive robust neuromodulatory monoaminergic afferents, including 5-HT, noradrenaline
(NA), and dopamine (DA)-containing terminals. In contrast to 5-HT and NA, little is known
about how DA modulates neuronal activity or synaptic transmission in the BST. DA-containing
afferents to the BST originate in the ventral tegmental area, the periaqueductal gray, and the
retrorubral field. They form a fairly diffuse input to the dorsolateral BST with dense terminal
fields in the oval (ovBST) and juxtacapsular (jxBST) nuclei. The efferent-afferent connectivity
of the BST suggests that it may play a key role in motivated behaviours, consistent with recent
evidence that the dorsolateral BST is a target for drugs of abuse. This study describes the effects
of DA on synaptic transmission in the ovBST. Whole-cell voltage clamp recordings were
performed on ovBST neurons in brain slices from adult rats in the presence or absence of
exogenous DA and receptor-targeted agonists and antagonists. The results showed that DA
selectively and exclusively reduced inhibitory synaptic transmission in the ovBST in a dose-
dependent and D2-like dopamine receptor-dependent manner. DA also modulated excitatory
synaptic transmission in a dose-dependent dependent manner. However, this effect was mediated
by α2-noradrenergic receptors. Thus, these data reveal a double-dissociation in
catecholaminergic regulation of excitatory and inhibitory synaptic transmission in the ovBST,
and may shed light on the mechanisms involved in neuropathological behaviours such as stress-
induced relapse to consumption of drugs of abuse.
Introduction

The bed nucleus of the stria terminalis receives adrenergic, noradrenergic, serotoninergic, and dopaminergic inputs (Freedman and Cassell 1994; Hasue and Shammah-Lagnado 2002; Meloni et al. 2006; Phelix et al. 1992). The distribution of axons immunoreactive for tyrosine hydroxylase (TH), DA-β-hydroxylase (DβH), phenylethanolamine-N-methyl transferase (PNMT) or 5-HT reveals that monoaminergic inputs to the BST are topographically organized with both overlapping and exclusive distributions, depending on specific BST subregions (Phelix et al. 1992).

5-HT immunoreactive terminals are broadly distributed rostrocaudally but mostly dorsal to the anterior commissure in the BST (Phelix et al. 1992). However, 5-HT immunoreactivity is more robust in the medial than the lateral parts of the BST and largely avoids the oval (ovBST) and juxtacapsular (jxBST), which are located dorsolaterally (Larriva-Sahd 2006; 2004; Phelix et al. 1992). 5-HT modulates neuronal activity and reduces excitatory synaptic transmission in the BST (Guo et al. 2009; Guo and Rainnie 2010; Hammack et al. 2009; Levita et al. 2004). The specific effects of 5-HT on BST neurons activity is dynamically modulated by, and causally related to, stress and anxiety (Hammack et al. 2009; Levita et al. 2004).

DβH (NA and/or adrenaline)-containing fibers are mostly concentrated in the ventrolateral BST although there is DβH immunoreactivity medially in the dorsal BST (Freedman and Cassell 1994; Kozicz 2001; Phelix et al. 1992). Although rare, adrenergic terminals are present in the dorsolateral region of the intermediate (from a rostro-caudal perspective) regions of the BST (Phelix et al. 1992). NA also modulates both neuronal excitability and synaptic transmission in the ventral and dorsal BST (Dumont and Williams 2004; Egli et al. 2005; McElligott et al. 2010; McElligott and Winder 2008; 2009; Shields et al.)
Noradrenergic modulation of BST neuron excitability and synaptic transmission is also dynamically altered by various physiological and pathological conditions (Dumont and Williams 2004; McElligott et al. 2010; McElligott and Winder 2008; 2009) including stress, anxiety, pain, reward, and addiction (Cecchi et al. 2002; Delfs et al. 2000; Deyama et al. 2009; Deyama et al. 2008; Dumont and Williams 2004; Leri et al. 2002).

In contrast to 5-HT and NA, little is known about the neurophysiology of DA in the BST (Kash et al. 2008). Although dopaminergic fibers form a fairly diffuse input to the dorsolateral BST, dense terminal fields can be observed in the ovBST and jxBST (Deutch et al. 1988; Freedman and Cassell 1994; Hasue and Shammah-Lagnado 2002; Meloni et al. 2006; Phelix et al. 1992). These dopaminergic inputs originate from the ventral tegmental area, the periaqueductal grey region, and the retrorubral field (Hasue and Shammah-Lagnado 2002; Meloni et al. 2006). A recent study on dopaminergic regulation of neuronal activity and synaptic transmission in the dorsolateral BST in the mouse (Kash et al. 2008) reported that DA produced a modest increase in spontaneous excitatory, but not inhibitory, synaptic transmission, an effect that was attributed to a D1/D2-like mediated depolarization of local CRFergic neurons.

However, Kash et al. did not specify the subregion of the dorsolateral BST examined in their study.

In addition to TH-positive axons, the ovBST contains several key components of the brain DA system including DA receptors (although the exact subtypes remains unclear) and DARPP-32, suggesting that DA may be an important neuromodulator of neuronal activity, synaptic transmission, or both, in this region (Freedman and Cassell 1994; Gustafson and Greengard 1990; Hasue and Shammah-Lagnado 2002; Meloni et al. 2006). The presence of multiple DA inputs as well as the connectivity of the ovBST is consistent with a role in
motivated behaviours (Dong et al. 2001a; Dong et al. 2001b; Larriva-Sahd 2006; McDonald et al. 1999). In fact, pharmacological manipulation of DA receptors in the dorsolateral BST alters natural and pharmacological rewards-motivated behaviours (Eiler et al. 2003; Epping-Jordan et al. 1998). Furthermore, the dorsolateral BST is ideally located to integrate stress and reward information and consequently, consistent with its reported role in stress-induced relapse to chronic voluntary drug intake in animal models of drug addiction (Erb et al. 2001; Leri et al. 2002). Because DA (reward) and NA (stress) response pathways co-localize in the dorsolateral BST, interaction between the DA and NA in this brain region might play a critical role in integrating stress- and reward-induced behaviours.

The objective of the present study was to elucidate the neuromodulatory effects of DA on synaptic transmission in the ovBST. Whole-cell voltage clamp recordings were done on ovBST neurons in brain slices from adult rats in the presence and absence of exogenous DA and selective receptor-targeted agonists and antagonists. DA reduced the amplitude of evoked GABA$_A$-inhibitory post-synaptic currents (IPSC) pre-synaptically in a dose- and D2 dopamine receptors (D2R)-dependent manner. In contrast, the effect of DA on excitatory transmission was absolutely dependent on functional $\alpha_2$-adrenergic receptors ($\alpha_2$R), a mechanism that overlapped substantially with the neuromodulatory mechanism of NA. DA did not produce measurable effects on passive neurophysiological properties of ovBST also suggesting pre-synaptic effects of DA. Immunohistochemical studies confirmed that D2R but not D1R are expressed in the ovBST. Together, our results demonstrate how DA modulates synaptic transmission in the ovBST, an important step in understanding the role of ovBST DA in motivated behaviours. Furthermore, the effect of DA on synaptic transmission in the ovBST could be dissociated from those of NA, revealing synaptic mechanisms that could help integrate stress- (NA) and reward-
(DA) related stimuli in the ovBST.

Methods

Subjects. Adult male Long Evans and Sprague-Dawley rats (Charles River Laboratories, Inc., www.criver.com) weighing 300-650g, were housed in a climate-controlled colony room. The animals were maintained on a 12 hr reversed light/dark cycle (09.00h lights off – 21.00h lights on) and fed ad libitum. All the experiments were conducted in accordance with the Canadian Council on Animal Care guidelines for use of animals in experiments and approved by the Queen’s University Animal Care Committee or the French (87-848, Ministère de l’Agriculture et de la Forêt) and European Economic Community (86-6091 EEC) guidelines for the care of laboratory animals and were approved by the Ethical Committee of Centre National de la Recherche Scientifique, Région Aquitaine.

Brain slices preparation and electrophysiology. Rats were anesthetized with isoflurane and their brains were rapidly removed. Coronal slices (250 µm) containing the BST were prepared in ice-cold physiological solution containing (in mM) 126 NaCl, 2.5 KCl, 1.2 MgCl₂, 6 CaCl₂, 1.2 NaH₂PO₄, 25 NaHCO₃ and, 12.5 D-glucose. Slices were incubated at 34°C for 60 minutes and transferred to a chamber that was constantly perfused (1.5ml/min) with physiological solution maintained at 34°C and equilibrated with 95%O₂/5%CO₂. Whole-cell voltage-clamp recordings were made using microelectrodes filled with a solution containing (in mM) 70 K⁺-gluconate, 80 KCl, 1 EGTA, 10 HEPES, 2 MgATP, 0.3 GTP, and 1 P-creatine.

All recordings were restricted to the ovBST, and more precisely, to the dorsal half of the ovBST (Figure 1a). The exact anteroposterior coordinates representing the brain slice used varies slightly from one Brain Atlas to the other (-0.26mm and -0.12mm in Swanson and Paxinos and...
Watson, respectively, Figure 1a). In practice, we used the slices where the posterior part of the anterior commissure (ac) decussates but where the lateral extension of the ac are still present. Recordings were restricted medially to an imaginary vertical line that would run through the lateral ventricle. In addition, recordings were restricted to the area of the dorsolateral BST located dorsal to the halfway point between the tip of the lateral ventricle and the top of the ac (Figure 1a). Post-synaptic currents were evoked by local fiber stimulation with tungsten bipolar electrodes while ovBST neurons where voltage-clamped at -70mV. Stimulating electrodes were placed in the ovBST, 100-500µm lateral (IPSC) or dorsal (EPSC) from the recorded neurons (Figure 1a), and paired electrical stimuli (0.1ms duration, 50ms interval) were applied at 0.1Hz. GABA<sub>A</sub>-IPSC and AMPA-EPSC were pharmacologically isolated with DNQX (50 µM) or picrotoxin (100µM), respectively. Recordings were made using a Multiclamp 700B amplifier and a Digidata 1440A (Molecular Devices Scientific, www.mds.com). Data were acquired and analyzed with Axograph X (www.axographx.com) running on an Apple computer (www.apple.com).

**Drugs.** Stock solution of DA (10 mM), SFK-81297 (1mM), quinpirole (1mM), SCH-23390 (10mM), methysergide (10mM), 5-HT (10mM), and propranolol (10mM) were prepared in double distilled water. Stock solution of DNQX (100mM), sulpiride (1mM), yohimbine (1mM), and, prazosin (1mM) were prepared in DMSO (100%). Every drug was further dissolved in the physiological solutions at the desired concentration and the final DMSO concentration never exceeded 0.1%. All the drugs were obtained from Sigma-Aldrich (www.sigmaaldrich.com) or Tocris (Tocris, www.tocris.com).

**Statistical Analyses.** We measured drug-induced change in post-synaptic currents (PSC) peak amplitude from baseline in percentage (((Peak amplitude<sub>drug</sub>-Peak amplitude<sub>baseline</sub>)/Peak amplitude<sub>baseline</sub>)*100).
amplitude_{baseline})*100). Paired-pulse ratios (PPR) were calculated by dividing the second (S2) by
the first (S1) peak amplitude that we normalized to baseline. Peak amplitudes for S1 and S2 were
calculated from a baseline value measured 10ms after the end of a 1mV test pulse. Coefficient of
variation (CV) analyses was done by plotting r ((1/CV^2_{drug})/(1/CV^2_{baseline})) against \pi (Peak
amplitude_{drug}/Peak amplitude_{baseline}). We computed bivariate linear Fits of r by \pi. Data were all
expressed as Mean ± SEM. Drug effects were assessed using one-tailed t-tests with hypothesized
values of 0 (changes in amplitude) and 1 (changes in paired-pulse ratio). When multiple t-tests
were done on a single set of data we adjusted the significance level accordingly (0.05/number of
t-tests). When comparing multiple means, one-way ANOVAs where used with Dunnett’s test for
multiple comparison when ANOVA deemed significance. All statistical analyses were done with
JMP 8.0.

**Immunohistochemical detection of D1R and D2R.** The rats were deeply anesthetized with sodium
chloral hydrate (400 mg/kg, i.p.) and perfused transcardiacally with 50–100 ml of 0.9% NaCl
followed by 300 ml of fixative consisting of 2% paraformaldehyde (PFA) with 0.2%
glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Brains were quickly removed, left
overnight in 2% PFA at 4°C, and cut into 60µm-thick frontal sections with a vibrating blade
microtome (Leica, VT1000S, www.Leica.com). To enhance the penetration of the
immunoreagents, the sections were equilibrated in a cryoprotectant solution (0.05 M PB, pH 7.4,
containing 25% sucrose and 10% glycerol), freeze-thawed, and stored in PBS with 0.03%
sodium azide until needed. D1R was detected by immunohistochemistry according to previously
described and validated procedures (Berthet et al. 2009; Caille et al. 1996) using a monoclonal
antibody raised in rat against a 97 amino acid sequence corresponding to the C terminus of the
human D1R (Sigma-Aldrich) (Hersch et al. 1995; Levey et al. 1993). D2R were detected with an
affinity-purified rabbit polyclonal antiserum directed against a 28 amino acid sequence within
the third cytoplasmic loop from the human D2R corresponding to anti D2-284 peptide
(Millipore) that recognizes both short and long isoforms (Ahmed et al. 2010). The sections
containing the ovBST were incubated in 4% normal goat serum (NGS) or in normal donkey
serum (NDS) in PBS for 1h at room temperature (RT) and then in the antibodies against D1R
(1:1000) or D2R (1:500) supplemented with 1% NGS or NDS overnight at RT. After washing (3
x 10min) in PBS, for D1R detection, the sections were incubated in goat anti-rat IgG coupled to
biotin (Amersham; 1:200 in PBS for 1h30) For D2R detection, the sections were incubated in
Dako EnVision+™ system-horseradish peroxydase (HRP) labelled polymer anti-rabbit for 40
min at RT. After three rinses (3 x 10min) in PBS, for D1-DA receptor detection, the sections
were then incubated in avidin-biotin complex (Vector ABC Elite, Vector Laboratories,
www.vectorlabs.com) (1:200) in PBS for lh30 at RT and again rinsed (3 x 10 min) in PBS. The
immunoreactive sites were revealed by a combination of the glucose oxidase-diaminobenzidine
(DAB; Sigma-Aldrich, 0.05% in TB) method and the DAB-nickel method (Shu et al. 1988). The
reaction was stopped by several washes in H2O. The sections were mounted on glass slides,
Negative immunohistological control demonstrated the absence of signal when omitting the first
antibody.

Results

Passive properties of ovBST neurons

No agonist tested in the present study significantly changed passive properties
(membrane input resistance (Rin) and membrane holding currents (Hc)) of ovBST neurons
voltage-clamped at -70mV. In particular, DA (30µM) and NA (10µM), which produced the
largest effects on synaptic transmission, had virtually no effect on Hc and Rin of ovBST neurons (Figure 1b).

**DA decreased inhibitory transmission in the ovBST by activating pre-synaptic D2R**

Exogenously applied DA (0.1 to 100µM) dose-dependently decreased the amplitude of evoked and pharmacologically isolated GABA\textsubscript{A}-IPSC in ovBST neurons (Figure 2). GABA\textsubscript{A} - IPSC rapidly returned to baseline upon when DA was washed out of the perfusion chamber. At concentrations where DA decreased GABA\textsubscript{A}-IPSC, we observed statistically significant increases in paired-pulse ratio (PPR\textsubscript{50msec}) suggesting it acts pre-synaptically (Figure 2b).

Coefficient of variation (CV) analyses revealed a significant positive correlation, also suggesting a pre-synaptic effect of DA (Figure 2c). The D2-like agonist quinpirole dose-dependently and pre-synaptically mimicked the effects of DA on GABA\textsubscript{A}-IPSC (Figure 2b). In contrast, the D1R agonist SKF-81297 did not alter the amplitude of GABA\textsubscript{A}-IPSC (Figure 2b).

DA 30µM was co-applied with either the D1R antagonist SCH-23390 (10µM), the D2R antagonist sulpiride (10µM), a cocktail of noradrenergic antagonists (prazosin 0.1µM, yohimbine 1µM, and propranolol 1µM) or the 5-HT\textsubscript{1,2,7} receptor antagonist (methysergide 10µM). Only D2R blockade with sulpiride altered DA-induced reduction in GABA\textsubscript{A}-IPSC (F(4,19)=7.6, P=0.0008) (Figure 3). As a result, DA produced significant decreases in GABA\textsubscript{A}-IPSC when co-applied with SCH-23390, noradrenergic antagonists or methysergide (Figure 3b). Likewise, NA (10µM) modestly reduced GABA\textsubscript{A}-IPSC amplitude (Figure 3b). On average, 5-HT (10µM) had no significant effect on GABA\textsubscript{A}-IPSC amplitude in the ovBST (Figure 3b). However, 5-HT decreased (-26.0±6.8, n=8) or slightly increased (11.0±6.1, n=3) GABA\textsubscript{A}-IPSC in the ovBST.

Sulpiride and SCH-23390 did not produce any effects on their own (Figure 3b).

**DA decreased fast excitatory transmission in the ovBST by activating pre-synaptic \(\alpha2R\)**
Exogenously applied DA (0.1 to 30µM) dose-dependently decreased the amplitude of evoked and pharmacologically isolated AMPA-EPSC in ovBST neurons (Figure 4a). AMPA-EPSC rapidly returned to baseline when DA was washed out of the perfusion chamber. At concentrations where DA decreased AMPA-EPSC, we observed statistically significant increases in PPR$_{50\text{msec}}$ suggesting it acted pre-synaptically (Figure 4a1). CV analyses revealed a significant positive correlation, also suggesting a pre-synaptic effect of DA (Figure 4a1, inset). In contrast to the effects of DA on GABA$_{A}$-IPSC, bath application of D1R or D2R agonists did not mimic the effects of DA on AMPA-EPSC (Figure 4a1). Bath application of the D2R agonist quinpirole (0.1-1µM), of the D1/D5 full agonist SKF-81297 (0.1-1µM), or of the broad-spectrum DAR agonist apomorphine (30-100µM) did not significantly change the amplitude of evoked AMPA-EPSC (Figure 4a1). Likewise, 5-HT (10-100µM) did not affect the amplitude of AMPA-EPSC in any tested ovBST neurons (Figure 4a1).

However, exogenously applied NA (0.1-10µM) dose-dependently reduced the amplitude of evoked AMPA-EPSC by acting pre-synaptically as shown by significant changes in PPR$_{50\text{msec}}$ (Figure 4b1). CV analyses revealed a significant positive correlation, also suggesting a pre-synaptic effect of NA (Figure 4b1 inset). Furthermore, the $\alpha_2$R agonist clonidine dose-dependently reduced the amplitude of evoked AMPA-EPSC (Figure 4b1). Clonidine also decreased AMPA-EPSC pre-synaptically since we measured a significant increase in PPR$_{50\text{msec}}$ (Figure 4b1). Furthermore, the $\alpha_2$R antagonist yohimbine (5µM) completely abolished NA- ($t_{(15)}=-7.7$, $P<0.0001$) and DA- ($t_{(13)}=4.9$, $P=0.0001$) induced reduction in AMPA-EPSC (Figure 5). In contrast, the effect of NA was unaffected by $\alpha_1$- and $\beta$-adrenergic receptors blockade with prazosin (5µM) and propranolol (5µM) (Figure 5c).

**Localization of D1R and D2R in the ovBST**
We observed immunoreactivity for both D1R and D2R in the anterior BST (Figure 6). However, the distribution of immunoreactivity for both receptors was not homogeneous. Notably, the ovBST was completely devoid of D1R immunostaining (Figure 6a). In contrast, the ovBST contained immunoreactivity for D2R, which, at the light microscopy level, appeared to be in the neuropil and cytoplasm of some cells bodies (Figure 6b).

Discussion

This study examines the effects of DA in the ovBST of the rat, and demonstrates the involvement of differential mechanisms for its effects on inhibitory and excitatory synaptic transmission. DA reduced the amplitude of whole-cell evoked GABA$_A$-IPSC pre-synaptically in an exclusively D2R-dependent manner, while it reduced fast excitatory transmission, also pre-synaptically, but through $\alpha$2R. This conclusion is strongly supported by the use of specific D2R and $\alpha$2R agonists and antagonists, and immunohistochemical evidence for expression of $\alpha$2R (Shields et al. 2009) and D2R but not D1R in the ovBST (this study).

Although, the neurophysiological D2-like effect of DA on inhibitory synaptic transmission was clearly pre-synaptic, immunohistochemical data does not unequivocally demonstrate exclusive pre-synaptic localization of D2R. In fact, we detected perikarya-like immunolabeling of D2R in the ovBST. However, previous studies showed the absence of D2R mRNA in the dorsolateral BST (Bouthenet et al. 1991). As such, these perikarya-like structures could also be axonal envelopes of ovBST perikarya or local ovBST interneurons expressing D2R. In a 2006 study, Larriva-Sahd clearly demonstrated that there are several subtypes (11) of neurons in the ovBST, amongst which subpopulations of short axon neurons that do not exit the nucleus and could thus be considered interneurons (Larriva-Sahd 2006). However, our electrophysiological data did not reveal any D2-like post-synaptic responses ruling out a possible
reduction in lateral inhibition in the ovBST through hyperpolarization of GABA interneurons. Accordingly, the most parsimonious interpretation of our results would be that DA, acting on D2-containing GABAergic axons from extra-ovBST origin, reduces GABA release. To resolve this question, higher resolution studies of the expression of D2R mRNA in BST subnuclei or single-cell PCR experiments would be needed to confirm or infirm the single previous study regarding D2R mRNA absence.

Both our neurophysiological and immunohistochemical data suggest that the expression of D1R is low in the ovBST. This result is consistent with previous studies showing low expression of D1R mRNA and protein in the dorsolateral BST (Fremeau et al. 1991; Scibilia et al. 1992). Furthermore, no D1-like mediated effect on synaptic transmission in the ovBST was detected in this study, which contrasts with a previous report by Kash et al. (Kash et al. 2008). Kash et al. suggested that DA, through D1/D2-like receptors, depolarizes corticotropin releasing hormones (CRH)-containing neurons and facilitates glutamate release, which would explain the modest increase in the frequency of spontaneous EPSC observed in their study. This discrepancy could be explained by several differences in the experimental systems used in the present study and by Kash et al., including the use of 6-8 weeks old mice vs. 12 weeks and older rats. Furthermore, because Kash et al. did not restrict their study to the ovBST, their results may reflect synaptic activity in the dorsomedial or the more ventral part of the dorsal BST, where we detected low-level expression of D1R (see Figure 6). It should also be noted that there was virtually no detectable spontaneous EPSC in our experimental system. Accordingly, the effects of DA on evoked EPSC were measured by electrically stimulating the stria terminalis, the main route of excitatory entry to the ovBST (Larriva-Sahd 2006). As such, our study extends Kash et al. by revealing regional D1 effects within the dorsolateral BST.
In our system, the effect of DA on the amplitude of evoked EPSC was not mimicked or reproduced by D1R or D2R agonists, but was dependent on pre-synaptic α2R, as described previously for NA (Dumont and Williams 2004; Egli et al. 2005; McElligott et al. 2010; McElligott and Winder 2008; 2009; Shields et al. 2009). These results are consistent with the widespread distribution of α2R immunoreactivity in the BST, including the ovBST (Shields et al. 2009). Furthermore, α2R are ideally located to modulate excitatory inputs entering the BST since stria terminalis fibers, including those innervating the ovBST, co-express α2R, the vesicular glutamate transporter VGLUT1, and TH (which could contain either DA or NA) (Shields et al. 2009). Together, this evidence demonstrates that if DA had the ability to activate α2R in the ovBST, it would very likely modulate excitatory transmission, which is consistent with our observations. In contrast, our results do not support a role for α2R in modulating GABAergic transmission in the BST, which is also consistent with previous studies (Dumont and Williams 2004; Forray et al. 1999; Shields et al. 2009).

Cross talk between cathecolaminergic subpathways, including activation of α2R by DA, is not uncommon and occurs in other brain regions and species (Cornil and Ball 2008; Cornil et al. 2002; Guiard et al. 2008). Interestingly, the affinity of DA for α2R is approximately 3-fold lower than that of NA (Boyajian and Leslie 1987) and in fact, 10μM NA and DA 30μM were equipotent at reducing AMPA-EPSC in the ovBST (see Figure 4a1 and b1). However, it is yet unclear whether α2R-mediated regulation of excitatory transmission by DA is physiologically, behaviourally, or pathophysiologically significant. *In vivo*, DA can, at best, be measured in the nM range in the BST (Carboni et al. 2000). In brain slices, exogenous application of DA in the nM range does not produce significant effects (see Figures 2 and 4). We thus suggest a double-
dissociation between the effects of DA and NA on inhibitory and excitatory synaptic transmission in the ovBST (Figure 7). This double-dissociation is appreciable at lower concentration of both DA and NA (e.g. at 1µM). It is thus likely that in physiological conditions, the effect of DA is restricted to inhibitory transmission whereas NA should preferentially modulate excitatory synaptic transmission.

In contrast to DA and NA, 5-HT does not modulate excitatory transmission and is largely undetected immunohistochemically in the ovBST (Phelix et al. 1992). This observation contrasts with 5-HT-induced reduction in evoked EPSC reported by Guo et al. in the anterolateral BST (Guo and Rainnie 2010), and suggests that 5-HT plays little or no neuromodulatory role of excitatory transmission in the ovBST. Nonetheless, we observed bi-directional modulation of inhibitory transmission by 5-HT in the ovBST (see Results section). It is, however, unlikely that DA mediated its effect through 5-HT receptors since methysergide did not interfere with the DA-mediated reduction in IPSC. 5-HT modulation of inhibitory transmission in the ovBST or in the BST altogether has not been done and will require a thorough investigation such as those done for excitatory transmission (Guo and Rainnie 2010).

Anatomical evidence suggests that the ovBST plays a role in neurocircuits that influence ingestive behaviour including its motivational component (Dong et al. 2001b). Accordingly, the BST, and in particular its oval region, could be key within the neural circuits sensitive to drugs of abuse. The ovBST receives ascending inputs from the cerebrospinal trunk, horizontal inputs from basal forebrain-related regions, and top-down cortical inputs. Cortical projections, likely conveying excitatory inputs, mostly originate in the viscerosensory portion of the dysgranular insular cortex and the olfactory amygdalopiriform transitional area (McDonald et al. 1999), which could respectively convey internal and external cues triggering operant behaviours.
towards reward. How excitatory inputs into the ovBST affect intrinsic neural network function is currently unknown. However, Larriva-Sahd extensively described the local neuroanatomy of the ovBST which is compartmentalized into a shell and a core (Larriva-Sahd 2006). The shell seems to receive and transmit incoming information to the core which is home of projections neurons. ovBST projection is quite complex and widespread but should result in coordinated autonomic, neuroendocrine, and somatomotor responses (Dong et al. 2001b) that may drive foraging behaviour when body nutrients (or drugs) levels become low and external cues predict substance availability. Consistent with this proposition, pharmacological manipulations of the dorsal BST influence motivated behaviours towards natural or pharmacological rewards (Eiler et al. 2003; Eping-Jordan et al. 1998; Erb et al. 2001; Hyytia and Koob 1995; Leri et al. 2002; Walker et al. 2000). However, the specific contribution of the ovBST in such pathways has not been reproduced and/or analyzed experimentally, because of technical difficulties associated with selectively targeting this small brain region. Consequently, the exact contribution of DA-dependent effects in the ovBST to motivated behaviours is not yet understood.

Reward prediction correlates with release of DA from midbrain neurons (Fiorillo et al. 2003; Phillips et al. 2003). Therefore, salience-induced release of DA in the ovBST could activate D2R, selectively reduce inhibitory influence to promote neuronal activation and, perhaps, reward-seeking behaviours. In fact, microinjection of the D2R antagonist eticlopride in the dorsolateral BST reduced sucrose self-administration (Eiler et al. 2003). In contrast, NA, which is released in the BST in response to stress (Pacak et al. 1995), should inactivate excitatory drive to the ovBST, without affecting inhibitory transmission. This functional double-dissociation of the effects of DA and NA in the ovBST may help fine tune the neurological integration of stress and goal-directed behaviours (Bowers et al. 1999) (Figure 7).
References


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Legends to figures

**Figure 1.** Anatomical localization of recordings and effects of agonists on passive electrophysiological properties of ovBST neurons. a. Schematic illustrating the anatomical localization of recording pipettes and stimulating electrodes. ac: anterior commissure; ic: internal capsule. b. Dot plot showing the effects of DA 30µM (circles) or NA 10µM (triangles) on membrane holding current (Hc) and membrane input resistance (Rin).

**Figure 2.** Effects of DA, D1R, and D2R agonists on the amplitude of evoked GABA$_\text{A}$-IPSC in the ovBST. a. Representative traces showing the effects of bath application of DA on electrically evoked GABA$_\text{A}$-IPSC in the ovBST. Each trace is the average of 5 consecutive events. b. Bar graph summarizing the effects of DA agonists on the peak amplitude and paired-pulse ratios of evoked GABA$_\text{A}$-IPSC in the ovBST. c. Coefficient of variation analysis of the effects of DA (0.1-30µM) on evoked GABA$_\text{A}$-IPSC in the ovBST. S1: stimulus one. S2:
Stimulus 2. \( r \): \( \frac{(1/CV_{drug})}{(1/CV_{baseline})} \). \( \Pi \): Peak amplitude_{drug}/Peak amplitude_{baseline}. Asterix, Significantly different from 0; \( P<0.01 \).

**Figure 3. Pharmacological characterization of the effects of DA on the amplitude of evoked GABA_{A}-IPSC in the ovBST.**
a. Representative dot plot showing the time-course of the effects of DA on evoked GABA_{A}-IPSC in the ovBST in the absence and the presence of the D1R antagonist SCH-23390 or the D2R antagonist sulpiride. b. Bar chart summarizing the effect of monoaminergic agonists and antagonists on evoked GABA_{A}-IPSC in the ovBST. Asterix, Significantly different from 0; \( P<0.01 \). Dagger, Significantly different from DA 30µM; \( P<0.05 \).

**Figure 4. Catecholaminergic modulation of evoked AMPA-EPSC in the ovBST.**
a. Representative traces showing the effects of bath application of DA on electrically evoked AMPA-EPSC in the ovBST. Each trace is the average of 5 consecutive events. a1. Bar graph summarizing the effects of DA and 5-HT agonists on the peak amplitude and PPR_{50msec} of evoked AMPA-EPSC in the ovBST. Inset shows CV analyses. b. Representative traces showing the effects of bath application of noradrenergic agonists on electrically evoked AMPA-EPSC in the ovBST. Each trace is the average of 5 consecutive events. b1. Bar graph summarizing the effects of noradrenergic agonists on the peak amplitude and PPR_{50msec} of evoked AMPA-EPSC in the ovBST. Inset shows CV analyses. S1: stimulus one. S2: Stimulus 2. Asterix, Significantly different from 0; \( P<0.01 \).

**Figure 5. Pharmacological characterization of the effects of DA and NA on evoked AMPA-EPSC in the ovBST.**
a. Representative dot plot showing the time-course of the effects of DA on
evoked AMPA-EPSC in the ovBST in the absence and the presence of the α2R antagonist yohimbine. b. Representative dot plot showing the time-course of the effects of NA on evoked AMPA-EPSC in the ovBST in the absence and the presence of the α2R antagonist yohimbine. c. Bar chart summarizing the effects of noradrenergic antagonists on dopaminergic and noradrenergic modulation of evoked AMPA-EPSC in the ovBST. Asterix, Significantly different from 0; P<0.01. Dagger; P<0.05.

Figure 6. Immunohistochemical localization of D1R and D2R in the ovBST. Light micrographs of the rat brain immunostained to reveal immunoreactivity for D1R (A and A’) and D2R (B and B’). The black dotted line delineates the anterior BST whereas the red dotted line delineates the ovBST. ac, anterior commissure; ic, internal capsula; ov, oval nucleus. Scale bars: A and B, 0.3mm; A’, 0.1 mm; B’, 50 μm.

Figure 7. Double-dissociation of the catecholaminergic modulation of synaptic transmission in the ovBST. The ovBST receives robust glutamatergic projection from insular cortex, piriform cortex, ventral subiculum and basolateral amygdala as well as DA inputs from the midbrain and NA inputs from the brainstem. GABAergic inputs to ovBST are from local short-axon GABAergic neurons or from the central nucleus of the amygdala. Functional evidences from this study demonstrate that: 1) release of DA in the ovBST could activate D2R and selectively reduce inhibitory influence to promote neuronal activation and 2) release of NA could activate α2R and selectively inactivate excitatory drive to the ovBST. This functional double-dissociation of the effects of DA and NA in the ovBST may be involved in processing both stress- and reward-related stimuli.
Krawczyk et al., 2010, Figure 1
Krawczyk et al., 2010, Figure 2
Krawczyk et al., 2010, Figure 3

(a) Graph showing GABA\textsubscript{A}-IPSC (Normalized) over time with different treatments.

(b) Bar graph showing the change in GABA\textsubscript{A} IPSC (%) with various treatments.

Legend:
- DA 30μM
- DA 30μM + Sulpiride 10μM
- DA 30μM + SCH-23390 10μM

Key:
- + + + + + - - - - - -
- + + + + + - - - - - -
- + + + + + - - - - - -
a. Baseline → DA 30μM → Wash

b. Baseline → NA 10μM → Wash

a1. △ AMPA-EPSC (%)

b1. △ Paired-pulse ratio (Normalized)

Krawczyk et al., 2010, Figure 4
Krawczyk et al., 2010, Figure 5
Krawczyk et al., 2010, Figure 6
Krawczyk et al., 2010, Figure 7