Neuroscience 128 (2004) 583–596

5-HYDROXYTRYPTAMINE$_{1A}$-LIKE RECEPTOR ACTIVATION IN THE BED NUCLEUS OF THE STRIA TERMINALIS: ELECTROPHYSIOLOGICAL AND BEHAVIORAL STUDIES

L. LEVITA,[1]* S. E. HAMMACK, I. MANIA, X.-Y. LI,[2] M. DAVIS AND D. G. RAINNIE*

Department of Psychiatry and Behavioral Neuroscience, Emory University, Yerkes Neuroscience Building, 954 Gatewood Drive, Room 5220, Atlanta, GA 30322, USA

Abstract —The anteriorlateral bed nucleus of the stria terminalis (BNST$_{AL}$) and the serotonergic system are believed to modulate behavioral responses to stressful and/or anxiogenic stimuli. However, although the BNST$_{AL}$ receives heavy serotonergic innervation, the functional significance of this input is not known. Data obtained from *in vitro* whole-cell patch clamp recording in the rat BNST slice show that exogenous application of 5-hydroxytryptamine (5-HT) evoked a heterogeneous response in BNST$_{AL}$ neurons. The principal action of 5-HT in this region was inhibitory, evoking a membrane hyperpolarization (5-HT$_{Hyp}$) and a concomitant reduction in input resistance in the majority of neurons tested. The broad-spectrum 5-HT$_1$ agonist, 5-carboxamindotryptamine (5-CT), but not *R*(±)8-hydroxydipropylaminotetralin hydrobromide (8-OH-DPAT), mimicked the 5-HT$_{Hyp}$ response in the BNST. Moreover, the outward current mediating 5-HT$_{Hyp}$ was inwardly rectifying and sensitive to the G protein activated inwardly rectifying K$^+$ (G$_{IRK}$) channel blocker, tertiapin-Q. In the CNS 5-HT$_{1A}$ receptors are thought to couple to G$_{IRK}$ channels, suggesting that 5-HT$_{Hyp}$ in BNST$_{AL}$ neurons was mediated by activation of 5-HT$_{1A\text{-}like}$ receptors. This was confirmed by the blockade of both 5-HT$_{Hyp}$ and 5-CT$_{Hyp}$ by the specific 5-HT$_{1A}$ receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate salt (WAY100635 200nM). Furthermore, an *in vivo* examination of the functional consequences of 5-HT$_{1A\text{-}like}$ induced inhibition of BNST neurons revealed that infusion of 5-CT into the BNST significantly reduced the acoustic startle response, without affecting the general motor activity of the animals. These data point to the possibility that 5-HT$_{1A}$ mediated inhibition of the BNST$_{AL}$ could contribute to an anxiolytic action. Hence, we propose that in response to stressful stimuli, enhanced levels of 5-HT in the BNST$_{AL}$ plays a critical homeostatic role in feedback inhibition of the anxiogenic response to these stimuli.

Key words: serotonin, inhibition, extended amygdala, anxiety, patch clamp, acoustic startle.

The bed nucleus of the stria terminalis (BNST) is thought to play a key role in modulating an organism's adaptive response to unpredictable stressful events. Thus, electrical stimulation of the BNST evokes cardiovascular and behavioral responses similar to those initiated by stressful stimuli (Casada and Dafny, 1991; Dunn and Williams, 1995; Shaikh et al., 1986). Similarly, exposure to a stressor increases neuronal activation in the BNST (Chung et al., 2000; Duncan et al., 1993; Lino-de-Oliveira et al., 2001; Martinez et al., 1998). In contrast, pharmacological inhibition or lesions of the BNST reduce behavioral indices of anxiety (Brutus et al., 1988; Crown et al., 2000; Fendt et al., 2003; Hammack et al., 2004; Henke, 1984; Schulz and Canbeyli, 1999; Walker and Davis, 1997b).

Stressful stimuli that activate neurons of the BNST also activate serotonergic (5-HT) neurons of the midbrain raphé (Casada and Dafny, 1991; Dilts and Boadle-Biber, 1995; Grahn et al., 1999; Lowry, 2002). Significantly, these two regions are reciprocally connected (Petit et al., 1995; Vertes et al., 1994), and serotonergic axons from the dorsal raphe nucleus preferentially target neurons of the anterior BNST (Commons et al., 2003; Phelix et al., 1992a,b). Moreover, 5-HT levels increase in forebrain regions in response to stress (Amat et al., 1998; Fujino et al., 2002; Funada and Hara 2001; Umriukhin et al., 2002). Consequently, stress-induced activation of the dorsal raphé could significantly elevate 5-HT levels in the BNST. However, there is little consensus regarding the role of 5-HT in modulating BNST function. Thus, c-fos expression in the BNST is increased by *in vivo* manipulations that either enhance, or deplete, 5-HT levels (Chung et al., 1999; Li and Rowland, 1996; Morelli and Pinna, 1999). One explanation for this apparent contradiction would be that 5-HT has differential effects in the BNST depending on the type of 5-HT receptor subtypes involved. Indeed, at least four 5-HT receptor subtypes are expressed in the BNST (Cornea-Hebert et al., 1999; Heidmann et al., 1998; Sari et al., 1999; Waeber and Moskowitz, 1997). In a preliminary

[1] Present addresses: Howard Hughes Medical Institute, Center for Neurobiology and Behavior, Columbia University, 1051 Riverside Drive, Room 668, New York, NY 10032, USA.
[2] Present address: Department of Psychiatry, 151A, Boston VA Health Care System, Harvard Medical School, Brockton, MA 02301, USA.
*Corresponding author. Tel: +1-404-712-9714; fax: +1-404-727-3233.
E-mail address: drainni@emory.edu (D. Rainnie).

Abbreviations: ACSF, artificial cerebrospinal fluid; BNST, bed nucleus of the stria terminalis; BNST$_{AL}$, anteriorlateral bed nucleus of the stria terminalis; CGP 52432, 3-[[3,4-dichlorophenyl)methyl]amino]propyl] diethoxymethyl)phosphinic acid; DAB, 3,3′-diaminobenzidine; E$_{5\text{-}HT}$, reversal potential of 5-HT; G$_{IRK}$, G protein-activated inwardly rectifying K$^+$ channels; ISI, interstimulus interval; LTDg, lateral tegmental nucleus; PnC, nucleus reticularis pontis caudalis; PPTg, pedunculopontine tegmental nucleus; Rm, input resistance; TTX, tetrodotoxin; WAY100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate salt; 5-CT, 5-carboxamidotryptamine; 5-CT$_{Hyp}$, 5-CT-evoked membrane hyperpolarization; 5-HT, 5-hydroxytryptamine; 5-HT$_{Dep}$, 5-HT-evoked membrane depolarization; 5-HT$_{Hyp}$, 5-HT-evoked membrane hyperpolarization; 5-HT$_{Hyp\text{-}Dep}$, 5-HT-evoked membrane hyperpolarization followed by depolarization; 8-OH-DPAT, *R*(±)8-hydroxydipropylaminotetralin hydrobromide.

0306-4522/04$30.00+0.00
doi:10.1016/j.neuroscience.2004.06.037

study, we demonstrated that exogenous 5-HT application evokes a heterogeneous response profile in neurons of the anterior BNST (Rainnie, 1999a). The majority of neurons were inhibited by 5-HT, although a small population of neurons was excited by 5-HT. However, the anterior BNST is comprised of several functionally distinct subregions (De-Olmos et al., 1985; Ju and Swanson, 1989). Consequently, in the present study we restricted our recording to the anteriorlateral BNST subdivision ($BNST_{AL}$), as this region is thought to be critical for modulating the response to stressful stimuli (Alheid, 2003; Commons et al., 2003; Dunn and Williams, 1995; Ju et al., 1989). If the majority of $BNST_{AL}$ neurons are inhibited by 5-HT, this action could be anxiolytic, based on the finding that manipulations that inhibit BNST function have been shown to block anxiety-induced enhancement of the acoustic startle response (Gewirtz et al., 1998; Lee and Davis, 1997; Walker and Davis, 1997b). In the CNS, 5-HT induced inhibition is often associated with activation of $5\text{-}HT_{1A}$ receptors (Craven et al., 2001; Kelly et al., 1991; Stein et al., 2000), and it is thought that forebrain activation of $5\text{-}HT_{1A}$ receptors may contribute to the clinical profile of many anxiolytic agents (Gross et al., 2002). However, the precise region/s in which they exert their effect is not known. We have previously suggested that $5\text{-}HT_{1A}$ receptor activation might mediate the inhibitory response of BNST neurons to 5-HT (Rainnie, 1999a).

Consequently, this study was designed to 1) examine the channel properties mediating the inhibitory response to 5-HT in neurons of the $BNST_{AL}$, 2) establish an agonist profile for the receptor subtype mediating this response, and 3) use local $BNST_{AL}$ application of a receptor-specific agonist to probe the behavioral consequences of activation of this receptor.

EXPERIMENTAL PROCEDURES

In vitro experiments

Slice preparation and patch clamp recording. Slices used for whole-cell patch clamp recording, containing the $BNST_{AL}$, were obtained from anesthetized 24–40-day old male Sprague–Dawley (Charles River, Raleigh, NC, USA) rats. Under deep isoflurane (Fisher Scientific, Hanoverpark, IL, USA) anaesthesia rats were decapitated and the brain exposed. A section of the forebrain containing the BNST was blocked and mounted on the stage of a Leica VTS-1000 vibratome (Leica Microsystems, Heerbrugg, Switzerland), bathed with ice-cold artificial cerebrospinal fluid (ACSF) of the following composition: (in mM) NaCl 130; KCl 3.5; KH_2PO_4 1.10; $MgCl_2$ 1.3; $CaCl_2$ 2.5; $NaHCO_3$ 30; and glucose 5; and gassed with a 95%–5% oxygen/carbon dioxide. Coronal sections (350 μm) containing the BNST were kept in oxygenated ACSF at room temperature for a minimum period of 1 h before recording. Individual slices were transferred to a Warner Series 20 recording chamber (0.5 ml volume; Warner Instruments, Hamden, CT, USA) mounted on the fixed stage of a Leica DM-LFS microscope (Leica Microsystems). The slices were maintained fully submerged and continuously perfused with ACSF heated to 32 °C, and gassed with a 95%–5% oxygen/carbon dioxide mixture. In this study, our recording site was restricted to the $BNST_{AL}$. Whole-cell recordings were obtained as previously described (Rainnie, 1999b). Patch-clamp recording pipettes were fabricated from borosilicate glass (resistance 6–9 MΩ) and filled with a recording solution of the following composition (in mM): 130 K-gluconate, 2 KCl, 10 HEPES, 3 $MgCl_2$, 2 K-ATP, 0.2 NaGTP, and 5 phosphocreatine. Data acquisition and analysis were performed using an Axpoatch-1D amplifier (Axon Instruments, Union City, CA, USA) in conjunction with pClamp 8.0 software and a DigiData 1322A interface (Axon Instruments).

Drug application. Drugs were applied in the ACSF using a continuous gravity fed bath application. Active drug concentrations applied to the tissue were from (1) Sigma (St. Louis, MO, USA), 5-HT (2.5–160 μM); baclofen (10, 30 μM); bicuculline methiodide (30 μM); tetrodotoxin (TTX, 1 μM); *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate salt (WAY100635, 200nM), and (2) Tocris (Ellisville, MO, USA), 5-carboxamidotryptamine (5-CT, 10 μM); *R*(±)8-hydroxydipropylaminotetralin hydrobromide (8-OH-DPAT; 1–50 μM); 3-[[(3,4-dichlorophenyl) methyl]amino]propyl]diethoxymethyl)phosphinic acid (CGP 52432; 1 μM); and tertiapin-Q (0.1 μM). All drugs were made as concentrated stock solutions in distilled H_2O, except baclofen and TTX that were made in 0.05M HCl, and 30% acetic acid, respectively. Stock solutions were kept as frozen aliquots at −20 °C until required. Stock solution of tertiapin-Q also contained 0.1% bovine serum albumin (Sigma) as a carrier protein. Tertiapin-Q was bath applied for between 10 and 40 min before testing the effects of agonist application. Experiments in the presence of TTX commenced only after a complete block of current-evoked action potentials was observed.

Electrophysiological recording techniques. Junction potentials were balanced at the start of each experiment to ensure accurate measurement of the membrane potential. Whole cell patch clamp configuration was established in current clamp mode, and all test protocols were performed at a holding potential of −60 mV to allow a direct comparison of voltage responses between different neurons. Only those BNST neurons that had a stable membrane potential more negative than −55 mV, and an action potential that overshot 0 by >5 mV were considered for further analysis. In current clamp, the firing properties of each neuron were examined by injection of transient (750 ms) depolarizing current steps of increasing amplitude. Voltage-dependent input resistance (Rm) and time constant of membrane charging (τ) were examined by injection of five incrementally increasing hyperpolarizing current steps, 750 ms in duration. The Rm of each neuron was calculated from the largest voltage excursion in the linear portion of the current-voltage relationship. In voltage clamp, voltage-dependent steady state conductance was determined using a "ramp" protocol before, during, and after drug application. Here, the command potential applied to BNST neurons was "ramped" from −100 to −40 mV at a rate of approximately 10 mV/s. Any drug-induced conductance changes could then be determined by subtraction of the ramp protocol run in control ACSF from that obtained during the drug application. To examine long-duration drug effects, neural responses were recorded on a chart recorder (Kipp and Zonen, Bohemia, NY, USA) for the duration of each experiment. All current and voltage clamp paradigms were computer controlled using the pClamp 8.0 data acquisition program (Axon Instruments). Data were low pass filtered at 5 kHz during current clamp acquisition and at 1 kHz during voltage clamp analysis, and sampled at a frequency determined by the speed of the response that was to be measured.

Histochemical visualization of recorded neurons. Biocytin (0.3%) was included in the recording solution to confirm the localization of recorded neurons in the $BNST_{AL}$ and to determine their morphological characteristics. After recording, slices were removed from the recording chamber and placed in 4% paraformaldehyde overnight. The following day the slices were washed with 0.05 M phosphate buffer/0.15 M NaCl solution (PBS, 3×10 min), and re-sectioned to 70 μM using the OTS 4000 vibratome. The tissue sections were then placed in 0.6% H_2O_2 and 0.5% Triton X-100 in PBS for 1 h to quench endogenous peroxidase activity,

washed in PBS (3×10 min) before being incubated in an avidin–biotin peroxidase complex for 2 h (ABC Vectorstain Elite kit; Vector Laboratories, Inc., Burlingame, CA, USA). Slices were then washed in PBS (3×10 min), and visualized using 3,3′-diaminobenzidine (DAB; Sigma) as the chromogen (DAB 0.7 mg/ml and H_2O_2 0.2 mg/ml in 0.06 M Tris buffer). The sections were placed in DAB until adequate staining had developed, and the reaction was stopped by transferring the sections to PBS. After two more washes in PBS the sections were mounted onto gelatinized slides, air dried, then dehydrated in an ascending series of alcohols, cleared in histoclear (2×3 min), and cover-slipped with Permount mounting medium (Fisher Scientific).

Statistical analysis. Statistical analysis was performed using SPSS Version 11 (SPSS, Chicago, IL, USA). Data are presented as means±S.E.M, and were analyzed using either a *t*-test for paired or unpaired observations or the Kruskal-Wallis one-way ANOVA. For all comparisons, the criterion for significance was $P<0.05$.

In vivo behavioral experiments

Subjects. Male Sprague–Dawley rats ($n=12$; Charles River) weighing 310–360 g at the start of the experiments were used. The rats were housed in groups of four for a minimum of 1 week before the start of the experimental procedure. The rats were tested during the light phase of a 12-h light/dark cycle (artificial white light on at 7:30 a.m.). The rats had unrestricted access to food and water and all experiments were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals. Experimental procedures were designed to minimize animal numbers and discomfort, and all protocols were approved by Emory University IACUC.

Surgical procedure. Rats were anesthetized with Avertin (10 g of 99% 2,2,2-tribromoethanol, 5 g of tertiary amyl alcohol, 40 ml absolute alcohol in 450 ml phosphate-buffered saline; 1.5 ml/100 g body weight i.p.), and placed in a flat skull position in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). The incisor bar was set at −3.3 mm relative to the interaural line. A mid-line incision was made along the scalp to expose the skull surface and using a handheld drill four small holes were made for placement of bone screws. Two additional holes were drilled through the skull to expose the dura at the implantation points of the guide cannula (22-gauge, Model C313G; PlasticOne, Roanoke, VA, USA). To avoid the ventricles, guide cannulas were implanted bilaterally at a 20° angle to the vertical axis of the target implantation sites, according to the following stereotaxic coordinates (from bregma in mm): −0.26 AP, ±3.82 ML and −4.6 DV from dura. Dura was pierced with the tip of a 25 G syringe needle and the guide cannula lowered to its target depth, 2 mm above the final injection site. Dental cement was placed around the guide-post over and under the screws until a secure mount was formed.

Apparatus. Rats were tested in four identical Plexiglas and wire mesh cages (8 cm long, 15 cm wide, 15 cm high). The floor of each cage consisted of four 6-mm diameter stainless steel bars spaced 18 mm apart. Each cage was suspended between compression springs within a steel frame and located within a custom-designed, ventilated, sound-attenuating chamber (internal dimensions=81 cm long, 41 cm wide, 48 cm high). Background noise (60 dB wideband) was provided by a General Radio Type 1390-B noise generator (Concord, MA, USA) and delivered through high-frequency speakers (Radio Shack Supertweeter; Tandy, Fort Worth, TX, USA) located 5 cm from the front of each cage. Sound level measurements were made with a Brüel and Kjaer (Marlborough, MA, USA) Model 2235 sound-level meter with the microphone located 7cm from the center of the speaker, which approximates the distance of the rat's ear from the speaker during testing.

Startle responses were evoked by 50 ms white-noise bursts (5 ms rise-decay). Noise bursts were generated by a Macintosh G3 computer sound file (0–22 kHz), amplified by a Radio Shack amplifier (100 W; Model MPA-200), and delivered through the same speakers as those used to provide background noise. Startle response amplitudes were quantified with an accelerometer (Model U321AO2; PCB Piezotronics, Depew, NY, USA) affixed to the bottom of each cage. Thus, cage movement produced by the rats' startle response resulted in displacement of the accelerometer, which after being integrated, produced a voltage output proportional to the velocity of cage movement. This output was amplified (Model 483B21; PCB Piezotronics) and digitized on a scale of 0–2,500 units by an InstruNET device (Model 100B; GW Instruments, Somerville, MA, USA) interfaced to the Macintosh computer. Startle amplitude was defined as the maximal peak-to-peak voltage that occurred during the first 200 ms after onset of the startle-eliciting stimulus. The presentation and sequencing of all stimuli were controlled by a computer with custom-designed software (The Experimenter; Glassbeads, Newton, CT, USA). During the test sessions the cage displacements produced by the spontaneous activity of the animals 10 s prior to presentation of each startle stimulus were also recorded. This provided a measure of the activity of the animals during both the pre- and post drug infusion periods.

Test procedure and drug administration. Post-surgery matching: One week after surgery, the rats were placed in the startle chambers and given a matching session that consisted of a 5-min acclimatization period, after which the animals were presented with 30 startle-eliciting noise bursts at 105 dB (duration 50 ms; interstimulus interval (ISI)=30 s). The animals were subsequently divided into two groups of six rats each that had similar mean startle amplitudes across the 30 startle stimuli presented during this matching session. This test was used to make sure that rats assigned in a counterbalanced manner would begin testing with equivalent baseline startle amplitudes, which we have found to be useful, even under conditions where the same animals are tested under both drug conditions.

Testing and drug administration: The test procedure was run over 2 days, starting the day after the matching session. On day 1, six rats in one group received bilateral intra-BNST infusions of 5-CT (10 mM), and the other six rats were infused with ACSF (vehicle). On day 2, these infusions were reversed in each group. Each test session consisted of a 20-min pre-infusion baseline test, during which a 5-min acclimatization period was followed by presentations of startle-eliciting stimuli (30 noise bursts, 105 dB; ISI=30 s). Immediately after this test session the rats were removed from the startle cages and infused with either the vehicle (ACSF) or drug. The infusions (0.5 μl) were made at a flow rate of 0.25 μl/min through a 28-gauge internal cannula that extended 2 mm from the end of the guide cannula. The injectors were connected to a 2-μl micro-syringe mounted on a Harvard PHD 2000 infusion pump (Harvard Apparatus, Inc. Holliston, MA, USA), using PE tubing (i.d, 0.012 inches). Following the infusions, the injectors were left in place for a further 2 min, to restrict diffusion along the needle tract. Animals were then placed back in the startle chambers for the post-infusion test session consisting of 80 startle-eliciting noise bursts (105 dB, 50 ms, ISI=30 s). We have used this procedure frequently to evaluate either excitatory or inhibitory effects of various treatments on acoustic startle amplitude using a pre-post, crossover, within-subjects design because it has proven to be a sensitive way to evaluate treatment effects on startle amplitude in the face of large individual differences in startle amplitude between rats. The vehicle used was ACSF containing 0.1% bovine serum albumin. The drug, 5-CT was dissolved in this vehicle, and infused at a dose of 10 mM, which is equivalent to 1.6 ng/0.5μl, respectively. Aliquots of the drug and the vehicle were kept at −20 °C, until immediately before use.

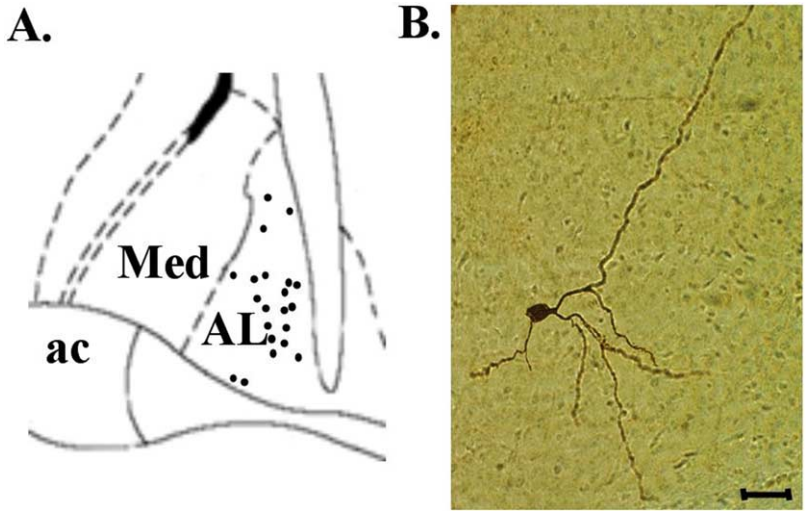

Fig. 1. Anatomical localization and morphology of neurons in the $BNST_{AL}$. (A) Schematic diagram showing the location of biocytin labeled neurons visualized with post hoc histochemistry in a coronal section through the anterior BNST. (B) Photomicrograph of a typical neuron in the $BNST_{AL}$. ac, anterior commissure; AL, $BNST_{AL}$; MED, medial BNST. Scale bar=50 μm.

Histological verification of cannula placement. Rats were anesthetized with chloral hydrate (i.p., 1 ml/100 g) and perfused intracardially with 0.9% (wt/vol) saline (4 min), followed by 10% (wt/vol) formalin (4 min). The brains were removed and post-fixed for 2 h, before immersion overnight in 30% (wt/vol) sucrose solution made in phosphate buffered saline for cryoprotection. The following day 60 μm coronal sections were cut through the area of interest using a freezing microtome. Every second section was mounted onto gelatinized slides, and allowed to dry overnight, and then stained with Cresyl Violet. The sections were then dehydrated in a series of alcohols, cleared in Histoclear (2×3 min; Sigma), and cover-slipped with Permount mounting medium (Fisher Scientific).

Statistical analysis. For each animal the 110 acoustic startle responses, 30 acquired during the baseline test session and 80 acquired during the post-infusion test session (drug or vehicle), were collapsed into 11 5-min blocks. The mean score of each 5-min block was designated as the raw startle score, on which all statistical analyses were performed. For data presentation the raw startle scores were converted into percentage scores with respect to the mean of the last 5-min pre-infusion baseline startle because by this time period startle generally has habituated to a stable asymptotic level (see Walker and Davis, 2002). All statistical analyses were performed using SPSS Version 11 (SPSS). Post hoc tests were performed where appropriate, using GB-Stat for windows (Dynamic Microsystems, Silver Spring, MD, USA). The acoustic startle data were analyzed using a repeated measures ANOVA, run separately for the pre-infusion, and post infusion test sessions. Subsequent paired *t*-tests compared startle amplitude during the last block of the pre-infusion baseline test with the first block post-infusion separately for both the ACSF and 5-CT conditions. For all comparisons, the criterion for significance was $P<0.05$.

RESULTS

In vitro characterization of the effect of 5-HT on $BNST_{AL}$ neurons

Neuron location and morphology. The $BNST_{AL}$ is easily distinguishable from the adjacent medial subdivision using low power (4×) stereomicroscope, allowing accurate targeting of neurons in this region in our *in vitro* slice preparation. Moreover, we confirmed that recorded neurons were confined to the $BNST_{AL}$ by including biocytin in the patch recording solution in many experiments. All of the 21 biocytin-filled neurons successfully recovered were located in the $BNST_{AL}$, dorsal to the anterior commissure between 0.0 and −0.6 mm to bregma. A schematic diagram of the position of recorded neurons and a typical example of a filled neuron are shown in Fig. 1. In agreement with McDonald (1983), neurons of the $BNST_{AL}$ had a characteristic oval soma (approximately 22×15 μm) from which emanated two to four primary spine-sparse dendrites. The length of the primary dendrites ranged from 126 to 501 μm. Unfortunately, full morphometric reconstruction on several of the recovered cells was not possible due to the loss of some of the dendritic arbor during tissue processing. Consequently, the sample of fully reconstructed neurons is too small at present to make any definitive statements about the possible correlation between $BNST_{AL}$ neuronal morphology and a particular 5-HT response.

Response of $BNST_{AL}$ neurons to exogenous application of 5-HT. Here we confirm and expand on our previous observations (Rainnie, 1999a), and show that 5-HT evoked a heterogeneous response in neurons of the $BNST_{AL}$ (see Fig. 2A), similar to that found when recording from the entirety of the anterior BNST. For a direct comparison with our previous study, we initially examined the response of $BNST_{AL}$ neurons to exogenous application of 50 μM 5-HT. The response profile of $BNST_{AL}$ neurons ($n=175$) is illustrated in Fig. 2A. 5-HT evoked a membrane hyperpolarization ($5\text{-}HT_{Hyp}$) in 37% of neurons tested, a hyperpolarization followed by a depolarization ($5\text{-}HT_{Hyp\text{-}Dep}$) in 19%, a depolarization ($5\text{-}HT_{Dep}$) in 25%, and had no effect on the resting membrane potential in the remaining 19% of neurons. The response profile of $BNST_{AL}$ neurons

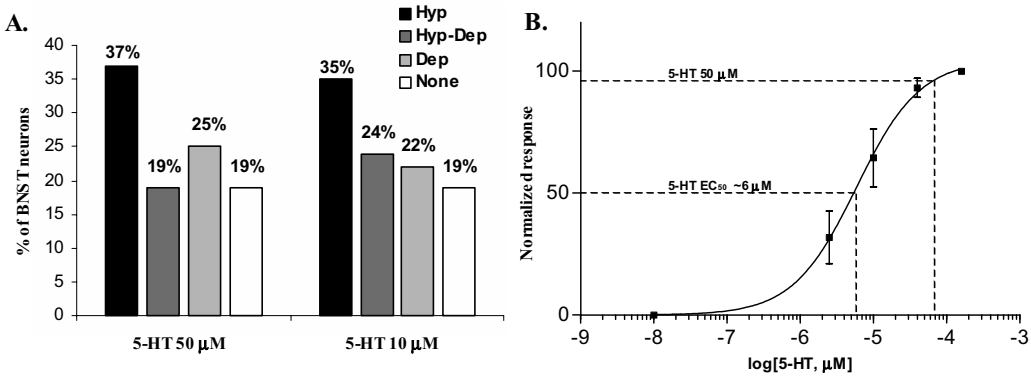

Fig. 2. Exogenous 5-HT evokes a heterogeneous response profile in $BNST_{AL}$ neurons. (A) Application of 50 μM 5-HT evoked either a monophasic membrane hyperpolarization (Hyp), a biphasic hyperpolarization followed by a depolarization (Hyp-Dep), a monophasic membrane depolarization (Dep), or had no effect on the resting membrane potential of $BNST_{AL}$ neurons (None). A five-fold reduction in 5-HT concentration (10 μM) did not alter the response profile. (B) Dose-response curve for 5-HT evoked membrane hyperpolarization, each point shows the average of four experiments±S.E.M. All responses were normalized to the peak response activated by 160 μM 5-HT.

to 5-HT was insensitive to pretreatment with TTX (1 μM, $n=24$) supporting a direct post-synaptic action of 5-HT on these neurons.

In agreement with our earlier observation, both the 5-HT_{Hyp} and 5-HT_{Dep} were associated with a decrease in Rm (37±2.4%), and a concomitant increase in conductance. The mean change in Rm, the amplitude of the response, and the reversal potential associated with each response are summarized in Table 1. Further analysis of this data revealed no correlation between the response to 5-HT and the passive membrane properties of $BNST_{AL}$ neurones ($\chi^2=2.7$, 3.3, 4.5 analysis of RMP, Rm and τ, respectively, $df=3$; Kruskal-Wallis one-way ANOVA).

It should be noted that the predominant response was membrane hyperpolarization, irrespective of the 5-HT concentration applied. Hence, even in those neurons that showed a biphasic response to 5-HT, in which an initial hyperpolarizing response was followed by a depolarization, the depolarization was rarely of sufficient magnitude to overcome the preceding inhibition. Indeed, in only four of 34 neurons did the depolarizing component of the $5\text{-HT}_{Hyp\text{-}Dep}$ overshoot the resting membrane potential. Hence, for most $BNST_{AL}$ neurons the net effect of 5-HT receptor activation was a reduction of membrane excitability.

The majority of 5-HT receptor subtypes expressed in the BNST are G-protein-coupled receptors, activation of which could result in desensitization, and or internalization (Albert and Tiberi, 2001; Marchese et al., 2003; Sadja et al., 2003). Consequently, the delayed depolarization observed in $BNST_{AL}$ neurons in which 5-HT evoked a biphasic response ($5\text{-HT}_{Hyp\text{-}Dep}$) could result from a delayed desensitization of the 5-HT_{Hyp} response. Desensitization of the 5-HT_{Hyp} response would be expected to significantly attenuate the response of these neurons to subsequent agonist exposure. However, no significant alteration in either the magnitude ($t=0.51$, $df=11$) or the pattern of response (Hyp, Hyp-Dep, or Dep) was observed between the first and second applications of 5-HT ($n=12$). These data suggest that the $5\text{-HT}_{Hyp\text{-}Dep}$ response observed in $BNST_{AL}$ neurons did not result from desensitization of the receptors mediating 5-HT_{Hyp} response, but more likely reflected a delayed activation of an independent depolarizing conductance.

The reproducibility of the 5-HT response allowed us to construct a dose-response relationship to 5-HT in the

Table 1. Physiological response profile of $BNST_{AL}$ neurons to exogenous 5-HT application

	Membrane response	*N*	ΔRMP	Decease in ΔRm%	$E_{5\text{-HT}}$	RMP[2]	Rm MΩ	τ
5-HT 50 μM[1]	Hyp	64	−5.2±0.3 mV −14.6±3.2 pA	−35.0±3.0	−77±1	−61±0.9	453±36	38±3.7
	Hyp/Dep	34	−12.2+1.9/+7.1±2.6 pA	−40.0±4.0	−71±2	−61±1.0	359±25	30.5±2.5
	Dep	44	+5.3±0.6 mV +13.7±2.7 pA	−23.0±4.0	−44±2	−63±1.0	413±38	32.5±3.1
	None	33	–	–	–	−60±1.0	382±41	27.5±2.5

[1] 5-HT application in ACSF and TTX.

[2] This resting membrane potential reflects the membrane potential at the start of the experiment. Note that the membrane potential at which all test protocols were run was −60 mV. RMP, resting membrane potential; ΔRMP, change in resting membrane potential; ΔRm, change in the Rm; τ, time constant of membrane charging; Hyp, hyperpolarization; Hyp-Dep, hyperpolarization followed by a depolarization; Dep, depolarization.

same neuron (Fig. 2B). As 5-HT_{Hyp} was the most frequently occurring response of $BNST_{AL}$ neurons, we examined the 5-HT dose-response relationship in a subgroup of neurons where an initial application of 5-HT evoked a clear monophasic 5-HT_{Hyp}. In these experiments, four concentrations of 5-HT (2.5, 10, 40 and 160 μM) were applied in order of increasing concentration. A sigmoidal best-fit curve (GraphPad Prism, version 3.03; San Diego, CA, USA) of the dose-response data revealed an EC_{50} for 5-HT of 6 μM, with a correlation of coefficient, 0.99. Moreover, the dose-response relationship revealed that our test concentration of 50 μM was close to a maximal response for 5-HT_{Hyp} (approximately 95%). It was possible, therefore, that the heterogeneous response of $BNST_{AL}$ neurons to 5-HT was biased in favor of a 5-HT_{Hyp} response due to the high test concentration of 5-HT. Consequently, we next examined the effects of a 5-HT concentration close to the EC_{50}, 10 μM.

As shown in Fig. 2A, exogenous application of 10 μM 5-HT evoked a heterogeneous response profile in BNST neurons that was qualitatively similar to that observed at the higher concentration. Thus, 10 μM 5-HT evoked a 5-HT_{Hyp} in 35% of neurons tested, a 5-$HT_{Hyp\text{-}Dep}$ in 24%, 5-HT_{Dep} in 22%, and had no effect in the remaining 19% of the neurons tested. No difference was observed in the 5-HT response of $BNST_{AL}$ neurons in control ACSF when compared with ACSF containing TTX, and hence the data presented were combined from experiments run in ACSF (n=35), or in TTX (n=11).

Cellular mechanisms underlying 5-HT_{Hyp}. Analysis of the 5-HT reversal potential ($E_{5\text{-}HT}$) in $BNST_{AL}$ neurons showing only a 5-HT_{Hyp} response, revealed two distinct subpopulations. The majority of neurons (75%) showed a $E_{5\text{-}HT}$ of −74±0.8 mV (n=35). This reversal potential was close to that predicted by the Nernst equation for a $GABA_A$ receptor-mediated increase in chloride conductance and hence, the 5-HT_{Hyp} in these $BNST_{AL}$ neurons may have resulted from an indirect action of 5-HT via an increase in TTX-resistant GABA release. An indirect action of 5-HT is possible as neurons of the anterior BNST are primarily GABAergic (Sun and Cassell, 1993), and we have shown that 5-HT excites approximately 23% of $BNST_{AL}$ neurons. However, pretreatment with a cocktail of the $GABA_A$ receptor antagonist, bicuculline methiodide (30 μM), and the $GABA_B$ receptor antagonist, CGP 52432 (1 μM), failed to block the 5-HT_{Hyp} response and did not alter $E_{5\text{-}HT}$ (data not shown). If the 5-HT_{Hyp} response was due to indirect release of GABA and subsequent activation of postsynaptic $GABA_{A/B}$ receptors, this response should have been blocked by the addition of the GABA receptor antagonists, which was clearly not the case.

These data suggest that even in those $BNST_{AL}$ neurons that exhibit a 5-HT_{Hyp} response, exogenous 5-HT may activate more than one 5-HT receptor subtype. Consequently, bidirectional modulation of $BNST_{AL}$ neurons could occur in the majority of 5-HT_{Hyp} neurons and not only in neurons that show a 5-$HT_{Hyp\text{-}Dep}$ response.

The remaining 25% of 5-HT_{Hyp} neurons (12/47) showed an $E_{5\text{-}HT}$ of −89±1.1 mV. This reversal potential was identical to that predicted by the Nernst equation for K^+ (E_K) using intracellular and extracellular K^+ concentrations derived from our ACSF and patch recording solutions. Hence, an increase in K^+ conductance mediates the monophasic outward current underlying the 5-HT_{Hyp} in this subgroup of $BNST_{AL}$ neurons.

Data from our preliminary study suggested that the 5-HT_{Hyp} observed in BNST neurons might be mediated by activation of a K^+ current that showed inward rectification (Rainnie, 1999a). Here we confirm this observation and show that the 5-HT_{Hyp} in $BNST_{AL}$ neurons is mediated by a K^+ conductance carried by G protein-activated inwardly rectifying K^+ (G_{IRK}) channels. Firstly, the 5-HT-induced current-voltage relationship was determined in voltage clamp by subtraction of the control steady-state current from the 5-HT-induced steady state current over the voltage range −100 to −40 mV. The 5-HT-induced chord conductance was then calculated using the following equation: $g5\text{-HT}=I_{5\text{-HT}}/(E-E_{5\text{-HT}})$; where $I_{5\text{-HT}}$ is the residual 5-HT-induced current, E is the holding potential, and $E_{5\text{-HT}}$ is the 5-HT reversal potential (approximately −89 mV). A typical plot showing the inwardly rectifying property of the 5-HT chord conductance is illustrated in Fig. 3A. Secondly, the activation of G_{IRK} channels by 5-HT was confirmed by examining the sensitivity of the 5-HT response to tertiapin-Q, a specific inhibitor of G_{IRK} channels (Jin et al., 1999; Jin and Lu, 1999). Only neurons in which 5-HT evoked a 5-HT_{Hyp} response were used to test the effect of tertiapin-Q. Here, the initial response to 5-HT was determined and the slice was then perfused with tertiapin-Q (0.1 μM) for 10–40 min, before 5-HT was re-applied. A 20 min pre-exposure to tertiapin-Q (n=3) attenuated the peak 5-HT_{Hyp} response (50%), whereas a 40 min pre-exposure to tertiapin-Q completely blocked the 5-HT_{Hyp} response (n=2). Incubation times of 10 min were ineffective in blocking the 5-HT_{Hyp} response (n=2).

Response of $BNST_{AL}$ neurons to exogenous application of 8-OH-DPAT and 5-CT. The 5-HT receptor subtype that is most often associated with activation of G_{IRK} channels is the 5-HT_{1A} receptor (e.g. Luscher et al., 1997). Hence, we next examined the response of $BNST_{AL}$ neurons to exogenous application of the 5-$HT_{1A/7}$ receptor agonist, 8-OH-DPAT, and the more broad-spectrum 5-HT_1 receptor agonist, 5-CT. The aim of these experiments was two-fold: (1) to verify the 5-HT receptor subtype mediating the $5HT_{Hyp}$ response, and (2) to find a relatively specific agonist that would, ideally, mimic only the inhibitory response to 5-HT, which we could then use to test the functional consequences of the inhibitory action of 5-HT *in vivo*.

We first examined the response of $BNST_{AL}$ neurons to the active (R) enantiomer of 8-OH-DPAT (Cornfield et al., 1991; Hadrava et al., 1996). In these experiments, we first compared the response of neurons to 5-HT with that of 8-OH-DPAT. Only those neurons showing 5-HT_{Hyp} (n=8), or a 5-$HT_{Hyp\text{-}Dep}$ (n=2) were used as comparators for the

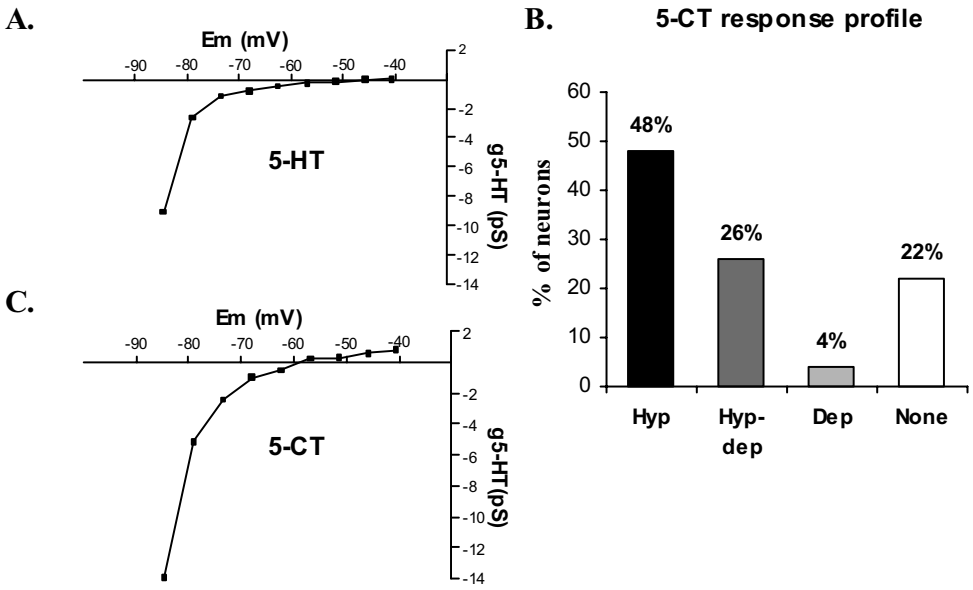

Fig. 3. The 5-HT_1 receptor ligand 5-CT activates G_{IRK} channels and mimics the inhibitory response of $BNST_{AL}$ neurons to 5-HT. Plots of the chord conductance evoked by 5-HT (A) and 5-CT (C) show similar voltage-dependent inward rectification. Chord conductance was calculated using the equation $g5\text{-HT}=I_{5\text{-HT}}/(E-E_{5\text{-HT}})$, where $I_{5\text{-HT}}$ was determined using voltage ramps from −100 mV to −40 mV before and during drug application. (B) The response profile of $BNST_{AL}$ neurons to exogenous 5-CT (10μM) application largely mimicked the inhibitory effect of 5-HT in $BNST_{AL}$ neurons.

response to 8-OH-DPAT. Unexpectedly, 8-OH-DPAT (1–50 μM) failed to elicit a response in nine out of the 10 $BNST_{AL}$ neurons tested.

Hence, we next examined the response of $BNST_{AL}$ neurons to 5-CT (10 μM), a broad-spectrum 5-HT1 receptor agonist with a high affinity for 5-HT_{1A} receptors (Palacios et al., 1996; Thomas et al., 1999). Unlike 8-OH-DPAT, 5-CT fully mimicked the inhibitory response to 5-HT in $BNST_{AL}$ neurons (Fig. 3B and 3C). In 23 neurons examined, 5-CT evoked a hyperpolarization in 48%, a hyperpolarization followed by a depolarization in 26%, or had no effect on the resting membrane potential in the remaining neurons tested (22%). Like the 5-HT_{Hyp}, the 5-CT induced hyperpolarization was associated with a decrease in membrane Rm, and an outward current in voltage clamp. The mean change in Rm, the amplitude of the response, and the reversal potential associated with each response are summarized in Table 2. In one neuron, 5-CT evoked a monophasic depolarizing response. Interestingly, this neuron exhibited a physiological phenotype resembling that of an interneuron, as defined in brain regions such as the basolateral amygdala (see Rainnie et al., 1993).

We then compared the response to 5-CT and 5-HT in the same neuron ($n=8$); a typical example is illustrated in Fig. 4A. Here, 50 μM 5-HT evoked an outward current followed by a delayed inward current in voltage clamp mode, which is characteristic of the 5-$HT_{Hyp\text{-}Dep}$ observed in current clamp mode. Subsequent application of 10 μM 5-CT in the same neuron evoked only an outward current. Significantly, the magnitude of the 5-CT response was approximately 43% greater than that evoked by 5-HT in neurons that showed only a 5-HT_{Hyp} response, or an outward current ($n=4$, $t=10$, $P<0.05$). The effects of 5-CT were longer lasting than those observed for 5-HT. Thus, the response to 5-HT returned to pre-5-HT baseline within 5–10 min of washout. In contrast, 5-CT response returned

Table 2. Physiological response profile of $BNST_{AL}$ neurons to exogenous 5-CT application

	Membrane response	*N*	ΔRMP	Decease in ΔRm%	$E_{5\text{-CT}}$	RMP[2]	Rm MΩ	τ
5-CT 10 μM[1]	Hyp	11	−7.0±1.4 mV −25±9pA	−34.6±6.1	−84±3	−61±1.8	589±73	36±5.4
	Hyp-Dep	6	−8.0/+3.5 mV −15.0±3.5/+9.5±4.5 pA	−32±7.3	−71±2	−60±0.4	412±119	22±10.5
	Dep	1	+15.0 pA	–	−45	−61	702	66
	None	5	–	–	–	−64±5.6	527±137	35±10

[1] 5-CT application in ACSF and TTX.

[2] This resting membrane potential reflects the membrane potential at the start of the experiment. Note that the membrane potential at which all test protocols were run was −60 mV. RMP, resting membrane potential; ΔRMP, change in resting membrane potential; ΔRm, change in the Rm; τ, time constant of membrane charging; Hyp, hyperpolarization; Hyp-Dep, hyperpolarization followed by a depolarization; Dep, depolarization.

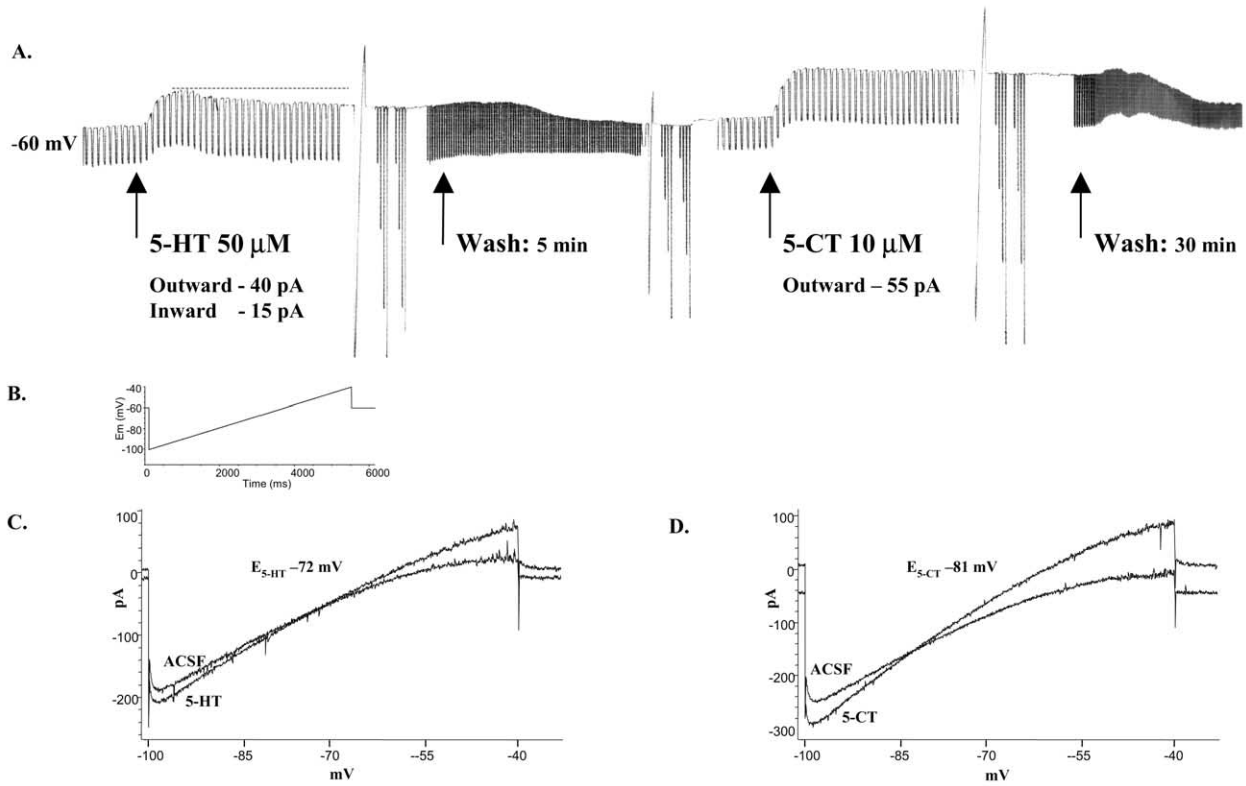

Fig. 4. In the same $BNST_{AL}$ neuron, 5-CT mimics the outward current evoked by 5-HT but not the inward current. (A) Voltage clamp records showing the response of a $BNST_{AL}$ neuron to sequential application of 5-HT and 5-CT. Here, 5-HT (50 μM) evoked a rapid outward current followed by an inward current. After washout, 5-CT (10 μM) evoked only a monophasic outward current that was 37% larger than that evoked by a higher concentration of 5-HT. (B) The ramp protocol used in voltage clamp experiments to generate the steady-state current records shown in C and D. (C) Superimposing the steady-state currents evoked before, and during 5-HT application show a reversal potential of approximately −72 mV. (D) In contrast, superimposition of the steady-state currents evoked before, and during 5-CT application show a reversal potential of approximately −81 mV. Holding potential=−60 mV.

to baseline only after 30–40 min of washout. However, despite the differences in the amplitude and duration of the 5-CT response compared with the 5-HT response, the reversal potential ($E_{5\text{-CT}}=-84\pm3$ mV; $n=11$) and the chord conductance were identical to that of 5-HT (Fig. 3C). Consequently, these data suggest that the 5-CT response was also mediated by activation of an inwardly rectifying K^+ conductance. Neurons that were unresponsive to 5-HT were also unresponsive to 5-CT ($n=3$).

5-HT_{Hyp} and 5-CT-evoked membrane hyperpolarization (5-CT_{Hyp}) are blocked by the 5-HT_{1A} antagonist, WAY100635. The 5-HT_{Hyp} observed in our experiments had the hallmark characteristic of a 5-HT_{1A} response, despite the inability of 8-OH-DPAT to mimic it. Consequently, we next examined the effect of prior application of WAY100635 (200nM), a specific 5-HT_{1A} antagonist, on the 5-HT_{Hyp} and 5-CT_{Hyp} evoked responses in $BNST_{AL}$ neurons. In these experiments, we first determined the response to 5-HT (50 μM), or 5-CT (10 μM), and then, after washout, the slice was perfused with WAY100635 (200 nM) for 10 min before reapplying the agonists. In agreement with our hypothesis, prior incubation with WAY100635 fully blocked the outward current evoked by either 5-HT ($n=11$) or 5-CT ($n=6$). Consequently, despite the insensitivity of $BNST_{AL}$ neurons to 8-OH-DPAT, the data above supports the hypothesis that a direct postsynaptic activation of 5-HT_{1A} receptors mediates the inhibitory response of $BNST_{AL}$ neurons to both 5-HT and 5-CT.

In vivo behavioral experiment

Previous *in vivo* studies had reported that systemic administration of 5-HT receptor ligands have anxiolytic actions as measured with either the fear-potentiated startle test (Davis et al., 1986; Mansbach and Geyer, 1988) or light-enhanced startle (Walker and Davis, 1997a; de Jongh et al., 2002). However, because of the route of drug administration used in these experiments it was impossible to determine the site of action of these compounds. Because non-specific activation of BNST neurons by *in vivo* electrical stimulation is believed to be anxiogenic (ibid), we hypothesized that the inhibitory response of $BNST_{AL}$ neurons to 5-HT would reduce acoustic startle amplitude under conditions where startle tends to increase as a result of dishabituation following handling. As a first test of this hypothesis, we examined the effects of bilateral infusion of 5-CT into the BNST on the acoustic startle response, using a paradigm where we normally see dishabituation of the startle reflex that

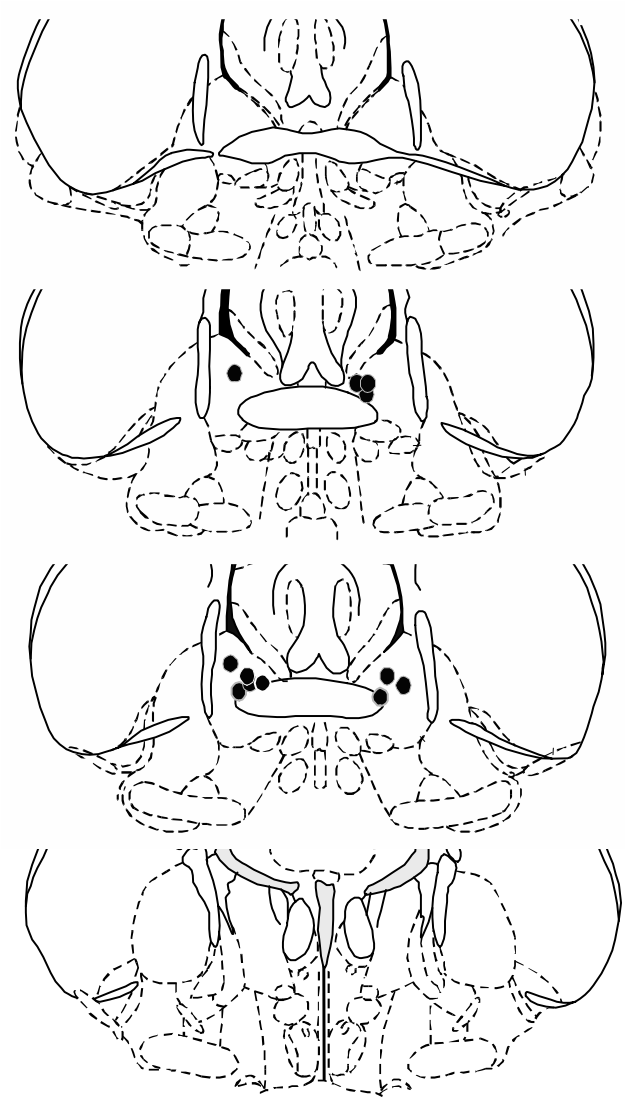

Fig. 5. A schematic representation of the placement of the injection cannula tip in the BNST. Tip placement in animals receiving 10 mM 5-CT ($n=6$) infusions. Coronal sections are shown from −0.26 to −0.8 relative to bregma.

occurs with handling, following a modest amount of prior habituation. 5-CT was chosen in preference to 5-HT as it mimics the inhibitory action of 5-HT, has a long duration of response, and is not affected by local degradation and reuptake processes that could affect local 5-HT concentrations.

Histology. To ensure that any behavioral response observed was due to activation of 5-HT receptors in the BNST, the position of the injection cannula was verified at the end of the experimental procedure. From the original cohort of 12 rats tested, data from six rats were excluded due to one or both injection sites being outside of the BNST (either in the lateral ventricles, or the septum). Fig. 5 shows a schematic of the cannula placements marking the final location of the injection site in the BNST.

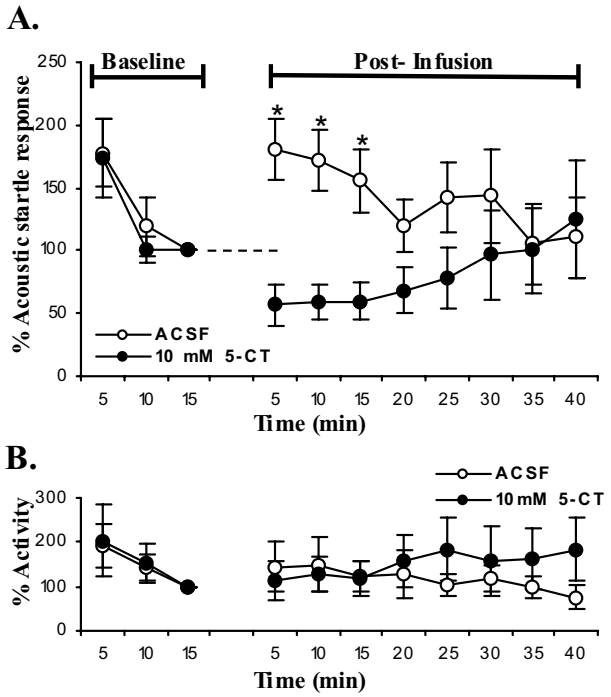

Fig. 6. *In vivo* infusion of 5-CT into the $BNST_{AL}$ attenuates the acoustic startle response. (A) Startle amplitude was significantly reduced when rats received 5-CT 10 mM bilateral intra-BNST infusions. The figure shows the percentage mean startle score (mean of 10 startle stimuli±S.E.M.) of rats receiving intra-BNST infusion of ACSF or 5-CT, during the pre-infusion (baseline) and post-infusion test sessions. In contrast, 5-CT had no effect on motor activity of the rats. (B) Mean motor activity scores±S.E.M.

The effect intra-BNST 5-CT infusions on the acoustic startle response. As expected from the electrophysiology, local infusions of 5-CT (10 mM) into the BNST produced a decrease in the amplitude of the acoustic startle response. The group data are illustrated in Fig. 6A. Here, local infusion of 5-CT into BNST significantly reduced startle amplitudes, starting immediately at the onset of the post-infusion test session, which commenced approximately 4 min after the 5-CT infusion protocol, and lasting for at least 15 min post-infusion. A repeated measures ANOVA of the data demonstrated a significant effect of Treatment ($F_{1,10}=4.8$, $P<0.05$), and a significant Treatment×Time interaction ($F_{7,70}=3.9$, $P<0.05$), with post hoc Tukey/Kramer tests revealing a significant difference in startle amplitudes in the first 15 min post-infusion.

Importantly, the difference in acoustic startle amplitudes following 5-CT infusion were dependent on the manipulation the rats received, and did not result from differences in their baseline acoustic startle response on the 2 days in which this experiment was run. Thus, analysis of the baseline acoustic startle response (before being normalized to a percentage score) showed that there was no significant difference between the two test groups in their pre-infusion baseline startle amplitudes across the 2 experimental days ($F_{1,5}=1.3$), and that these animals showed significant and yet equivalent levels of habituation

of the startle response during the pre-infusion test sessions ($F_{1,5}=4.1$, $P<0.5$).

As often observed in this behavioral paradigm, cessation of the startle stimuli, removal of the rat from the test cage, and the handling that takes place during infusion of ACSF can lead to significant dishabituation of the startle response ($t(5)=3.4$, $P<0.05$), comparing the last block of the preinfusion baseline to the first block of the post-infusion test. In contrast, on the day the same rats received 5-CT infusions there was no dishabituation. However, the effect of 5-CT could not be attributed entirely to a blockade of dishabituation because the level of startle right after 5-CT infusion was actually lower than that seen at the end of habituation during the baseline period (see Fig. 6A). Thus, the amplitude of startle during the first block of 10 startle stimuli in the 5-CT condition was significantly less than that during the last block of the preinfusion baseline ($t(5)=2.7$, $P<0.05$).

Interestingly, rats in which only one cannula was correctly placed in the BNST showed a trend toward a reduction in startle amplitude, but the effect did not reach threshold for significance ($n=3$). Moreover, analysis of startle amplitudes in those rats which were also excluded from the study because both of the injection cannulas were misplaced, either in the lateral ventricle, or the septum ($n=3$), showed that 5-CT infusions in these rats had no effect on acoustic startle amplitudes, supporting a discrete effect of 5-CT within the BNST.

Motor activity. Because of its connection with areas such as the ventromedial caudate-putamen (Dong et al., 2000), the BNST is in a position to modulate somatomotor outflow, and hence motor activity. Startle amplitude is negatively correlated with the degree of motor activity preceding the startle stimulus (Hoffman and Ison, 1980; Plappert et al., 1993), and hence our 5-CT manipulations could have affected general motor activity and thus indirectly attenuated the acoustic startle response. Consequently, we also examined the effect of local infusion of 5-CT into the $BNST_{AL}$ on motor activity. A repeated measures ANOVA demonstrated no significant effect on motor activity of 5-CT during the post-infusion period ($F_{1,10}=1.4$, Fig. 6B). Moreover, the motor activity of the animals also did not differ during the pre-infusion baseline session across the two days ($F_{1,10}=0.6$). Thus, the decrease in startle amplitude as a consequence of 5-CT BNST infusion does not seem to be an indirect effect on motor activity.

DISCUSSION

We have used both *in vitro* and *in vivo* experimental paradigms to examine the functional consequences of 5-HT receptor activation in the $BNST_{AL}$. Exogenous application of 5-HT can both excite and inhibit $BNST_{AL}$ neurons by a direct action at postsynaptic 5-HT receptors. Nevertheless, our studies demonstrate that although 5-HT can activate more than one postsynaptic 5-HT receptor on $BNST_{AL}$ neurons, the net response to 5-HT release in this region would be inhibition, and this local inhibition might have an anxiolytic behavioral effect. The inhibitory response to 5-HT was observed in approximately 56% of $BNST_{AL}$ neurons, had a reversal potential of approximately −89 mV, was attenuated by the G_{IRK} channel blocker, tertiapin-Q, mimicked by the broad-spectrum 5-HT_1 receptor agonist, 5-CT, but not by 8-OH-DPAT, and blocked by the 5-HT_{1A} receptor antagonist WAY100635. Together, these data suggest that the inhibitory response of $BNST_{AL}$ neurons to 5-HT was mediated by activation of 5-$HT_{1A\text{-like}}$ receptors coupled to G_{IRK} channels.

Moreover, we delineated 5-CT as a prototypical agonist for the inhibitory response to 5-HT, mediated by activation of 5-HT_{1A} receptors, because 5-CT_{Hyp} was fully blocked by WAY100635. Consequently, we then used 5-CT as an *in vivo* tool to probe the behavioral consequences of local inhibition of $BNST_{AL}$ neurons. Here, local activation of 5-$HT_{1A\text{-like}}$ receptors in the $BNST_{AL}$ caused a significant reduction in the acoustic startle response without affecting motor activity.

Data from previous *in vivo* single unit recording studies have suggested that 5-HT, noradrenaline and dopamine, and the endogenous opioid peptides all function to inhibit neuronal activity in the BNST (Casada and Dafny, 1993b; Dalsass and Siegel, 1990; Matsui and Yamamoto, 1984; Sawada and Yamamoto, 1981). In contrast, acetylcholine was the only neurotransmitter reported to increase the firing rate of BNST neurons (Casada and Dafny, 1993a,b). While our results partially confirm these early observations, it is clear that 5-HT plays a much more complex modulatory role on the activity of BNST neurons, as would be predicted by the expression of multiple 5-HT receptor subtypes in this region (Cornea-Hebert et al., 1999; Heidmann et al., 1998; Sari et al., 1999; Waeber and Moskowitz, 1997).

At opposite ends of the postsynaptic 5-HT response spectrum are two subpopulations of $BNST_{AL}$ neurons, one that was inhibited by 5-HT and the other that was excited by 5-HT. The factors determining the relative expression of these two opposing responses in individual BNST neurons remain unknown. Nevertheless, the majority of $BNST_{AL}$ neurons were inhibited by 5-HT and this action was mimicked by the broad-spectrum 5-HT_1 receptor agonist, 5-CT. Moreover, 5-HT and 5-CT both activate 5-HT receptors that are coupled to inwardly rectifying potassium (G_{IRK}) channels. As previously noted, the 5-HT receptor subtype most often associated with activation of G_{IRK} channels is the 5-HT_{1A} receptor. It was somewhat surprising therefore that 8-OH-DPAT did not mimic the 5-HT_{Hyp} response. However, while 8-OH-DPAT acts a full agonist at presynaptic 5-HT_{1A} autoreceptors, it can display partial agonist properties in other systems (Varrault and Bockaert, 1992; Rueter et al., 1998), and it is possible that 8-OH-DPAT also acts as a partial agonist at 5-HT_{1A} receptors in the $BNST_{AL}$. 5-HT and 5-CT also have a high affinity for 5-HT_7 receptors that are expressed at high levels in the BNST (Heidmann et al., 1998), and for which both are full agonists. Although 8-OH-DPAT has a high affinity for 5-HT_7 receptors, it acts only as a partial agonist (Krobert et al., 2001; Thomas et al., 1999; Wood et al., 2000). Consequently, this rank order of potency for 5-HT_7 receptors

could explain the agonist profile for the 5-HT_{Hyp} response. However, two observations would argue against this conclusion: 1) the specific 5-HT1A receptor antagonist, WAY100635 (200 nM), fully blocked the outward currents evoked by both 5-HT and 5-CT, and 2) 5-HT_7 receptor activation in the thalamus elicits a membrane depolarization and not a hyperpolarization (Chapin and Andrade, 2001). However, because of the inconsistency with the 8-OH-DPAT data we have referred to the receptor mediating the 5-HT_{Hyp} as a 5-$HT_{1A\text{-like}}$ receptor.

Neurons of the $BNST_{AL}$ have a high Rm (Rm=416.0±20.0 MΩ, but see Egli and Winder, 2003) when compared with the medium spiny GABAergic neurons of the adjacent dorsal striatum (Rm=59.1±2.1 MΩ; Delgado et al., 2000). Consequently, $BNST_{AL}$ neurons are highly responsive to afferent input. Thus, the 5-HT_{Hyp} response not only moves the membrane potential away from the threshold for action potential generation, but by additionally reducing Rm by 35% would significantly dampen the response of these neurons to afferent input. However, the significance of the voltage-dependency of the 5-HT_{Hyp} response should not be overlooked. At membrane potentials close to rest (>−60 mV) G_{IRK} channels are functionally closed (for review see, Mark and Herlitze, 2000), and hence excitatory afferent input could still activate $BNST_{AL}$ neurons, despite continued 5-HT_{1A} receptor activation. This property ensures that excitatory input of sufficient magnitude to overcome a 5-HT_{Hyp} response could drive the membrane potential to action potential threshold unopposed.

Stimulation of the BNST mimics many of the behavioral and cardiovascular responses that are activated in response to stressful/anxiogenic stimuli (ibid). In contrast, BNST lesions or pharmacological inactivation are reported to have anxiolytic actions (Brutus et al., 1988; Fendt et al., 2003; Hammack et al., 2004; Walker and Davis, 1997b). Our *in vivo* study shows that local infusions of 10 mM 5-CT into the BNST significantly reduced startle amplitude, without affecting the general motor activity, and recent evidence suggests that such a reduction in baseline-startle may reflect a reduction in anxiety state (Baas et al., 2002; for review see, Grillon, 2002). Consistent with these results, inactivation of the BNST following infusion of muscimol, a $GABA_A$ agonist, has no effect on general motor activity while simultaneously blocking freezing to an aversive predator odor (Fendt et al., 2003).

Activation of the serotonergic system is associated with the behavioral response to stress (Clement et al., 1993; Duncan et al., 1993; Summers et al., 2003), and extracellular levels of 5-HT increase in several forebrain regions in response to stressful stimuli/conditions (Amat et al., 1998; Fujino et al., 2002; Funada and Hara 2001). Consequently, 5-HT release in the $BNST_{AL}$ would result in 5-$HT_{1A\text{-like}}$ receptor-mediated inhibition of the majority of neurons, and as a result dampen stress-induced activation of the BNST. We propose that, as the BNST and dorsal raphé are reciprocally connected, this may represent a feedback inhibitory loop, which acts as an essential part of the normal homeostatic mechanism that controls the intensity and duration of stress-induced anxiety.

The precise neural circuitry by which inhibition of $BNST_{AL}$ activity could modulate anxiety and acoustic startle is not known. Neurons of the $BNST_{AL}$ are primarily GABAergic, with more than 75% of neurons expressing mRNA for the GABA synthetic enzyme, GAD_{67} (Cullinan et al., 1993; Erlander et al., 1991). Hence, output neurons of the $BNST_{AL}$ would be expected to inhibit their target sites and, by extrapolation, 5-HT-induced inhibition of $BNST_{AL}$ neurons would act to disinhibit these target sites. The startle response is mediated by a pathway that can be traced from the cochlear root neurons to neurons of the nucleus reticularis pontis caudalis (PnC), whose fibers synapse onto spinal motor neurons that mediate the startle reflex (Lee et al., 1996). It is at the PnC that higher sensory and cognitive processes are thought impact the reflex circuit (for review see, Koch and Schnitzler, 1997). A potential route by which the BNST might influence startle amplitude is via its connection with cholinergic neurons of the pedunculopontine and lateral tegmental areas (pedunculopontine tegmental nucleus [PPTg] and lateral tegmental nucleus [LTDg]; Semba and Fibiger, 1992). Activation of these cholinergic neurons inhibits neurons of the PnC and attenuates the acoustic startle response (Fendt and Koch, 1999; Koch et al., 1993). Hence, inhibition of BNST output neurons by 5-HT could disinhibit the PPTg and LTDg, thereby enhancing acetylcholine release in the PnC and attenuating the startle response. It should be noted, however, that many BNST neurons co-localize peptide neurotransmitters and that the effects of peptide release at target sites has yet to be established.

Furthermore, recent behavioral studies suggest that the BNST is also a key structure involved in stress-induced drug recidivism (Erb et al., 2001; Erb and Stewart, 1999; Leri et al., 2002; Koob and Le Moal, 2001; Macey et al., 2003), possibly through its direct connections with the dopaminergic system (Georges and Aston-Jones, 2001, 2002). Co-morbidity of anxiety states and substance abuse are well documented (Barlow, 1997; Marshall, 1997), and many drugs of abuse increase brain serotonin levels (Weiss et al., 2001). Thus, individual vulnerability to drug taking and subsequent recidivistic behavior might reflect an underlying dysfunction of 5-HT-mediated feedback inhibition in the BNST.

Moreover, the BNST is a highly sexually dimorphic region (Stefanova and Ovtscharoff, 2000), and it is more than likely that gender differences exist in the process of 5-HT mediated feedback inhibition. Hence, in future studies we will examine gender differences in the response of BNST neurons to 5-HT. Such studies could have significant clinical import given the marked gender differences that are observed in the prevalence, phenotype and etiology of mood disorders, as well as in the response to therapy and the comorbidity observed between psychiatric and substance abuse disorders (Palanza, 2001; Pigott, 1999; Zilberman et al., 2003).

In summary, here we have suggested a mechanism by which 5-HT release in the BNST can adaptively modulate

the response of an organism to anxiogenic events. We propose that a time-dependent enhancement of 5-HT levels in the BNST has a role in feedback inhibition of the anxiogenic response to stressful stimuli. Importantly, a neuropathology in this feedback circuit may underlie that the phenotype and etiology of anxiety disorders and vulnerability to drug abuse.

Acknowledgments—This work was supported by a Whitehall Foundation grant 200-12-09-A to D.G.R.; NIMH grants MH47840, MH 57250, MH59906 to M.D.; and the STC Program, Center for Behavioral Neuroscience, NSF Agreement IBN-9876754.

REFERENCES

Albert PR, Tiberi M (2001) Receptor signaling and structure: insights from serotonin-1 receptors. Trends Endocrinol Metab 12:453–460.

Alheid GF (2003) Extended amygdala and basal forebrain. Ann NY Acad Sci 985:185–205.

Amat J, Matus-Amat P, Watkins LR, Maier SF (1998) Escapable and inescapable stress differentially and selectively alter extracellular levels of 5-HT in the ventral hippocampus and dorsal periaqueductal gray of the rat. Brain Res 797:12–22.

Baas JM, Grillon C, Bocker KB, Brack AA, Morgan CA 3rd, Kenemans JL, Verbaten MN (2002) Benzodiazepines have no effect on fear-potentiated startle in humans. Psychopharmacology (Berl) 161:233–247.

Barlow DH (1997) Anxiety disorders, comorbid substance abuse, and benzodiazepine discontinuation: implications for treatment. NIDA Res Monogr 172:33–51.

Brutus M, Zuabi S, Siegel A (1988) Effects of D-Ala2-Met5-enkephalinamide microinjections placed into the bed nucleus of the stria terminalis upon affective defense behavior in the cat. Brain Res 473:147–152.

Casada JH, Dafny N (1991) Restraint and stimulation of bed nucleus of the stria terminalis produce similar stress-like behaviors. Brain Res Bull 27:207–212.

Casada JH, Dafny N (1993a) Muscarinic receptors mediate the effect of acetylcholine (ACh) on neurons of the bed nucleus of the stria terminalis (BNST). Brain Res 631:124–128.

Casada JH, Dafny N (1993b) Responses of neurons in bed nucleus of the stria terminalis to microiontophoretically applied morphine, norepinephrine and acetylcholine: evidence for two different afferent pathways carrying stress-related information (noxious and amygdala stimulation) to the bed nucleus of the stria terminalis. Neuropharmacology 32:279–284.

Chapin EM, Andrade R (2001) A 5-HT(7) receptor-mediated depolarization in the anterodorsal thalamus: I. Pharmacological characterization. J Pharmacol Exp Ther 297:395–402.

Chung KK, Martinez M, Herbert J (1999) Central serotonin depletion modulates the behavioural, endocrine and physiological responses to repeated social stress and subsequent c-fos expression in the brains of male rats. Neuroscience 92:613–625.

Chung KK, Martinez M, Herbert J (2000) C-fos expression, behavioural, endocrine and autonomic responses to acute social stress in male rats after chronic restraint: modulation by serotonin. Neuroscience 95:453–463.

Clement HW, Schafer F, Ruwe C, Gemsa D, Wesemann W (1993) Stress-induced changes of extracellular 5-hydroxyindoleacetic acid concentrations followed in the nucleus raphe dorsalis and the frontal cortex of the rat. Brain Res 614:117–124.

Commons KG, Connolley KR, Valentino RJ (2003) A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders. Neuropsychopharmacology 28:206–215.

Cornea-Hebert V, Riad M, Wu C, Singh SK, Descarries L (1999) Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. J Comp Neurol 409:187–209.

Cornfield LJ, Lambert G, Arvidsson LE, Mellin C, Vallgarda J, Hacksell U, Nelson DL (1991) Intrinsic activity of enantiomers of 8-hydroxy-2-(di-*n*-propylamino)tetralin and its analogs at 5-hydroxytryptamine1A receptors that are negatively coupled to adenylate cyclase. Mol Pharmacol 39:780–787.

Craven RM, Grahame-Smith DG, Newberry NR (2001) 5-HT1A and 5-HT2 receptors differentially regulate the excitability of 5-HT-containing neurones of the guinea pig dorsal raphe nucleus in vitro. Brain Res 899:159–168.

Crown ED, King TE, Meagher MW, Grau JW, McLemore S, Sieve AN, Joynes RL (2000) Shock-induced hyperalgesia: III. Role of the bed nucleus of the stria terminalis and amygdaloid nuclei. Behav Neurosci 114:561–573.

Cullinan WE, Herman JP, Watson SJ (1993) Ventral subicular interaction with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. J Comp Neurol 332:1–20.

Dalsass M, Siegel A (1990) Opioid peptide regulation of neurons in the bed nucleus of the stria terminalis: a microiontophoretic study. Brain Res 531:346–349.

Davis M, Cassella JV, Wrean WH, Kehne JH (1986) Serotonin receptor subtype agonists: differential effects on sensorimotor reactivity measured with acoustic startle. Psychopharmacol Bull 22:837–843.

de Jongh R, Groenink L, van Der Gugten J, Olivier B (2002) The light-enhanced startle paradigm as a putative animal model for anxiety: effects of chlordiazepoxide, flesinoxan and fluvoxamine. Psychopharmacology (Berl) 159:176–180.

Delgado A, Sierra A, Querejeta E, Valdiosera RF, Aceves J (2000) Inhibitory control of the GABAergic transmission in the rat neostriatum by D2 dopamine receptors. Neuroscience 95:1043–1048.

De-Olmos J, Alheid G, Betramino C (1985) AmygdalaIn: The rat nervous system (Paxinos G, ed), pp. 223–334. Orlando: Academic Press.

Dilts RP, Boadle-Biber MC (1995) Differential activation of the 5-hydroxytryptamine-containing neurons of the midbrain raphe of the rat in response to randomly presented inescapable sound. Neurosci Lett 199:78–80.

Dong H, Petrovich GD, Swanson LW (2000) Organization of projections from the juxtacapsular nucleus of the BST: a PHAL study in the rat. Brain Res 859:1–14.

Duncan GE, Johnson KB, Breese GR (1993) Topographic patterns of brain activity in response to swim stress: assessment by 2-deoxyglucose uptake and expression of Fos-like immunoreactivity. J Neurosci 13:3932–3943.

Dunn JD, Williams TJ (1995) Cardiovascular responses to electrical stimulation of the bed nucleus of the stria terminalis. J Comp Neurol 352:227–234.

Egli RE, Winder DG (2003) Dorsal and ventral distribution of excitable and synaptic properties of neurons of the bed nucleus of the stria terminalis. J Neurophysiol 90:405–414.

Erb S, Salmaso N, Rodaros D, Stewart J (2001) A role for the CRF-containing pathway from central nucleus of the amygdala to bed nucleus of the stria terminalis in the stress-induced reinstatement of cocaine seeking in rats. Psychopharmacology (Berl) 158:360–365.

Erb S, Stewart J (1999) A role for the bed nucleus of the stria terminalis, but not the amygdala, in the effects of corticotropin-releasing factor on stress-induced reinstatement of cocaine seeking. J Neurosci 19:RC35.

Erlander MG, Tillakaratne NJ, Feldblum S, Patel N, Tobin AJ (1991) Two genes encode distinct glutamate decarboxylases. Neuron 7:91–100.

Fendt M, Endres T, Apfelbach R (2003) Temporary inactivation of the bed nucleus of the stria terminalis but not of the amygdala blocks

freezing induced by trimethylthiazoline, a component of fox feces. J Neurosci 23:23–28.
Fendt M, Koch M (1999) Cholinergic modulation of the acoustic startle response in the caudal pontine reticular nucleus of the rat. Eur J Pharmacol 370:101–107.
Fujino K, Yoshitake T, Inoue O, Ibii N, Kehr J, Ishida J, Nohta H, Yamaguchi M (2002) Increased serotonin release in mice frontal cortex and hippocampus induced by acute physiological stressors. Neurosci Lett 320:91–95.
Funada M, Hara C (2001) Differential effects of psychological stress on activation of the 5-hydroxytryptamine- and dopamine-containing neurons in the brain of freely moving rats. Brain Res 901:247–251.
Georges F, Aston-Jones G (2001) Potent regulation of midbrain dopamine neurons by the bed nucleus of the stria terminalis. J Neurosci 21:RC160.
Georges F, Aston-Jones G (2002) Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. J Neurosci 22:5173–5187.
Gewirtz JC, McNish KA, Davis M (1998) Lesions of the bed nucleus of the stria terminalis block sensitization of the acoustic startle reflex produced by repeated stress, but not fear-potentiated startle. Prog Neuropsychopharmacol Biol Psychiatry 22:625–648.
Grahn RE, Will MJ, Hammack SE, Maswood S, McQueen MB, Watkins LR, Maier SF (1999) Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. Brain Res 826:35–43.
Grillon C (2002) Startle reactivity and anxiety disorders: aversive conditioning, context, and neurobiology. Biol Psychiatry 52:958–975.
Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R (2002) Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. Nature 416:396–400.
Hadrava V, Blier P, de Montigny C (1996) Partial agonistic activity of *R*- and *S*-enantiomers of 8-OH-DPAT at 5-HT1A receptors. J Psychiatry Neurosci 21:101–108.
Hammack SE, Richey KJ, Watkins LR, Maier SF (2004) Chemical lesion of the bed nucleus of the stria terminalis blocks the behavioral consequences of uncontrollable stress. Behav Neurosci 118:443–448.
Heidmann DE, Szot P, Kohen R, Hamblin MW (1998) Function and distribution of three rat 5-hydroxytryptamine7 (5-HT7) receptor isoforms produced by alternative splicing. Neuropharmacology 37:1621–1632.
Henke PG (1984) The bed nucleus of the stria terminalis and immobilization-stress: unit activity, escape behaviour, and gastric pathology in rats. Behav Brain Res 11:35–45.
Hoffman HS, Ison JR (1980) Reflex modification in the domain of startle: I. Some empirical findings and their implications for how the nervous system processes sensory input. Psychol Rev 87:175–189.
Jin W, Klem AM, Lewis JH, Lu Z (1999) Mechanisms of inward-rectifier K^+ channel inhibition by tertiapin-Q. Biochemistry 38: 14294–14301.
Jin W, Lu Z (1999) Synthesis of a stable form of tertiapin: a high-affinity inhibitor for inward-rectifier K^+ channels. Biochemistry 38: 14286–14293.
Ju G, Swanson LW (1989) Studies on the cellular architecture of the bed nuclei of the stria terminalis in the rat: I. Cytoarchitecture. J Comp Neurol 280:587–602.
Ju G, Swanson LW, Simerly RB (1989) Studies on the cellular architecture of the bed nuclei of the stria terminalis in the rat: II. Chemoarchitecture. J Comp Neurol 280:603–621.
Kelly JS, Larkman P, Penington NJ, Rainnie DG, McAllister-Williams H, Hodgkiss J (1991) Serotonin receptor heterogeneity and the role of potassium channels in neuronal excitability. Adv Exp Med Biol 287:177–191.
Koch M, Kungel M, Herbert H (1993) Cholinergic neurons in the pedunculopontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. Exp Brain Res 97:71–82.
Koch M, Schnitzler HU (1997) The acoustic startle response in rats-circuits mediating evocation, inhibition and potentiation. Behav Brain Res 89:35–49.
Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97–129.
Krobert KA, Bach T, Syversveen T, Kvingedal AM, Levy FO (2001) The cloned human 5-HT7 receptor splice variants: a comparative characterization of their pharmacology, function and distribution. Naunyn Schmiedebergs Arch Pharmacol 363:620–632.
Lee Y, Davis M (1997) Role of the hippocampus, the bed nucleus of the stria terminalis, and the amygdala in the excitatory effect of corticotropin-releasing hormone on the acoustic startle reflex. J Neurosci 17:6434–6446.
Lee Y, Lopez DE, Meloni EG, Davis M (1996) A primary acoustic startle pathway: obligatory role of cochlear root neurons and the nucleus reticularis pontis caudalis. J Neurosci 16:3775–3789.
Leri F, Flores J, Rodaros D, Stewart J (2002) Blockade of stress-induced but not cocaine-induced reinstatement by infusion of noradrenergic antagonists into the bed nucleus of the stria terminalis or the central nucleus of the amygdala. J Neurosci 22:5713–5718.
Li BH, Rowland NE (1996) Effect of chronic dexfenfluramine on Fos in rat brain. Brain Res 728:188–192.
Lino-de-Oliveira C, Sales AJ, Del Bel EA, Silveira MC, Guimaraes FS (2001) Effects of acute and chronic fluoxetine treatments on restraint stress-induced Fos expression. Brain Res Bull 55:747–754.
Lowry CA (2002) Functional subsets of serotonergic neurones: implications for control of the hypothalamic-pituitary-adrenal axis. J Neuroendocrinol 14:911–923.
Luscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA (1997) G protein-coupled inwardly rectifying K^+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. Neuron 19:687–695.
Macey DJ, Smith HR, Nader MA, Porrino LJ (2003) Chronic cocaine self-administration upregulates the norepinephrine transporter and alters functional activity in the bed nucleus of the stria terminalis of the rhesus monkey. J Neurosci 23:12–16.
Mansbach RS, Geyer MA (1988) Blockade of potentiated startle responding in rats by 5-hydroxytryptamine1A receptor ligands. Eur J Pharmacol 156:375–383.
Marchese A, Chen C, Kim YM, Benovic JL (2003) The ins and outs of G protein-coupled receptor trafficking. Trends Biochem Sci 28:369–376.
Mark MD, Herlitze S (2000) G-protein mediated gating of inward-rectifier K^+ channels. Eur J Biochem 267:5830–5836.
Marshall JR (1997Alcohol and substance abuse in panic disorder. J Clin Psychiatry 58(Suppl 2):46–49.
Martinez M, Phillips PJ, Herbert J (1998) Adaptation in patterns of c-fos expression in the brain associated with exposure to either single or repeated social stress in male rats. Eur J Neurosci 10:20–33.
Matsui H, Yamamoto C (1984) Neuronal sensitivity to opiate and opioid peptides in the bed nucleus of the stria terminalis: effects of chronic treatment with morphine. Neuropharmacology 23:755–762.
McDonald AJ (1983) Neurons of the bed nucleus of the stria terminalis: a Golgi study in the rat. Brain Res Bull 10:111–120.
Morelli M, Pinna A (1999) Antidepressants and atypical neuroleptics induce Fos-like immunoreactivity in the central extended amygdala. Ann NY Acad Sci 877:703–706.
Palacios JM, Raurich A, Mengod G, Hurt SD, Cortes R (1996) Autoradiographic analysis of 5-HT receptor subtypes labeled by [^{3}H]5-CT ([^{3}H]5-carboxamidotryptamine). Behav Brain Res 73:239–243.
Palanza P (2001) Animal models of anxiety and depression: how are females different?Neurosci Biobehav Rev 25:219–233.
Petit JM, Luppi PH, Peyron C, Rampon C, Jouvet M (1995) VIP-like immunoreactive projections from the dorsal raphe and caudal linear raphe nuclei to the bed nucleus of the stria terminalis demon-

strated by a double immunohistochemical method in the rat. Neurosci Lett 193:77–80.
Phelix CF, Liposits Z, Paull WK (1992a) Monoamine innervation of bed nucleus of stria terminalis: an electron microscopic investigation. Brain Res Bull 28:949–965.
Phelix CF, Liposits Z, Paull WK (1992b) Serotonin-CRF interaction in the bed nucleus of the stria terminalis: a light microscopic double-label immunocytochemical analysis. Brain Res Bull 28:943–948.
PigottTA (1999) Gender differences in the epidemiology and treatment of anxiety disorders. J Clin Psychiatry 60(Suppl 18):4–15.
Plappert CF, Pilz PK, Schnitzler HU (1993) Acoustic startle response and habituation in freezing and nonfreezing rats. Behav Neurosci 107:981–987.
Rainnie DG (1999a) Neurons of the bed nucleus of the stria terminalis (BNST): electrophysiological properties and their response to serotonin. Ann NY Acad Sci 877:695–699.
Rainnie DG (1999b) Serotonergic modulation of neurotransmission in the rat basolateral amygdala. J Neurophysiol 82:69–85.
Rainnie DG, Asprodini EK, Shinnick-Gallagher P (1993) Intracellular recordings from morphologically identified neurons of the basolateral amygdala. J Neurophysiol 69:1350–1362.
Rueter LE, de Montigny C, Blier P (1998) In vivo electrophysiological assessment of the agonistic properties of flibanserin at pre- and postsynaptic 5-HT1A receptors in the rat brain. Synapse 29:392–405.
Sadja R, Alagem N, Reuveny E (2003) Gating of GIRK channels: details of an intricate, membrane-delimited signaling complex. Neuron 39:9–12.
Sari Y, Miquel MC, Brisorgueil MJ, Ruiz G, Doucet E, Hamon M, Verge D (1999) Cellular and subcellular localization of 5-hydroxytryptamine1B receptors in the rat central nervous system: immunocytochemical, autoradiographic and lesion studies. Neuroscience 88:899–915.
Sawada S, Yamamoto C (1981) Postsynaptic inhibitory actions of catecholamines and opioid peptides in the bed nucleus of the stria terminalis. Exp Brain Res 41:264–270.
Schulz D, Canbeyli R (1999) Freezing behavior in BNST-lesioned Wistar rats. Ann NY Acad Sci 877:728–731.
Semba K, Fibiger HC (1992) Afferent connections of the laterodorsal and the pedunculopontine tegmental nuclei in the rat: a retro- and antero-grade transport and immunohistochemical study. J Comp Neurol 323:387–410.
Shaikh MB, Brutus M, Siegel HE, Siegel A (1986) Regulation of feline aggression by the bed nucleus of stria terminalis: forebrain structures regulating flight behavior in the cat. Brain Res Bull 16:179–182.
Stein C, Davidowa H, Albrecht D (2000) 5-HT(1A) receptor-mediated inhibition and 5-HT(2) as well as 5-HT(3) receptor-mediated excitation in different subdivisions of the rat amygdala. Synapse 38:328–337.
Stefanova N, Ovtscharoff W (2000) Sexual dimorphism of the bed nucleus of the stria terminalis and the amygdala. Adv Anat Embryol Cell Biol 158:III–X, 1–78.
Summers CH, Kampshoff JL, Ronan PJ, Lowry CA, Prestbo AA, Korzan WJ, Renner KJ, van der Vegt BJ, Lieuwes N, van de Wall EH, Kato K, Moya-Albiol L, Martinez-Sanchis S, de Boer SF, Koolhaas JM (2003) Monoaminergic activity in subregions of raphe nuclei elicited by prior stress and the neuropeptide corticotropin-releasing factor: activation of serotonergic neurotransmission during the performance of aggressive behavior in rats. J Neuroendocrinol 15:1122–1133.
Sun N, Cassell MD (1993) Intrinsic GABAergic neurons in the rat central extended amygdala. J Comp Neurol 330:381–404.
Thomas DR, Middlemiss DN, Taylor SG, Nelson P, Brown AM (1999) 5-CT stimulation of adenylyl cyclase activity in guinea-pig hippocampus: evidence for involvement of 5-HT7 and 5-HT1A receptors. Br J Pharmacol 128:158–164.
Umriukhin AE, Wigger A, Singewald N, Landgraf R (2002) Hypothalamic and hippocampal release of serotonin in rats bred for hyper- or hypo-anxiety. Stress 5:299–305.
Varrault A, Bockaert J (1992) Differential coupling of 5-HT1A receptors occupied by 5-HT or 8-OH-DPAT to adenylyl cyclase. Naunyn Schmiedebergs Arch Pharmacol 346:367–374.
Vertes RP, Kocsis B, Fortin WJ, Crane AM (1994) Projections of the dorsal raphe nucleus to the brainstem: PHA-L analysis in the rat. J Comp Neurol 340:11–26.
Waeber C, Moskowitz MA (1997) 5-Hydroxytryptamine1A and 5-hydroxytryptamine1B receptors stimulate [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate binding to rodent brain sections as visualized by in vitro autoradiography. Mol Pharmacol 52:623–631.
Walker DL, Davis M (1997a) Anxiogenic effects of high illumination levels assessed with the acoustic startle response in rats. Biol Psychiatry 42:461–471.
Walker DL, Davis M (1997b) Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. J Neurosci 17:9375–9383.
Walker DL, Davis M (2002) Quantifying fear potentiated startle using absolute versus proportional increase scoring methods: implications for the neurocircuitry of fear and anxiety. Psychopharmacology 164:318–328.
Weiss F, Ciccocioppo R, Parsons LH, Katner S, Liu X, Zorrilla EP, Valdez GR, Ben-Shahar O, Angeletti S, Richter RR (2001) Compulsive drug-seeking behavior and relapse: neuroadaptation, stress, and conditioning factors. Ann NY Acad Sci 937:1–26.
Wood M, Chaubey M, Atkinson P, Thomas DR (2000) Antagonist activity of meta-chlorophenylpiperazine and partial agonist activity of 8-OH-DPAT at the 5-HT(7) receptor. Eur J Pharmacol 396:1–8.
Zilberman ML, Tavares H, Blume SB, el-Guebaly N (2003) Substance use disorders: sex differences and psychiatric comorbidities. Can J Psychiatry 48:5–13.

(Accepted 23 June 2004)
(Available online 3 September 2004)