Available online at www.sciencedirect.com
SCIENCE DIRECT
Behavioural Brain Research 170 (2006) 219–223
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

Electrolytic lesions of a discrete area within the nucleus accumbens shell attenuate the long-term expression, but not early phase, of sensitization to cocaine

Heather C. Brenhouse *, Stefanie Montalto, James R. Stellar

Department of Psychology, Northeastern University, 115 Mill St, Belmont, MA 02478, United States

Received 15 November 2005; received in revised form 13 February 2006; accepted 17 February 2006
Available online 3 April 2006

Abstract

Repeated exposure to cocaine leads to behavioral sensitization, which is the augmentation of the locomotor response to a subsequent exposure to the drug. The nucleus accumbens (NAc), a major termination site of dopaminergic neurons, is believed to be involved in behavioral sensitization and studies have demonstrated that the NAc shell can be split into five zones of analysis; the vertex, arch, cone, intermediate and ventrolateral zones [Todtenkopf MS, Stellar JR. Assessment of tyrosine hydroxylase immunoreactive innervation in five subregions of the nucleus accumbens shell in rats treated with repeated cocaine. Synapse 2000;38:261–70]. Several reports show cocaine-induced c-fos expression particularly in the intermediate zone after 14, but not 2, drug-free days following repeated cocaine administration, suggesting that this region may be involved in sensitization and particularly in the later phase of expression, versus the earlier phase of sensitization. Bilateral electrolytic lesions of the intermediate zone were made in two groups of rats, which were then repeatedly exposed to cocaine (15 mg/kg, twice/day for 5 days). One group was subsequently given a single cocaine challenge injection (15 mg/kg) after 14 drug-free days, while the other group was challenged after only 2 drug-free days. Two sham surgery groups in which an electrode was lowered but no current was passed served as controls. Results show that lesioned animals as well as sham controls exhibited behavioral sensitization to the drug. However, following a 14-day drug-free period, the lesioned animals showed significant reduction in sensitization, compared to sham controls. Together these findings suggest that the intermediate zone of the NAc shell is indeed involved in the expression phase of behavioral sensitization to cocaine.

Keywords: Psychostimulants; Sensitization; Electrolytic lesion; Nucleus accumbens shell

Repeated exposure to psychostimulants produces a characteristic augmented locomotor response to a subsequent challenge injection in rodents. Increased ambulation as well as stereotypic behaviors sensitize with repeated exposure. This phenomenon has been found to present in two distinct stages, namely induction and expression [23]. The induction of sensitization is defined as the transient sequence of cellular and molecular events triggered by psychostimulant administration that leads to the enduring changes in neural function responsible for behavioral augmentation. Expression is the manifestation of long-lasting cellular alterations that arise from the induction phase and directly mediate the augmented behavioral response [23]. Induction is thought to occur during repeated drug administration and possibly last 1–2 days after repeated administration has ceased, while any changes that are seen weeks after the cessation of treatment are associated with expression.

It is now thought that the mesolimbic DA system, comprised largely of the nucleus accumbens (NAc) and ventral tegmental area (VTA) plays a central role in the mechanisms responsible for the induction and expression of sensitization (see Ref. [15] for review). In this model, the NAc has traditionally been associated with the expression phase of sensitization and the VTA with induction. During the first week of withdrawal from repeated injections or self-administration of psychostimulants, no change, decreases and increases in the ability of amphetamine or cocaine to elevate extracellular DA levels in the NAc have been reported [7,14,22,29,35]. After more lengthy withdrawal periods from repeated cocaine (14 days or longer), increased DA transmission in the NAc is consistently associated with the expression of behavioral sensitization [14,16,27,36,37].

* Corresponding author. Tel.: +1 617 549 4549; fax: +1 617 855 3479.
E-mail address: hbrenhouse@mclean.harvard.edu (H.C. Brenhouse).

0166-4328/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2006.02.029

Anatomically, the NAc is a heterogeneous structure, as evidenced by immunohistochemical staining and neuronal projection patters [39]. The major recognized subdivisions are a medioventral shell and a dorsolateral core in the caudal NAc, and a rostral pole. Recent evidence suggests that the ventromedial shell of the NAc is itself a heterogeneous structure that can be further subdivided into five regions: the vertex (NAc_{VERT}), arch (NAc_{ARCH}), cone (NAc_{CONE}), and the intermediate (NAc_{INT}) and ventrolateral (NAc_{LAT}) zones [13,30]. These subregions can be identified by the differential histological staining for tyrosine hydroxylase (TH) [13], substance P [33,39], calbindin D28k [8], enkephalin and DA [33,34], as well as by differential connectivity patterns [9,38,39]. The heterogeneous nature of the NAc shell likely has functional significance. For example, electrolytic lesions of the dorsomedial region of the shell, comprising the NAc_{VERT}, NAc_{ARCH} and NAc_{CONE}, abolish the induction, but not expression, of sensitization [31]. Other reports from our laboratory reveal diminished c-Fos expression in the rostral tip of the shell and the NAc_{LAT} after a challenge dose of cocaine following 2 days of withdrawal [32], and a significant increase in c-Fos immunoreactivity in the NAc_{INT} after a challenge dose following 14 days, but not 2 days, of withdrawal [1,32].

Given the evidence that the NAc_{INT} is active during the expression, but not induction, of sensitization, we hypothesize that ablation of this subregion should reduce the long-term expression of sensitization, but have no effect on the early phase after repeated exposure. Here, we performed bilateral electrolytic lesions in the NAc_{INT} prior to any cocaine exposure and examined the effects of these lesions on behavioral sensitization to repeated cocaine exposure.

1. Materials and methods

1.1. Subjects

Adult male Sprague–Dawley rats ($N = 48$; 250–300 g; Taconic Farms, Germantown, NY, USA) were allowed free access to food and water and housed individually in plastic cages on a reversed 12-h light/12-h dark cycle (lights on at 19:00 h) at an ambient temperature of 22–24 °C with a controlled relative humidity of 55%. All procedures were performed according to NIH guidelines (NIH Publications No. 80-23) and approved by the Institutional Animal Care and Use Committee at Northeastern University.

1.2. Surgery

Rats were randomly assigned to undergo either a bilateral electrolytic lesion of the NAc_{INT} or sham surgery. Rats were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (12 mg/kg) in a 1 ml/kg volume (0.5 mg/kg) followed by a SC injection of Atropine sulfate (0.1 cm^3) to reduce bronchial secretions. Single monopolar electrodes purchased from Plastics One (Roanoke, VA, USA) were used. The electrodes were insulated except for the tip and the bottom 10.0 mm were unembedded so as to be able to be lowered into the brain. The tip of the electrode was beveled to enable easier insertion through the dura mater. The electrode was then lowered into the NAc_{INT} of one hemisphere of the brain (AP, +1.6; ML, ±1.3; DV, −8.1 from dura; [41]). A constant cathodal current (0.5 mA) was passed through the rat from the electrode to the ground, which was a rectal probe, for 20 s. The electrode was left in place for an additional 15 s allowing for the dispersal of ions to be complete. This procedure was then repeated on the contralateral hemisphere. The procedure for the sham lesions was identical except that the electrode was lowered only 2 mm below the dura, and no current was passed. All animals were allowed a recovery period of 1 week prior to behavioral testing.

1.3. Behavioral testing

Animals received twice daily injections of cocaine (15 mg/kg, i.p.; Research Biochemicals International, Natick, MA, USA) separated by approximately 6 h for 5 consecutive days. Cocaine was prepared in sterile 0.9% isotonic saline. Rats were separated into two groups such that one group received a challenge injections of cocaine (15 mg/kg, i.p.) after 2 days without treatment or handling, while the other group received a challenge injection (15 mg/kg, i.p.) after 14 days without treatment or handling.

All locomotor testing was done in a dimly-lit room with a white noise generator (San Diego Instruments, San Diego, CA, USA) to mask any external sounds. On the day prior to the first day of treatment, animals were habituated for 1 h to one of four Plexiglas activity monitor chambers (43.2 cm × 43.2 cm × 30.5 cm; Med-Associates, St. Albans, VT, USA) that were demarcated into equal sized quadrants. All injections (15 mg/kg, i.p.) were performed in each animal's assigned locomotor chamber after a 20 min baseline. The locomotor activity of the animals was measured for 40 min after the first injection on the first and last day of treatment, as well as the after the challenge injection. Total centimeters traveled were recorded over the 40 min period.

1.4. Histology

Animals were anesthetized with a ketamine/xylazine mixture and intracardially perfused with 50 ml of ice-cold 0.01 M phosphate buffered saline (PBS, pH 7.4) followed by 300 ml of ice-cold 4% paraformaldehyde solution in 0.1 M phosphate buffer (PB, pH 7.4). The brains were then removed and post-fixed in the same solution overnight, then placed in 20% glycerol until sunk. The anterior portion of each brain was blocked and mounted onto a vibratome. Hundred micrometers sections were taken and alternate sections were mounted on slides. The slides were dried overnight, then stained in cresyl violet solution and coverslipped using Cytoseal 60 mounting medium (VWR Scientific; West Chester, PA, USA) the following day.

1.5. Data analysis

Lesion placement and volume was verified under a Nikon light microscope, using a section from a similar anterior–posterior level that had been stained for TH [30] for reference to locate the NAc_{INT}. The lesion size at four anterior–posterior levels (Bregma +2.7, +1.4, +0.7 and +0.2; [41]) was traced onto an atlas plate, and any lesion that included at least 1/2 of the NAc_{INT} was included in analysis. Seven of the 26 total lesions performed were excluded for poor placement.

Behavioral data was analyzed using two-way analysis of variance (ANOVA), with day as a repeated measure using SigmaStat Version 3.11 statistics package (Systat Software Inc). Holm–Sidak pairwise multiple comparison procedures were performed to analyze group differences on each day. Three outliers were identified using the Dixon test for outliers ($\alpha = 0.01$). Behavioral scores were eliminated if either the locomotion during habituation and/or the post-injection locomotion were significantly outlying values.

2. Results

2.1. Histological verification

Experimental animals that were included in behavioral analyses had 1.5–2.25 mm^2 lesions at their maximum at the electrode tip. Anatomically, this point was at the level of the Island of Calleja that encompassed the NAc_{INT} (see Fig. 1). Animals were excluded from analysis if the lesion encroached significantly into another region, or if less than half of the NAc_{INT} was ablated. Seven of 26 total lesions were rejected for these reasons. Of