Behavioural Brain Research 216 (2011) 673–680

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Nucleus accumbens carbachol disrupts olfactory and contextual fear-potentiated startle and attenuates baseline startle reactivity

Graham A. Cousens*, Cheryl G. Skrobacz, Anna Blumenthal

Department of Psychology, Drew University, 36 Madison Avenue, Madison, NJ 07940, United States

ARTICLE INFO

Article history:
Received 27 May 2010
Received in revised form
13 September 2010
Accepted 13 September 2010
Available online 18 September 2010

Keywords:
Olfaction
Conditioned fear
Nucleus accumbens
Carbachol
Acoustic startle

ABSTRACT

Although the nucleus accumbens (NAc) typically is not considered a primary component of the circuitry underlying either the acquisition or retrieval of conditioned fear, evidence suggests that this region may play some role in modulating fear-related behaviors. The goal of the present study was to explore a potential role for NAc cholinergic receptors in the expression of fear-potentiated startle (FPS) and baseline startle reactivity. Intra-NAc infusion of the broad-acting cholinergic receptor agonist, carbachol, suppressed FPS elicited by re-exposure to both a discrete odor previously paired with footshock and the conditioning context. Although carbachol elevated spontaneous motor activity, activity bouts did not account for startle suppression in carbachol-treated Ss. In addition, intra-NAc carbachol suppressed baseline startle over a range of acoustic pulse intensities in the absence of explicit fear conditioning. Collectively, these findings suggest that NAc cholinergic receptors play a role in the modulation of baseline startle reactivity, rather than in the retrieval of learned fear, and that this role is independent of overt motor activity.

1. Introduction

Pavlovian fear conditioning paradigms have been utilized widely to examine basic mechanisms of associative learning in the mammalian nervous system. In these paradigms, an initially neutral conditional stimulus (CS) is paired with an aversive unconditional stimulus (US), usually mild footshock, and subsequently comes to elicit a range of behavioral and physiological responses thought to reflect a central state of fear. The acquisition and expression of conditioned fear is supported by a distributed network that prominently includes lateral and central amygdaloid nuclei, closely associated thalamic and cortical regions, and brain stem nuclei involved in the generation of fear-related behaviors [7,9,26,33].

Although the ventral striatum typically is not considered a primary component of the circuitry underlying conditioned fear, evidence suggests that ventral striatal territories, particularly the nucleus accumbens (NAc), may play some role in fear acquisition or retrieval. This notion is suggested by anatomical evidence demonstrating that shell and core subregions are densely innervated by areas, including the amygdaloid complex [52] and the hippocampal formation [11], broadly implicated in either discrete-cue or contextual conditioned fear. Further, re-exposure to fear-eliciting stimuli has been shown to enhance expression of the immediate early gene *c-fos* [4] and to elevate dopamine concentration within the NAc [30,37,53,54; though see 27]. In some studies, pharmacological manipulation of NAc signaling or experimentally-induced lesions have been associated with disruptions in fear-related conditional responses (CR), including freezing behavior [14,18,34,40,46,50] and fear-potentiated startle (FPS) [44,46]. However, these findings need to be reconciled with those of other studies showing null effects of a number of NAc manipulations [18,24,45]. Interpretation of these data is complicated by procedural differences across studies, including the specific behavioral index of fear assessed, the distribution and extent of influence of manipulations within the NAc, and the timing of those manipulations relative to fear acquisition and expression.

A series of influential recent reports [44–46] demonstrated that intra-NAc infusions of either the sodium channel blocker, tetrodotoxin, or the broad-acting cholinergic receptor agonist, carbachol, immediately prior to either training or testing, blocked the expression of FPS conditioned to a discrete visual CS. In contrast, neither amphetamine nor haloperidol were with effect. These findings are consistent with those of other reports suggesting that manipulation of dopaminergic signaling within the NAc has no affect on the expression of FPS [18,24]. Cholinergic markers, choline-acetyltransferase and acetylcholinesterase, are expressed at high levels throughout the striatum [55], suggesting that acetylcholine has a strong modulatory influence on striatal output by directly or indirectly influencing the activity of projection neurons. However, the notion that cholinergic signaling mechanisms may play a role in either the acquisiton or retrieval of conditioned fear has not been systematically studied.

* Corresponding author. Tel.: +1 973 408 3353; fax: +1 973 408 3781.
E-mail address: gcousens@drew.edu (G.A. Cousens).

0166-4328/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2010.09.011

The goal of the present study was to explore further a potential role for NAc cholinergic signaling in the modulation of conditioned fear, using an olfactory FPS paradigm that also permits examination of contextual conditioning. Our primary question was whether carbachol would impair the retrieval of either olfactory or contextual conditioned fear, or whether any disruption in fear expression could be explained by effects unrelated to conditioning. The use of a cholinergic agonist was motivated in part the finding that presentation of conditioned aversive cues elevates NAc acetylcholine [28]. Carbachol was used in the present study to facilitate comparison with previous research [46] and because of evidence linking it to appetitive states. Rats will self-administer carbachol directly into the NAc [15], and intra-NAc infusion of carbachol elicits high-frequency vocalizations thought to reflect positive affective states [10]. Startle suppression has been observed following exposure to appetitive stimuli [42,43] and may reflect the activation of a positive affective states [25], so we were interested in determining whether carbachol would suppress startle in both conditioned and unconditioned Ss.

An initial experiment sought to establish suitable training and testing procedures for olfactory FPS within our laboratory as a substrate for these and subsequent experiments. A second experiment evaluated the effects of pre-test intra-NAc carbachol infusions on the expression of both olfactory and contextual FPS and on spontaneous motor activity (SMA). A third experiment evaluated the effects of intra-NAc carbachol on baseline startle reactivity.

2. Materials and methods

2.1. Subjects

Seventy-eight male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing between 250 g and 350 g at study onset served as Ss. All Ss were housed individually in clear plastic cages in a temperature- and humidity-controlled vivarium maintained on a 12-h light:dark cycle with lights on at 07:00 h. Behavioral sessions were conducted at the same time each day during the light phase of the cycle. Ss were afforded free access to water and standard rat chow throughout the study. All procedures were approved by the Drew University Institutional Animal Care and Use Committee and are in accordance with current US Public Health Service guidelines.

2.2. Apparatus

Behavioral procedures were conducted using two identical custom-built startle systems, each consisting of a steel grid animal chamber (16.5 cm × 9.0 cm × 9.0 cm; Med-Associates, St. Albans, VT) housed within a sound-attenuating enclosure (39.5 cm × 15.5 cm × 27.5 cm). The floor and rear wall of each chamber consisted of 6 stainless steel rods spaced 1.5 cm on center. The opposing front wall and the ceiling were constructed from clear plexiglass, and the remaining walls were constructed from white acrylic. Each enclosure was lined with 1.3 cm thick plexiglass to reduce odor absorption and was continuously ventilated by negative pressure using a variable-speed exhaust blower situated in an adjacent room and connected to a room outlet duct. The airflow rate was adjusted to produce background noise level of 67 dB measured in the inner chamber.

When appropriate, white-noise pulses were delivered through a high-frequency speaker fitted to the rear wall of the enclosure, 3 cm from the chamber rear grid wall, and scrambled current was delivered to the chamber floor using a precision shocker (model H13-16; Coulbourn Instruments, Whitehall, PA). Odorized air was delivered to the subject using a custom-built olfactometer similar to that described previously [32]. During odor presentation, pressurized room air (1.5 l/min) was passed through a 50 ml reservior containing 20 ml 100% amyl acetate and into the chamber through a T-shaped port in the chamber ceiling. At all other times, air was diverted through a reservior containing distilled water and then into the chamber through the same port. Thus, airflow into the chamber was continuous, and residual odor in the chamber inlet tubing was rapidly evacuated after odor offset. Subjective assessment of odor quality by a human observer revealed that odor was not detectable 5 s after odor offset. In order to eliminate any possible behavioral impact of auditory cues associated with valve operation, olfactometers were situated in an adjacent room with fluid reservoirs positioned immediately adjacent to the enclosures. All paradigmatic events were coordinated by a dedicated microcomputer running MedPC IV software (Med-Associates).

Motor activity was transduced by an accelerometer (model 338B35; PCB Piezotronics, Depew, NY) mounted on the lower housing of each animal chamber. Voltage output proportional to vertical chamber displacement was amplified 100× by a signal processor (model 482A20; PCB Piezotronics), digitized at 2 kHz, and continuously recorded to a dedicated microcomputer equipped with an A/D processing card (model PCI-6070E; National Instruments Corp., Austin, TX) and physiological data acquisition software (Recorder, Plexon, Inc., Dallas, TX). Corollary stumulus trigger events were sent through a separate recording channel and were saved for purposes of time-locking accelerometer output to auditory pulses. Trigger events were isolated using OfflineSorter software (Plexon, Inc.), and behavioral responses were analyzed using NeuroExplorer (Nex Technologies, Littleton, MA) and Matlab (The Mathworks, Natick, MA) software.

2.3. Procedures

2.3.1. Experiment 1

Experiment 1 verified the efficacy of training and testing procedures for olfactory and contextual FPS and evaluated the effects of control procedures that eliminate CS-US contingency. Prior to the onset of behavioral procedures, Ss were gently handled for 5 min on each of 3 consecutive days. On each of the next 3 days, Ss received a baseline startle session consisting of a 5 min acclimation period followed by ten white-noise pulses (20 ms duration; 4 ms rise; 30 s interstimulus interval, ISI) at each of four intensities (75 dB, 85 dB, 95 dB, 105 dB) presented according to a pseudorandom sequence held constant across all baseline sessions.

On the following day, Ss were administered a single 27 min conditioning session. Ss were assigned to matched groups according to startle response amplitudes averaged over the ten 95 dB trials of the third baseline session and received one of the following procedures: (1) six odor-shock pairings (group *odor-shock*; $n = 9$) consisting of odor (4 s duration) coterminating with 0.5 mA shock (0.5 s duration); (2) six presentations of odor without shock (group *odor-alone*; $n = 9$); (3) six explicitly unpaired presentations of odor and shock (group *unpaired*, $n = 8$) with a 2 min odor-shock onset latency; and (4) a single odor-shock conditioning trial (Group *odor-shock*-1; $n = 8$). Odor and shock stimulus duration and intensity were identical, if presented, across all conditions, and the odor interstimulus interval was 4 min for each of the first three groups. Conditioning groups were counterbalanced across chambers and time of day, and Ss were run in batches of 6–15 rats with each group distributed as evenly as possible within each batch. The exception to this procedure was the *odor-shock*-1 group, which received training and testing sessions as a single cohort separately from and following all other groups.

Twenty-four hours after conditioning, all Ss received a startle test session modeled after "long interval" procedures initially described by Paschall and Davis [35]. The test session consisted of a 5 min acclimation period followed by seventy 95 dB white noise pulses (20 ms duration; 4 ms rise; 30 s ISI). The first 30 pulses were presented in the absence of odor and served both to minimize effects of within-session habituation and to enable evaluation of contextual FPS, as described below. Odor (4 s duration) was presented prior to pulses 31, 35, 39, and every fourth pulse thereafter, with a 3.5 s odor-pulse onset latency corresponding to the conditioned odor-shock onset latency.

2.3.2. Experiment 2

Experiment 2 evaluated the effects of intra-NAc carbachol on the expression of conditioned olfactory and contextual FPS. Prior to all behavioral procedures, Ss were implanted with a bilateral 27 ga guide cannula (Plastics One, Roanoke, VA) positioned immediately dorsal to the border of the NAc core and medial shell (bregma: 3.0 mm anterior; 1.2 mm lateral; 6.5 mm ventral). Ss were anesthetized (i.m.; ketamine hydrochloride, 50 mg/kg; acepromazine maleate, 0.65 mg/kg; xylazine hydrochloride, 2.65 mg/kg) and mounted in a stereotaxic frame with the tooth bar positioned 5 mm dorsal to the interaural line. The crainium was exposed, and two small burr holes were drilled in the frontal bones overlying the NAc. The cannula was slowly lowered into position, and the base was affixed to the skull with dental acrylic and skull screws placed in the nasal, parietal, and occipital bones. A stylet was inserted to maintain cannula patency, and a headcap was affixed to the guide cannula base. The plane of anesthesia was regularly monitored by verifying the absence of hind-limb reflex, and isoflurane vapor was administered as needed. Following surgery, Ss were administered a topical antibiotic along with 4% lidocaine solution and were placed on a heating pad where they remained until they were alert and could be returned to the colony. Ss were afforded a 10–14 day post-surgical recovery period.

Following recovery, Ss were administered behavioral procedures as in Experiment 1. Ss were gently handled for 5 min on each of 3 consecutive days, followed by a baseline startle session on each of the next 3 days. Ss were acclimatized to infusion procedures by gentle hand-restraint for 5 min immediately prior to each of the first two baseline sessions, and a sham infusion procedure was conducted prior third baseline session. The sham procedure included removal of the headcap and stylet, insertion and removal of a bilateral 33 ga infusion cannula with the tip positioned 9.5 mm ventral to skull surface (3 mm beyond the guide cannula tip), mock infusion of drug including turning on and off the infusion pump, and replacement of the stylet and headcap. Twenty-four hours after the third baseline session, all Ss received a single 6-trial *odor-shock* conditioning session identical to that described in Experiment 1.

In order to evaluate the effects of carbachol on FPS expression, infusions were conducted immediately prior to the FPS test session conducted 24 h after conditioning. Ss were assigned to one of two groups matched on startle response amplitudes on the ten 95 dB trials of the third baseline session. Group *carbachol* ($n = 12$) received