Behavioural Brain Research 221 (2011) 314–319

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

Excitatory conditioning to the interoceptive nicotine stimulus blocks subsequent conditioning to an exteroceptive light stimulus

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ARTICLE INFO

Article history:
Received 10 February 2011
Received in revised form 4 March 2011
Accepted 9 March 2011
Available online 17 March 2011

Keywords:
Appetitive conditioning
Blocking
Interoceptive cue competition
Drug discrimination
Nicotine
Overshadowing

ABSTRACT

Previous research has shown that a nicotine conditional stimulus (CS) can compete with (i.e., overshadow) a brief light CS. Another form of competition, blocking, has not yet been examined with the nicotine CS. Groups of rats were assigned to an element training condition. For the N+ group, during each daily 2 h element training session, there were ten intravenous nicotine infusions (0.03 mg/kg) followed 30 s later with 4 s access to sucrose. In the N− group, nicotine and sucrose presentations were explicitly unpaired. The chamber alone group (C alone) had no stimulus presentations. Element training was followed by compound training in all groups. A 30-s houselight was included during the time between the nicotine infusion and paired sucrose delivery. Non-reinforced element presentations assessed relative control of the goal tracking conditioned response (CR). The N+ group showed a higher proportion of CR control by the nicotine than the light. The opposite pattern was found in the N− and C alone groups indicating that nicotine CS controlled less of the CR than the light. Thus, excitatory conditioning with the nicotine CS blocked later conditioning to the light. This finding adds to literature examining the interaction between interoceptive drug CSs and other environmental stimuli.

The interoceptive effects of nicotine have been shown to serve as a contextual conditional stimulus (CS) in an appetitive Pavlovian conditioning task. In these studies, rats were injected subcutaneously with nicotine or saline before placement in a conditioning chamber. On nicotine sessions, liquid sucrose (the unconditioned stimulus; US) was intermittently available. On intermixed saline sessions, sucrose was not available. This discrimination is readily acquired as indicated by an increase in anticipatory head entries into the sucrose receptacle (i.e., goal-tracking; [1,2]) on nicotine compared to saline sessions (e.g., [3]). This discriminated goal-tracking task has been helpful in studying behavioral and neuropharmacological processes involving the nicotine stimulus (cf. [4,5]). However, the contextual nature of subcutaneous nicotine administration has limited each session to a single trial – the time before the first sucrose delivery on nicotine sessions (equivalent time on saline sessions) so that the index of anticipatory responding is not confounded by sucrose delivery. For example, in experiments in which there are 36 deliveries of sucrose in a single 20-min session – a standard number in our laboratory – the measure of conditioning can only be evaluated after every 36 nicotine–sucrose pairings. As assessment of conditioning is not possible after each nicotine–sucrose pairing, this arrangement poses some experimental limitations. One example comes from a set of studies examining possible dose-dependent differences in nicotine-evoked conditioned responding. In that research, we compared rate of acquisition of a 0.1, 0.2, and 0.4 mg/kg nicotine CS [6,7]. Although there were dose-dependent differences in several measures (e.g., extinction), the rate of acquisition was similar across groups (i.e., within 10–12 intermixed nicotine and saline sessions). This translates into at least 180 nicotine–sucrose pairings (5 sessions × 36 deliveries per session). Perhaps if conditioning could be assessed following each nicotine–sucrose pairing, dose-dependent differences in acquisition would have emerged. Further, the ability to measure conditioning after each trial would allow us to determine the effects of manipulating specific within-session trials on responding several times within a single session – an experimental design more analogous to 'traditional' conditioning research that uses discrete stimuli such as illumination of a light or onset of a tone.

With this and other limitations in mind that have been described previously [cf. 8], we developed a task that used short, intravenous (IV) nicotine infusions as a CS [4,8,9]. Briefly, rats were infused with IV nicotine or saline (36 μl over 1 s). On nicotine trials, 30 s after the infusion, liquid sucrose was available for 4 s; sucrose was not available on intermixed saline sessions. Goal-tracking increased in the 30 s that intervened between the nicotine infusion and sucrose; no such increase was seen after saline infusions. The goal-tracking conditioned response (CR) evoked by the IV nicotine CS was blocked by

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doi:10.1016/j.bbr.2011.03.020

the central and peripheral nicotinic acetylcholine receptor antagonist mecamylamine, but not the predominately peripheral nicotinic acetylcholine receptor antagonist hexamethonium [10,11], indicating the CS effects of IV nicotine are mediated by receptors in the central nervous system [8].

How the nicotine stimulus interacts with other co-presented stimuli is only beginning to be investigated. In humans, nicotine is rarely, if ever, experienced without the presence of other stimuli. These stimuli could include tactile cues of a cigarette or a lighter, the visual cues of an ashtray or cigarette pack, or the smell and taste of inhaled smoke. From this perspective, the stimulus effects of nicotine are part of a more complex multimodal compound stimulus with interoceptive and exteroceptive elements. Because of this potential complexity of a compound stimulus formed between nicotine and other stimulus elements, an important associative property to examine is competition for control of the CR between the interoceptive nicotine stimulus and an exteroceptive stimulus. Two important forms of cue competition include overshadowing and blocking. In overshadowing, two stimulus elements are trained as a compound stimulus. Each element of the compound can acquire some control over the CR. The amount of behavioral control exerted by each stimulus element is presumably based on the salience (perceptibility) of that element relative to the other element [12–14]. We have previously shown that the IV nicotine stimulus competes with an exteroceptive light stimulus when trained as a compound CS [15]. In those experiments, cue-lights near the dipper receptacle were presented during the 30-s interval between nicotine infusion and access to sucrose. Tests of the nicotine and light elements individually revealed that the goal-tracking CR evoked by the light, was dose-dependently reduced (i.e., overshadowed) by co-presentation with the nicotine stimulus.

In blocking, one of the stimulus elements is first paired with the US. Following acquisition of the CR, a second stimulus is added to the initial stimulus to create a compound CS. This compound CS is then repeatedly paired with the US. Subsequent testing on the different elements of the compound would result in the added stimulus controlling less responding than if the stimulus had been trained alone [16]. Additionally, the originally trained stimulus should control more conditioned responding than the stimulus that had been subsequently added. Investigation of blocking between nicotine and exteroceptive stimuli has not yet been conducted. The present experiment sought to fill this gap by assessing whether previous excitatory conditioning with the nicotine CS would block subsequent conditioning to an exteroceptive light CS. We expect the nicotine CS to block conditioning to the light CS given our recent demonstration of overshadowing using this light plus nicotine compound stimulus [15].

Male Sprague–Dawley rats (348 ± 2 g at surgery) from Harlan Industries (Indianapolis, IN) were housed individually in clear 48.3 × 26.7 × 20.3 cm ($l \times w \times h$) polycarbonate tubs lined with aspen shavings. The colony was humidity- and temperature-controlled and maintained on a 12-h light:dark cycle; sessions were conducted during the light portion of the cycle. Water was continuously available in the home cage. Food was restricted to 20 g after each daily session except when noted.

Sessions were conducted in twenty conditioning chambers (ENV-008CT; Med Associates, Inc., Georgia, VT) measuring 30.5 × 24.1 × 21.0 cm ($l \times w \times h$). Each chamber was enclosed in a light- and sound-attenuating cubicle fitted with a fan to provide airflow and mask noise. A houselight with two bulbs (28 V, 100 mA each) was mounted on the back wall of the cubicle. The houselight was centered side-to-side, 23.5 cm above the top of the conditioning chamber, and 5 cm below the ceiling of the cubicle. Chamber sidewalls were aluminum; the ceiling and front and back walls were clear polycarbonate. Chambers were equipped with a recessed receptacle (5.2 × 5.2 × 3.8 cm; $l \times w \times d$) on the right sidewall. A dipper arm raised a 0.1 ml cup of 26% sucrose solution (w/v) in the receptacle. An infrared emitter/detector unit, located 1.2 cm into the receptacle and 3 cm from the floor, monitored head entries into the dipper. A second infrared emitter/detector unit was mounted 14.5 cm from the sidewall containing the receptacle and was positioned 4 cm above the rod floor. This unit provided a measure of chamber activity. Each chamber had a computer-controlled variable-speed syringe pump (Med-Associates, PMH-100VS) that allowed nicotine to be delivered IV. Pumps were located outside the sound-attenuating cubicle. Each chamber contained a spring leash attached to a balanced metal arm with a swivel. Tygon® tubing (AAQ04103; VWR, West Chester, Pennsylvania) extended from a 5-ml syringe mounted on the syringe pump through the leash to attach to the catheter. A personal computer with Med Associates interface and software (Med-PC for Windows, version IV) controlled stimulus presentations and recorded dipper entries and chamber activity.

Before surgery, rats were handled for at least 3 min per day for 3 days. Food was removed after handling on the last day. Dipper training in the conditioning chambers began the following day. Three 50 min sessions were conducted on three consecutive days with each session not starting until a rat's first dipper entry. The probability of receiving 4 s access to sucrose decreased from 0.167 to 0.05 per 60 s over the three sessions (ca. from 2.5 to 0.75 sucrose deliveries per min).

Catheter surgery occurred within two days of the last preliminary training session. Each rat was anesthetized with an intraperitoneal (IP) injection (1 ml/kg) of ketamine hydrochloride (100 mg/ml) followed by an IP injection (0.6 ml/kg) of xylazine hydrochloride (20 mg/ml) purchased from Midwest Veterinary Supply (Des Moines, IA). One end of a silicon catheter was implanted into the external left jugular. The other end was positioned under the skin such that it exited just below the scapula via a back-mount. The catheter was accessible by a metal cannula. To manage post-surgical pain, buprenorphine hydrochloride (0.1 mg/kg; Sigma, St. Louis, MO) was injected SC immediately following surgery and once more approximately 12 h after surgery. The catheter was flushed twice a day for the duration of the experiment with 0.1 ml of sterile saline mixed with heparin (30 U/ml; Midwest Veterinary Supply). The first five post-surgical flushes also contained streptokinase (ca. 7000 U/ml) to break down any clots that may start to form in the catheter. Rats were allowed five to six days of recovery in their home cage with free access to food before beginning the experiment. Catheter patency was assessed with a 0.05 ml IV infusion of xylazine (20 mg/ml) at pre-established points in each study [cf. 15,17]. Only rats with patent catheters were included in analyses.

Following the surgical recovery period, rats were assigned to a training group ($n = 14$ for C alone; $n = 10$ for N+; $n = 11$ for N−) irrespective of preliminary training performance. Nicotine is denoted by N. Chamber is denoted by C. The + indicates the stimulus was paired with sucrose, and the − indicates the stimulus was explicitly unpaired with sucrose during element training. More specifically, during the element phase of training, the N+ group received a 1 s nicotine infusion [(−)-nicotine hydrogen tartrate (Sigma), mixed in 0.9% sterile saline, adjusted to pH 7.0 ± 0.2 with NaOH, infusion of 36 μl over 1 s at 0.03 mg (base)/kg/infusion] followed 30 s later by 4 s access to sucrose; 10 such nicotine CS-sucrose US pairings occurred in each 2 h session. Stimulus presentations were separated by an average of 11 min (range 8–14 min) [cf. 8]. The N− group received the same number of nicotine and sucrose presentations as group N+ except that a nicotine infusion did not occur within 4 min of any sucrose presentations (i.e., unpaired control). This control was used to control for exposure to the CS and the US in a manner that does not produce excitatory conditioning. The C alone group was transported and exposed to the chamber like the