Behavioural Brain Research 257 (2013) 83–89

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Environmental manipulations alter age differences in attribution of incentive salience to reward-paired cues

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HIGHLIGHTS

- Food restriction enhanced sign-tracking behavior in isolate-housed adolescents.
- Food restriction increased goal-tracking behavior in pair-housed adolescents.
- Food restriction increased overall sign- and goal-tracking behavior in adults.
- As seen before with pair housing, adults sign-tracked more than adolescents.

ARTICLE INFO

Article history:
Received 14 December 2012
Received in revised form 13 August 2013
Accepted 9 September 2013
Available online 16 September 2013

Keywords:
Pavlovian conditioned approach
Incentive salience
Adolescent
Rat
Sign-tracking
Isolate-housing
Food restriction

ABSTRACT

Cues repeatedly paired with rewards often themselves become imbued with enhanced motivational value, or incentive salience. During Pavlovian conditioned approach procedures, a cue repeatedly preceding reward delivery often elicits conditioned responses at either the reward delivery location ("goal-tracking") or the cue itself ("sign-tracking"). Sign-tracking behavior is thought to reflect the individual differences in attribution of incentive salience to reward-paired cues that may contribute to addiction vulnerability. Adolescent rats typically demonstrate less sign-tracking behavior than adult rats, a surprising finding given that adolescence is hypothesized to be a time of heightened addiction vulnerability. Given evidence that adult sign-tracking behavior can be influenced by environmental conditions, the present study compared the effects of isolate housing and food deprivation on expression of sign-tacking and goal-tracking behavior in adolescent and adult male rats across eight days of a Pavlovian conditioned approach procedure. Pair-housed adults exhibited more sign-tracking behavior than pair-housed adolescents; however, this age difference was not apparent in isolate-housed subjects. Adolescents often appeared more sensitive than adults to both food restriction- and isolate housing-induced changes in behavior, with food restriction promoting an increase in sign-tracking among isolate-housed adolescents and an increase in goal-tracking among pair-housed adolescents. For adults, food restriction resulted in a modest increase in overall expression of both sign- and goal-tracking behavior. To the extent that sign-tracking behavior reflects attribution of incentive salience to reward-paired cues, results from the present study provide evidence that reactivity to rewards during adolescence is strongly related to the nature of the surrounding environment.

1. Introduction

Cues repeatedly paired with rewards often themselves become imbued with incentive value. Incentive salience refers to the enhanced motivational value of stimuli repeatedly paired with reward delivery (see [1]). Attribution of incentive salience to reward-paired cues may reflect individual differences that underlie addiction vulnerability (reviewed by [2,3]). Pavlovian conditioned approach (PCA) procedures (sometimes referred to as 'autoshaping') have gained popularity as a means to assess the attribution of incentive salience to reward-paired cues in rodents (e.g., [4–7]). A typical PCA procedure involves repeated pairings of a cue (conditioned stimulus; CS) and a reward (unconditioned stimulus; US); such pairings often eventually elicit one or more conditioned responses (CR) during cue presentation. One CR involves approach to the reward delivery location (typically a food trough or liquid dipper arm area); this reward-directed response is referred to as goal-tracking [8]. An alternative CR involves approach and interaction with the CS itself, a response referred to as sign-tracking [9]. Sign-tracking behavior is hypothesized to reflect attribution

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http://dx.doi.org/10.1016/j.bbr.2013.09.021

of incentive salience to a reward-paired cue, resulting in the cue serving as a "motivational magnet" (see [2]).

Evidence supports the hypothesized relationship between sign-tracking and addiction vulnerability: animals that exhibit high levels of sign-tracking show greater sensitization to cocaine-induced psychomotor activation [10], more rapid acquisition of cocaine self-administration [5], higher breakpoints in progressive ratio operant responding for cocaine [11], and enhanced reinstatement of cocaine self-administration [11]. Likewise, prior amphetamine sensitization increases sign-tracking behavior [12]. Sign-tracking behavior is also positively correlated with other behaviors related to addiction vulnerability, including impulsive action [13,14] and novelty preference [5]. Release of corticosterone (CORT) also appears to correlate with sign-tracking behavior: animals that exhibit high levels of sign-tracking behavior during PCA sessions have significantly higher post-session CORT levels than animals that exhibit low levels of sign-tracking [2,15]. Evidence also strongly supports a role for CORT in addiction vulnerability (see [16,17]), with high levels of CORT associated with greater drug self-administration and psychomotor activation [18–21].

Adolescence is the developmental period that encompasses the transition from youth to maturity, during which individuals experience a host of neural, hormonal, and behavioral alterations that include increased peer affiliation, impulsivity, risk taking, and novelty seeking/preference [22–28]. These behavioral characteristics may contribute to the initiation of substance use and abuse that is prevalent during adolescence. Results from the 2011 Monitoring the Future study revealed that 70% of high school seniors have consumed alcohol, 40% have smoked cigarettes or marijuana, and 25% report having used other illicit drugs [29]. Indeed, adolescence is often considered a critical period for addiction vulnerability (see [30,31]). Adolescents demonstrate greater neural activation (indexed by c-fos protein expression) than adults in the nucleus accumbens in response to a cue previously paired with a food reward [32]. Among adult animals, elevated c-fos mRNA expression in the nucleus accumbens in response to presentation of a cue previously paired with a food reward is seen only in animals that exhibit high levels of sign-tracking behavior [33]. Given the hypothesized relationship between sign-tracking behavior and heightened drug abuse vulnerability, as well as the other behavioral and neurobiological correlates of sign-tracking, one might expect adolescents to exhibit more sign-tracking behavior than adults. Previous evidence from our lab, however, has revealed an opposite ontogenetic profile: adults typically exhibit greater levels of sign-tracking behavior than adolescents [4,12].

Although evidence supports a strong genetic component in sign-tracking and goal-tracking behavior (e.g., [13]), early environmental manipulations such as isolation rearing and deprivation of natural maternal care have recently been demonstrated to increase expression of sign-tracking behavior in adulthood [34,35]. These studies support a role for early life experiences in shaping attribution of incentive salience to reward-paired cues later in life, potentially contributing to differences in addiction vulnerability. The present study was designed to assess the effects of environmental manipulations on sign-tracking and goal-tracking behavior in adolescent and adult rats. Food restriction and isolate-housing were selected as the experimental manipulations due to evidence that each can influence drug reward and/or sensitivity [36–40]. Isolate-housing in particular may have different consequences for adolescents and adults (see [41]).

2. Materials and methods

2.1. Subjects

A total of 64 male Sprague-Dawley rats bred in our colony at Binghamton University were used in the present study. On postnatal day (P) 1, litters were culled to 8–10 pups, keeping a ratio of 6 males to 4 females when possible. Subjects were weaned on P21, at which time they were pair-housed with same-sex littermates and maintained in a temperature-controlled vivarium on a 12:12-h light:dark cycle (lights on at 7 AM), with ad libitum access to food (Purina lab chow, Lowell, MA) and water (except as specified below). All animals were treated in accordance with guidelines established by the National Research Council [62] and protocols approved by the Binghamton University Institutional Animal Care and Use Committee. Eight subjects were assigned to each of the groups defined by the 2 age (adolescents, adults) × 2 housing (isolated, paired) × 2 food condition (food-restricted, free-feeding) factorial design. In order to avoid confounding litter effects, no more than one animal per litter was assigned to the same experimental condition (see [42,43]). All testing was conducted between 1000 and 1600 h.

2.2. Apparatus

Twelve operant chambers measuring 30.5 cm × 24.1 cm × 21 cm (Med Associates, St. Albans, VT) housed within sound-attenuating boxes measuring 55.9 cm × 38 cm × 35.6 cm were used. A food receptacle with a dispenser for banana pellets (45 mg dustless precision banana-flavored pellets, Bio-Serv, Frenchtown, NJ) was mounted on the right wall of each chamber, along with a retractable illuminated lever on either the left or right side of the receptacle. Levers were illuminated only when extended out into the chamber, and not while retracted into the chamber wall. For the adults, the lever measured 4.8 cm wide, whereas a mouse-sized lever measuring 1.6 cm was used for adolescent animals. The receptacle and the lever were mounted 2.5 cm from the floor of the chamber for adolescents and 4.5 cm from the floor of the chamber for adults. Photosensors within the food receptacle were used to count nosepokes into the receptacle area. A red house light was mounted in the top right corner of the left wall and was illuminated throughout each session.

2.3. Procedure

Eleven days before the start of PCA testing, subjects were re-housed either alone or with a same-sex non-littermate on P21 (adolescents) or P65 (adults) in standard acrylic breeder tubs with wood shavings. Animals remained either isolate- or pair-housed for the duration of the study. Whenever possible, subjects were housed with a counterpart of similar body weight, resulting in average weight differences of 7.6 g (12% total body weight) among adolescents and 15.1 g (4% total body weight) among adults. To reduce potential neophobia to the banana pellets used during training, approximately 6.5 g of banana pellets were placed in the home cage of each animal (or 13 g per pair of animals) beginning on P28 or P72, for 2 consecutive days prior to pre-training. All animals assigned to free-feeding conditions had ad libitum access to food and water. Subjects in the food-restricted conditions had ad libitum access to water, but were given daily food allotments as described below.

2.3.1. Food restriction

Beginning the day prior to pre-training, adult subjects assigned to the food-restricted group were given 3–3.5 g of rat chow daily until they reached 85% of their free-feeding (pre-restriction) weight. When they reached this point, they were given approximately 14 g per day, with this amount increased as needed to maintain their target body weight. Adolescents assigned to the food-restricted group were given approximately 7–7.5 g of food initially, such that they gained little weight overnight (approximately 1–2 g). Each day thereafter, this amount was increased as needed to allow for 5–8 g of weight gain, thereby permitting maintenance of approximately 85% of the normal growth trajectory determined from the weights of their free-feeding counterparts. Food-restricted subjects received food each day after testing.

2.3.2. Pre-training

On each of the 2 days prior to onset of the PCA procedure (P30 or P74), animals were placed in the operant chambers with the levers in the retracted position. During each pre-training session, 25 pellets were delivered on a variable interval (VI) 90 s schedule over the course of 35–40 min.

2.3.3. Pavlovian conditioned approach

Beginning on P32 or P67, subjects were given daily PCA sessions for 8 days. Each session consisted of 25 8-s presentations of the lighted lever conditioned stimulus (CS) on a VI 90 s schedule, followed by delivery of a pellet (US) as the lever retracted. Sessions lasted for approximately 35–45 min, with the CS presentations provided on a VI 90-s schedule and with the 25 CS-US pairings occurring independently of the subjects' behavior. Number of nosepokes and lever presses were recorded during each 8-s lever presentation as measures of goal-tracking (GT) and sign-tracking (ST), respectively. Any remaining banana pellets after each daily session were counted and removed from the chamber. By day 8 of the PCA procedure, food-restricted adolescents and all adults consumed all banana pellets whereas free-feeding adolescents had an average of 1.87 (pair-housed) and 4.5 (isolate-housed) leftover pellets. Immediately following the final PCA session, subjects were sacrificed and trunk blood was collected and centrifuged. Plasma was stored at −80 °C until assayed for CORT using radioimmunoassay (see [64]).