



Sign Tracking in an Enriched Environment: A Potential Ecologically Relevant Animal Model of Adaptive Behavior Change

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Abstract

When an object conditioned stimulus (CS) is paired with a food unconditioned stimulus (US), anticipatory goal-directed action directed at the US location (goal tracking) is accompanied by behavior directed at the object CS (sign tracking). Sign-tracking behavior appears to be compulsive and habit-like and predicts increased vulnerability to the addictive potential of drugs in animal models. A large body of the literature also suggests that environmental enrichment protects against the development of addiction-prone phenotypes. Thus, we investigated whether compulsive-like sign tracking develops in environmentally enriched rats trained directly in their enriched home environment. We demonstrate that adolescent enriched-housed male Sprague-Dawley rats readily sign track a 5% ethanol bottle CS in their home environment and at a rate higher than adolescent standard-housed rats. We also show that enriched adolescent rats sign track less than enriched adult-trained rats and that acute isolation stress affects sign- and goal-tracking performance of adolescents and adults differently. Sign tracking increased more in the adult than the adolescent rats. Whereas the younger rats showed a decrease in goal tracking after the final stressor manipulation, the adults showed increased goal tracking. Our results are consistent with recent studies, which suggest that although sign tracking performance is compulsive-like, it is not as inflexible and habit-like as previously assumed. Testing in an enriched home environment with object CSs having greater affordance than “neutral” lever CSs may provide greater ecological relevance for investigating the development and expression of adaptive and compulsive-like behaviors in translational research.

Keywords Animal models · Motivation · Reward · Development

Introduction

In a Pavlovian or classical conditioning paradigm in which a tangible object conditioned stimulus (CS) (e.g., a retractable lever) is paired with a food unconditioned stimulus (US), sign tracking emerges as conditioned responding directed at the CS (Hearst & Jenkins, 1974). Sign-tracking rats respond to the inserted object CS by approaching and manipulating it. However, some rats do not sign track but react to the insertion of the object CS by directing their attention and behavioral responses toward the site of the impending US—a behavior labeled as “goal tracking” (Boakes, 1977). Thus, while goal trackers prefer to use the CS as a predictive signal for the

impending US, as revealed by conditioned headpoking into the food tray during the CS, sign trackers prefer to approach and interact with the object CS. Over the past decade, the finding that there are substantial individual differences in the sign-tracking behavior of outbred rats has been interpreted to reflect individual differences in the attribution of incentive salience to reinforcer-associated cues (Robinson & Flagel, 2009). Intriguingly, increased attribution of incentive salience to the object CS in the sign-tracking procedure is associated with increased responsiveness to drug-paired cues and an increased vulnerability to acquire addiction-like behaviors (Saunders & Robinson, 2011; Flagel, Akil, & Robinson, 2009; Yager, Pitchers, Flagel & Robinson, 2015).

The sign tracker’s attribution of incentive salience to an object CS also appears to capture behavioral characteristics that are comorbid with addiction in humans (Colaizzi et al., 2020). The sign tracking procedure evokes excessive and unproductive behavior because responding to the lever CS (biting, pawing, and gnawing) has no efficacy. The food US appears whether a response to the object CS occurs or not.

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Moreover, sign tracking appears to be maladaptive when responding to the object CS stubbornly persists in modified procedures where responding during a trial results in the loss of the US (e.g., negative automaintenance or omission procedures) (Chang & Smith, 2016; Hearst & Jenkins, 1974; Williams & Williams, 1969) and in extinction when the non-contingent US rewards are no longer presented (Ahrens et al., 2016; Flagel et al., 2009). Thus, for the sign tracker the US-paired cue object, initially “neutral,” appears to have been transformed into a “motivational magnet” that elicits a “wanting” or craving for the object (Berridge & Robinson, 2016). The behavior of sign trackers has been proposed as an animal model of the compulsive-like and habit-like behavior relevant to human psychiatric disorders, such as impulse control disorders and substance abuse (Colizzi et al., 2020; Morrow, 2018; Robinson & Berridge, 2008).

The success of the incentive-sensitization theory has generated substantial interest among behavioral neuroscientists to use the sign-tracking procedure as a “tool” to identify animals with an exaggerated predisposition to assign incentive salience to an object CS (Meyer et al., 2012). In these experiments, rats are trained (typically 5 training sessions) with a retractable lever CS and a food US. The animals are then separated into sign trackers and goal trackers for comparison, whereas animals that display a mixed profile of sign- and goal-tracking behaviors may be excluded from further study. Individual differences in sign- and goal-tracking behaviors are interpreted as indicative of a behavioral trait or phenotype that reflects differences in incentive salience attribution (Flagel, Akil, & Robinson, 2009; Robinson & Flagel, 2009). These studies have revealed that compared with goal trackers, sign trackers are more vulnerable to drug effects in experimental procedures designed to model human drug addiction (Saunders & Robinson, 2011; Yager, Pitchers, Flagel & Robinson, 2015). More recently, however, the view that sign trackers are distinctly more vulnerable to addictive-like behavior than goal trackers has been tempered by evidence that other important factors common to sign- and goal-trackers moderate addiction vulnerability (Kawa, Bentzley & Robinson, 2016).

Another, more traditional, conceptual understanding of sign-tracking behavior is as an acquired behavioral action directed toward the object CS that emerges partly, as is the case with any conditioning procedure, because of the constraints of the experimental contingencies. In this experimental approach, animals experience the object CS and US pairings daily until asymptotic performance is achieved where most, if not all, animals show at least some sign-tracking behavior (in these studies goal tracking may or may not be measured) (Conrad & Papini, 2018; Keefer et al., 2020; Srey, Maddux, & Chaudhri, 2015; Tomie, di Poce, Derenzo, & Pohorecky, 2002). From this learning perspective, individual differences in sign- and goal-tracking performance are not examined

experimentally as dichotomous traits with the goal of identifying individuals vulnerable to addiction, but as a continuum of sign-tracking/goal-tracking performance that are moderated by many factors including those factors theorized to contribute to drug and alcohol use and abuse (e.g., stress, drug availability, peer influence). We believe that there is strong potential for revealing the impact of environmental factors on individual differences in goal-directed and compulsive-like action when sign tracking is considered within the broader context of acquired expectancies (Deraman et al., 2018) and adaptive learning (Cabrera, Jiménez, & Covarrubias, 2019; Timberlake, 1984, 1994; Timberlake & Lucas, 1989).

The ostensibly compulsive, excessive, and unproductive behavior directed at the retractable lever during a sign-tracking procedure has contributed to the acceptance of this procedure as an animal model of compulsive behavior and drug use with good face validity (Flagel et al., 2009; Colaizzi et al., 2020). Alternative theoretical interpretations of behaviors that emerge in conditioning procedures emphasize that these seemingly unproductive behaviors reflect functional behavioral repertoires constrained by the experimental environment (Timberlake, 1994; Breland & Breland, 1961). Variations of the sign tracking procedure have been used that reveal the complex and functional aspects of sign-tracking behavior. The CS that is “tracked” can be any tangible object, even objects that offer more affordance than a retractable lever. A rolling ball bearing, for example, not only evokes the natural predatory behaviors of approach, pawing, biting, and gnawing that are elicited by a retractable lever, but also supports chasing, capturing, and transporting of the captured artificial “prey” (Timberlake & Melcer, 1988; Timberlake & Washburne, 1989). Behaviors directed at the ball bearing have characteristics of normal functional predatory behaviors, but in strong sign trackers they also have the quality of compulsive-like responding. When nonthirsty, nonhungry rats are trained with a water bottle CS and a sucrose pellet US sign tracking rats will paw, bite, and lick the bottle spout and even drink the water. Drinking is a productive consummatory behavior, but in this situation drinking, nonetheless, appears compulsive to the experimenter because the rats have no homeostatic need for water. When the bottle is filled with alcohol rather than water the animals also will drink compulsively, although in greater amounts than when the bottle contains water indicating that the sign tracking has gained some efficacy because of the experience with alcohol (e.g., pharmacological effects) (Tomie et al., 2004; Tomie, Mohamed, & Pohorecky, 2005; Tomie & Sharma, 2013). Thus, when an alcohol-containing bottle is the object CS compulsive alcohol consumption during sign tracking is not just about the effects of the alcohol itself but driven primarily by the exigencies of the situation (also see Falk & Tang (1988) on scheduled-induced polydipsia as a model of alcohol abuse). We concur with Tomie and colleagues that sign tracking of an ethanol bottle has potential for an animal model of alcohol abuse. We

further argue that this version of the sign-tracking procedure provides greater face validity for human compulsive-like behavior than sign tracking of a retractable lever, with an alcohol bottle CS specifically providing potentially valuable translational value to the broader issue of alcohol use, not just abuse and addiction. As is the case with humans, alcohol and drug taking behavior has the features of intentional productive behavior (e.g., drinking) and, as in the case of individuals with problems of alcohol abuse, the features of compulsive behavior. Indeed, even though the response-independent pairings the CS and US of the sign-tracking procedure meets the strict definition of a Pavlovian conditioning paradigm, the debate concerning whether sign tracking is a pure Pavlovian CR or also influenced by adventitious reinforcement (the procedure was originally called “auto shaping”) is a long-standing one. More recently, Domjan (2016) has argued that the distinction between traditional views of acquired motivated behavior as comprising distinctive responses that are elicited (sign tracking) or emitted (goal tracking) has outlived its usefulness and is not consistent with current conceptions of classical and operant conditioning.

While there is some promise in the translation of compulsive-like sign tracking in rodents to human behavior and addiction (Colazzi et al., 2020), others have questioned the applicability of animals tested in isolation and in the restricted laboratory environment to the complex issue of human drug use, abuse, and addiction (Field & Kersbergen, 2019). Moreover, critics of animal models of drug abuse and addiction have questioned the validity of any psychological construct that characterizes human addiction as inflexible, compulsive, and habit-like (Heather, 2017). Relevant to this latter issue is the finding from recent reinforcer devaluation studies that have revealed that sign tracking is not as inflexible and habit-like as previously assumed (Amaya et al., 2020; Derman et al., 2018). Thus, a reconceptualization of sign tracking behavior as compulsive-like yet more flexible than previously assumed may have more translational value for critics of animal models of habit-like drug abuse.

This study was designed to improve the face validity of the sign tracking procedure for translational research relating to compulsive-like behavior by using a bottle CS to provide greater affordance than the retractable lever and by training rats in a more complex and ecologically valid home environment. The housing of rats alone or in pairs in restrictive rodent cages is necessarily standard housing in most behavioral neuroscience laboratories to obtain greater experimental control. Compared with standard housing, the complex housing condition used in the present experiment is typically described as “enriched” and often manipulated as an independent variable in experiments on enrichment. From an ecological perspective, however, standard laboratory housing is more appropriately described as impoverished and “enriched” housing as more natural. Nevertheless, in the present paper we will continue to describe the housing condition as enriched housing.

Beckmann and Bardo (2012) reported that rats raised in enriched housing showed less sign tracking directed toward a lever CS compared with rats raised in isolation, suggesting that enriched housing reduces incentive salience attribution. However, these results are limited. Training was with a retractable lever CS and lasted for only 5 days. It is now well established that sign tracking behavior may emerge in most rats with more extensive training (Keefer et al., 2020; Srey, Maddux, & Chaudhri, 2015). To improve ecological validity, we raised rats in an enriched environment and trained them in a sign-tracking procedure with an alcohol bottle CS directly in their enriched home cage. If the Beckmann and Bardo study results applies broadly to all variants of sign-tracking procedures, we would not be able to develop a sign-tracking procedure with greater ecological relevance. Thus, in anticipation of a possible failure to observe sign-tracking performance in enriched rats, we included for comparison a group of comparably aged pair-housed rats raised in standard laboratory cages to determine if the Beckmann and Bardo results extended to our experimental procedure.

Prior sign tracking studies with an alcohol bottle CS demonstrate that although drinking is elevated relative to a water bottle control group, this elevation of alcohol consumption is not transferred to 2-bottle preference tests with alcohol and water outside of the sign-training context (Tomie et al., 2004; Tomie & Sharma, 2013; Tomie, Jeffers, & Zito, 2018). In the present study, we did not manipulate the content of the bottle CS (all groups were exposed to an alcohol bottle), but we were interested in determining whether the extensive exposure to alcohol during the sign-tracking procedure induced a strong preference for alcohol over water. We demonstrate that rats housed and trained in their enriched home environment during adolescence show robust sign tracking of an alcohol bottle CS at levels significantly greater than pair-housed rats tracking an alcohol bottle, but neither group developed a strong preference for alcohol over water outside of the training context (i.e., outside their home environment).

While individual differences in sign tracking is influenced by genetic variation of species-typical behavior of rats (Flagel, Akil, & Robinson, 2009), it is the contingencies of the sign-tracking procedure that constrains preorganized behavior to induce compulsive-like sign tracking behavior (Cabrera, Jiménez, & Covarrubias, 2019; Timberlake, 1993, 1994; Timberlake & Lucas, 1989). Maturation and varied experiences during ontogenetic development also are important factors. For example, adolescent rats show less sign tracking of a retractable lever CS than adults (Anderson, Bush, & Spear, 2013; DeAngeli, Miller, Myer, & Bucci, 2017). Once we had established that enriched-housed animals demonstrate robust sign tracking, we added a group of rats from the same cohort as the adolescent enriched-housed rats that began the sign-tracking procedure when they reached an adult age. We found that the age differences in sign tracking observed in rats

trained with retractable lever CS in isolated test chambers (Doremus-Fitzwater & Spear, 2011; Rode, Moghaddam & Morrison, 2019) also are seen in rats sign tracking an alcohol bottle CS in an enriched environment. Stressors can have amplifying effects on the compulsive-like characteristic of sign tracking. While adolescent rats show less sign tracking of a lever CS than adults, chronic stress increases sign tracking to adult levels or greater (Anderson et al., 2013; DeAngeli et al., 2017). We examined the impact of an acute stressor on asymptotic sign-tracking and goal-tracking performance in the enriched rats trained as adolescents or as adults by temporarily removing them from their home environment and placing them in restrictive cages. Upon returning the isolated rats for brief test sessions in their home cage, we were able to examine the effects of the acute stressor on sign tracking and goal tracking during the isolation stress and during recovery.

Methods

Subjects and Housing Conditions

The subjects were 24 male Sprague-Dawley rats purchased from Envigo™ (Indianapolis, IN) at 21 days of age and delivered to the laboratory on the same day. Sixteen rats were housed in groups of four in galvanized wire-mesh tower cages (Martin Cages, Inc, Nanticoke, PA, Model H-600HR) to provide an enriched housing experience. The middle shelves of two animal cage racks were removed to accommodate two cage towers per rack. The tower cages (45.7 W x 27.9 D x 61 H cm) sat in 7.6-cm-deep plastic pans that served as the bottom floor. The middle floor spanned the full width of the cage and included two ramps leading to a lower and an upper landing on opposite sides of the cage, each landing spanning approximately one third of the cage width. The mesh (1.3 x 2.5 cm) cage structure afforded the rats the opportunity to climb the walls, which they often did. For hiding, two enclosures were included in each cage, an untethered PVC pipe (10.2 Dia x 30.5 L cm) on the middle floor and a wood nesting box (30.5 W x 20.3D x 12.7H cm) on the lower floor. The rats accessed food pellets from two hoppers attached to a side wall on the lower floor, and two water bottles were affixed side-by-side to the front cage wall on the lower landing. The upper landing included a food tray and sipper access area for sign tracking (see apparatus description below). Although the rats could explore these areas on the top platform at any time, the sign tracking equipment was activated only during training sessions. Novel objects (e.g., commercially available hard wood chew toys, cardboard tubes) were scattered on all levels of the cage and rotated weekly. A black cloth curtain affixed to the top of the rack was always lowered to cover the front of the top two thirds of the cage tower except when transferring the rats from and to the cages. The remaining eight rats were

housed in pairs in standard clear polypropylene cages (42.7 L x 28.7 W x 19.8 H cm) with stainless-steel grill tops that held two water bottles and food pellets. A mix of corncob and dry cellulose material (Teklan 7087c) was used as the bedding for the standard and tower cages. Food (Teklan Rodent Diet, 7102) and water were provided for all animals *ad libitum* throughout the experiment. The animals were maintained on a 12-hour light-dark cycle, with the light turning on at 8 am. Testing took place in the mornings and early afternoon. All procedures were approved by Seton Hall's Institutional Animal Care and Use Committee and performed according to the animal care standards of the National Institutes of Health.

Apparatus

Each of the four cage towers were fitted with devices necessary to conduct sign tracking in the home environment. A retractable sipper device (Med Associated Inc., St. Albans, Vt, ENV-252M) was mounted on the outside of the upper landing on the left side. When activated the device made a noise that alerted the animals to the advancing bottle and placed the sipper tip flush with the wire-mesh wall. The wire between two neighboring mesh squares was cut to provide a 2.5- x 2.5-cm sipper access opening 3 cm above the floor. Contact lickometers (Med Associates, Model ENV-250B) monitored contacts/licks on the sipper until the bottle was retracted at the end of a trial. A 5- x 5-cm area of the mesh wall was cut away 7.6 cm to the right of the sipper tube opening to mount a metal food tray with clear plastic sides. An infrared head entry detector (Med Associates, model ENV-254) recorded headpoking responses into the food tray. A pellet dispenser (Ralph Gerbrands Co) filled with sugar pellets (Noyes, 45 mg) was mounted directly behind the food tray. A speaker located in the lower right-hand corner of the upper landing provided background white noise (Med Associates, model ENV-225SM) during sign tracking sessions. All equipment and experimental events were controlled by MedPC software (Med Associates Inc., St. Albans, VT).

Procedure

Phase 1: Acquisition of Sign Tracking.

The rats housed in the enriched tower cages were divided into two groups to be trained in the sign-tracking procedure as adolescents or as adults. The rats in both groups were maintained identically daily with the exception that exposure to sign tracking procedure and preexposure to ethanol was delayed in the adult group until they reached the target age. A generally accepted onset of adolescence in rats is postnatal day (P) 28 with adulthood beginning at P70 (Spear, 2000). The Adolescent-Enriched (Adol-E) group (n = 8) began

ethanol preexposure at P35 and sign tracking at P56 (21 days). The Adult-Enriched (Adult-E) group ($n = 8$) began ethanol preexposure at P98 and sign tracking at P112 (14 days). For Phase 1, we also included an Adolescent-Standard (Adol-S) group ($n = 8$) pair-housed in standard cages. As with the Adol-E group, the Adol-S group began ethanol pre-exposure at P35 and sign tracking at P56.

Ethanol Pre-exposure For 3 (Groups Adol-E and Adol-S) or 2 (Group Adult-E) weeks before the initiation of sign tracking, one of the water bottles on the home cages was replaced with ethanol, beginning with 2% ethanol, and gradually increased by 1% to 5%. The adult rats were given one less week of preexposure because they more readily consumed the ethanol than the adolescent rats. However, the number of days with access to ethanol was the same for both age groups during the subsequent acquisition phase (6 weeks) and acute stressor manipulation phase (2 weeks). We suspect that an additional week of ethanol preexposure was necessary for the adolescents to drive up free-access ethanol intake during preexposure because of an environment that was relatively novel and sufficiently complex to support many competing behaviors. When the adults were preexposed to ethanol, they were more familiar with the environment and they had much more experience with drinking from sipper tubes. Although it was not possible to determine how much ethanol each rat was consuming in the home cages, we tested each rats' preference for ethanol individually in a stainless-steel wire mesh test cage in a separate room once per week. These individual two-bottle tests were 20 minutes in duration and provided the rats with a choice of the current ethanol concentration and water by affixing two bottles to the front of the wire-mesh cage. These weekly two-bottle tests continued during the sign tracking training.

Sign tracking For the sign tracking sessions all rats were removed from their cage tower and temporarily placed with their cage mates into a standard cage in an adjoining room. The Adol-E and Adult-E groups were tested individually on the upper platform of their own home cage tower. Rats were confined to the upper platform by installing a plexiglass barrier that spanned the full length of the platform floor. The rats of the Adol-S group were also trained on the confined platform of a cage tower to which they were randomly assigned. Adaptation sessions to the confined platform took place during the third week of ethanol preexposure with 10 sucrose pellets in the food tray and the white noise turned on.

All groups were run in sign-tracking sessions every other day, excluding Sundays, for 6 weeks (i.e., Monday, Wednesday, & Friday or Tuesday, Thursday & Saturday). During a session, the animal was weighed, placed on the confined sign-tracking platform, and a white noise was turned on to indicate the start of the session. After 60 seconds, the sipper

bottle containing 5% ethanol was advanced to make the sipper accessible to the rat. After 10 seconds, the sipper was retracted, and a single sugar pellet delivered into the food tray to complete a trial. A single session consisted of 25 bottle-pellet trials separated by a 60-second intertrial interval. Two minutes after the last trial, the session ended. The bottle containing the ethanol was weighed before and after each session to measure the ethanol consumed while sign tracking. When all sessions for the day were completed, the plexiglass barriers on the upper platforms were removed, and the enriched groups were returned to their home towers.

Phase 2: Acute Stressor Manipulations and Recovery

Only the rats raised in the enriched home environment (Adol-E and Adult-E) were tested in this second phase of the experiment to determine the effects of acute isolation stress on subsequent sign-tracking and goal-tracking performance in adolescent-trained and adult-trained animals. In phase 2, the enriched-housed groups were administered an acute isolation stressor by transferring the rats from their enriched home environment to restrictive cages for 3 days followed by 3 days of recovery back in their home tower. The restrictive home cage was a standard polypropylene rat cage with stainless-steel grid top, but the exterior walls of the cage were covered with black contact paper to block visual access to the surrounding vivarium environment (food and water was provided *ad libitum*). During the acute stressor condition, the rats were tested in the sign-tracking procedure on the second and third day by returning them briefly to the platform of their home cage for a sign tracking session, and again 24 hours later when they were returned to their enriched home cage to begin a week of recovery. During the recovery week, the rats were again tested three more times on the sign-tracking platform. The stressor manipulation was then repeated the following week. Thus, there were two acute 3-day stressor manipulations (Stressor 1 & 2) each followed by three days of recovery (Recovery 1 & 2) when the rats were transferred back to their enriched home tower. Although isolation from the enriched home environment was the main stressor manipulation and administered twice, during one of the two stressor administrations the rat was alone and during the other stressor administration the rat was with one home companion. The order was counterbalanced so that half the rats in each age group were first stressed alone and then in pairs (alone-pairs), and vice versa for the other half (pairs-alone).

Free Range Access to the Home Cage Tower During Sign Tracking Sessions

The original plan of the study was to test various manipulations on sign-tracking performance when the rats were no longer confined by the plexiglass barrier to the home cage

platform and therefore free to roam the home cage, however the shutdown of Seton Hall University and the laboratories because of the Covid-19 pandemic caused the premature termination of the study. Nevertheless, we were able to collect some data demonstrating that the rats continued to sign track when they had free range of their home cage tower during a sign-tracking session. After the completion of Phase 2 by the Adol-E group, we continued to test the rats in sign-tracking sessions to maintain performance while the Adult-E rats completed Phase 1. The Adol-E and Adult-E groups were tested for three sessions with the barrier removed (i.e., free range access to the home cage) approximately 3 weeks and 1 week after their last sign-tracking session, respectively. No other changes in procedure were made; thus, all rats continued to sign track a bottle CS containing 5% ethanol as in Phases 1 and 2. The following week the two groups were again tested for three additional free-range, sign-tracking sessions; however, the ethanol solution was replaced with water. Finally, the behaviors of several rats were recorded during a sign-tracking session with a GoPro HERO8 camera and their behavior analyzed using the *Behavioral Observation Research Interactive Software (BORIS)*, an open-source event logging and behavior coding software (Friard & Gamba, 2016).

Data Analysis

The dependent variables for each phase of the experiment were analyzed with separate mixed factorial ANOVAs using JASP version 0.11.1. Significant interactions were followed when appropriate by additional ANOVAs, simple main effects, planned comparisons, or Bonferroni post-hoc comparisons. When Mauchly's tests indicated a violation of the sphericity assumption, degrees of freedom were corrected using the Greenhouse-Geisser estimates of sphericity.

Results

Phase 1: Acquisition

The acquisition of sign tracking for the Adol-E and Adult-E groups as well as the Adol-S group are shown in the top panel of Fig. 1. The 18 days of sign tracking training are presented as 2-day session blocks. Sign tracking performance was defined as the mean total licks per block. Note that the lickmeters measure contact with the drinking spout and may include bites, sniffs, and occasional pawing particularly early in training. Because the substantial number of contacts are licks, the dependent variable for sign tracking is referred to as licks. A 3 (Group; Adol-E, Adult-E, Adol-S) \times 9 (Block 1-9) mixed ANOVA on the mean total licks (Fig. 1, top panel) revealed significant main effects of Group, $F(2, 21) = 22.68$, $p < 0.001$, $\eta_p^2 = 0.684$, and Block, $F(3.7, 78.4) = 51.15$, $p <$

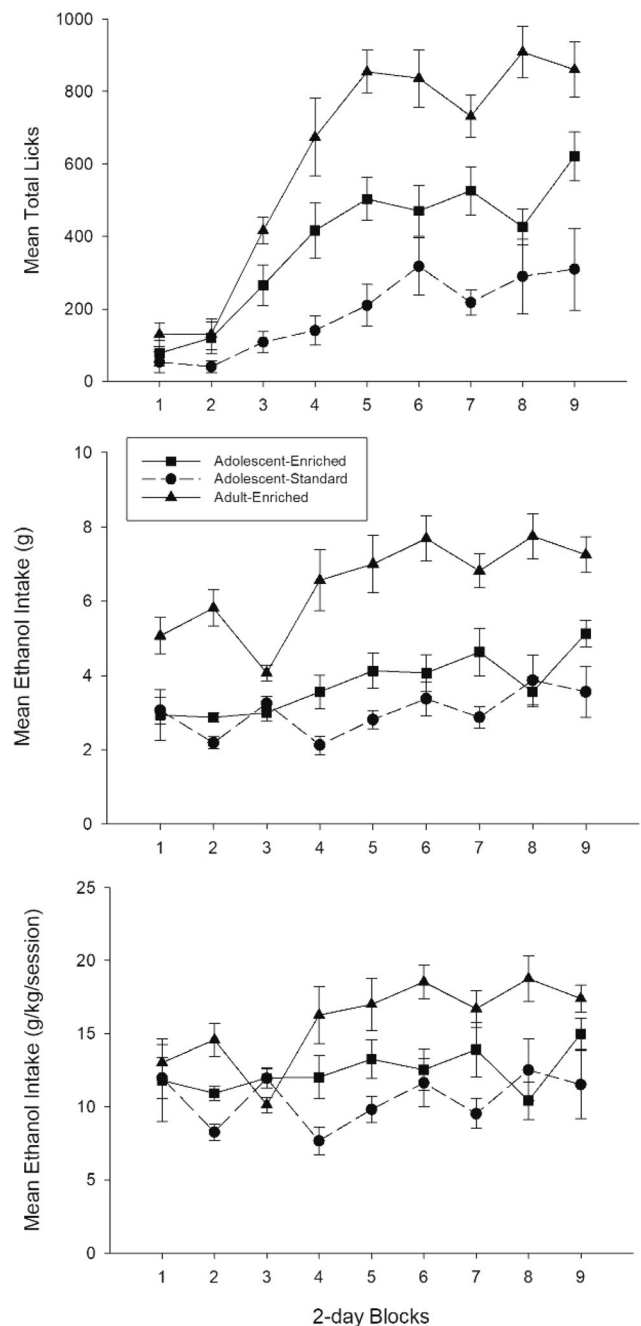


Fig. 1 Mean total licks (top panel), ethanol solution intake in grams (middle panel) and grams per kg to correct for body weight (bottom panel) averaged across 2-day blocks during the first 18 days of sign tracking for adolescent and adult rats raised in enriched housing and adolescent rats raised in standard housing

0.001, $\eta_p^2 = 0.709$. Post-hoc comparisons revealed that the mean total licks of the Adol-E group was significantly greater than the Adol-S group ($p = 0.006$) and that the Adult-E group engaged in significantly greater sign tracking than both the Adol-E ($p = 0.003$) and Adol-S groups ($p < 0.001$). A significant Groups \times Block interaction, $F(17.5, 78.4) = 5.98$, $p < 0.001$, $\eta_p^2 = 0.363$, revealed that these group differences did not emerge until block 3 and persisted until the end of the

acquisition phase ($ps < 0.001$). Among the two adolescent-trained groups, the enriched-housed rats had more licks than the standard-housed rats during blocks 3, 4, 5, 7, and 9 ($ps < 0.05$).

The middle panel of Fig. 1 shows the mean intake of the ethanol solution during the acquisition of sign tracking. Main effects of Group, $F(2, 21) = 44.54, p < 0.001, \eta_p^2 = 0.809$, and Block, $F(4.9, 102.2) = 8.20, p < 0.001, \eta_p^2 = 0.281$, and their interaction, $F(9.7, 102.2) = 2.72, p = 0.006, \eta_p^2 = 0.206$, demonstrated differences in EtOH intake. Post-hoc comparisons confirmed that the adult-trained rats consumed more ethanol than both adolescent-trained groups ($ps < 0.001$). Planned comparisons indicated that all three groups demonstrated significantly higher intake during at least one block later in training (Adult-E: blocks 4,5,6, & 8; Adol-E: blocks 5,7,& 9; Adol-S: block 8), demonstrating an escalation of ethanol intake especially in the enriched-housed groups. A Group \times Block mixed ANOVA on the two adolescent groups showed that the enriched-housed group consumed significantly more ethanol than the standard-housed group (main effects of Group, $F(1, 14) = 6.89, p = 0.02, \eta_p^2 = 0.330$, and Block, $F(4, 56.4) = 3.8, p = 0.009, \eta_p^2 = 0.212$; no interaction, $F(4, 56.4) = 2.0, p = 0.11, \eta_p^2 = 0.125$).

The age difference in alcohol consumption was also observed when intakes were corrected for differences in body weight (Fig. 1, lower panel). Averaged over all days of acquisition, the adult enriched rats ($M = 0.406$ kg, S.E. = 0.009) weighed more than the adolescent enriched rats ($M = 0.303$ kg, S.E. = 0.004). The grams per kilogram intake data revealed that the adult rats consumed more ethanol than the adolescents, $F(1, 14) = 10.01, p = 0.01, \eta_p^2 = 0.417$, with intake levels reaching 17.4 g/kg in the adults and 14.9 g/kg in adolescents during the last training block. Although the Age \times Blocks interaction did not reach statistical significance, $F(3.1, 43.1) = 2.45, p = 0.08, \eta_p^2 = 0.149$, there was a

significant main effect of Blocks, $F(3.1, 43.1) = 3.46, p = 0.02, \eta_p^2 = 0.198$, with intakes generally increasing over blocks. Beginning at Block 4 (data not shown), the adolescent groups also differed slightly in body weight with the Adol-E rats ($M = 0.303$ kg, S.E. = 0.004) overall slightly heavier during acquisition than the Adol-S rats ($M = 0.286$ kg, S.E. = 0.005). As a result, the group difference in ethanol intake in terms of grams per kg did not reach traditional levels of statistical significance: $F(1,14) = 3.78, p = 0.07, \eta_p^2 = 0.213$.

Rats engage in sign tracking and goal tracking behavior during a bottle CS presentation. Figure 2 shows mean total headpokes into the food tray during the 10-sec period before (Pre-CS), during (CS) and after (post-US) the insertion of the ethanol bottle CS during the 9 blocks of training. A comparison of data in the three panels demonstrates the general pattern of acquired headpoking behavior during a typical sign tracking trial. Headpoking performance is low during the pre-CS period but increases during the bottle presentation demonstrating conditioned goal tracking (i.e., anticipation of the impending food pellet US). The food pellet presentation ends a trial, but rats persist with some search behavior in the food tray during the post-US period—a natural predisposition allowing rats to “confirm” that the brief meal has ended—before reducing headpokes for the remainder of the ITI and just prior to the start of the next trial (i.e., pre-CS period). Mixed ANOVAs conducted on each group separately yielded significant Period (PreCS, CS) \times Block interactions for all three groups, confirming that goal tracking during the bottle CS is an acquired anticipatory response (all F s > 3.19 , all $ps < 0.005$). Simple main effects with blocks as the moderating factor yielded the same results for all three groups: significantly greater headpoking during the CS than pre-CS at all blocks except the first block (all $ps < 0.05$). Planned comparisons (difference contrasts) revealed that while the highest level of headpoking was early in training for Group Adult-E (Blocks 3

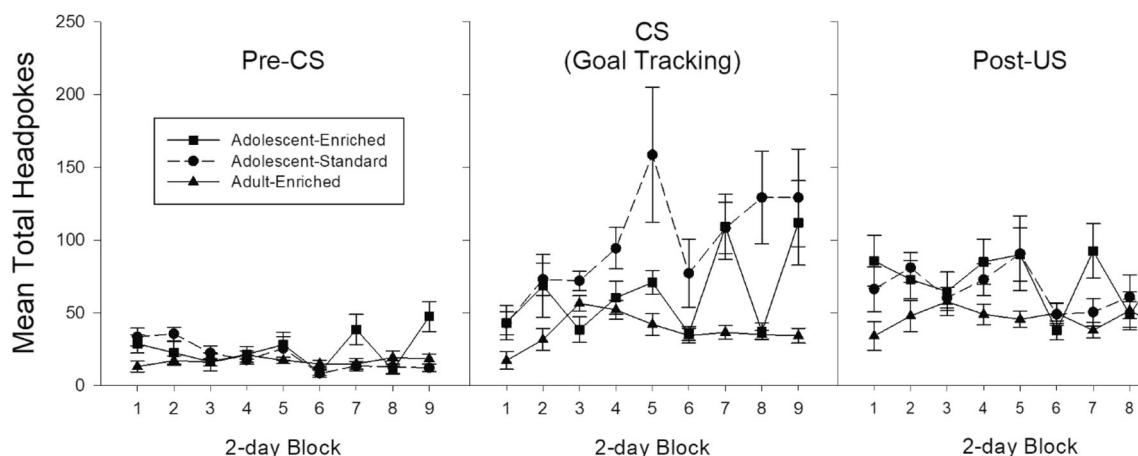


Fig. 2 Mean total headpokes during the 10-sec period before (Pre-CS), during (CS) and after (post-US) the insertion of the ethanol bottle averaged across 2-day blocks during the first 18 days of sign tracking for

adolescent and adult rats raised in enriched housing and adolescent rats raised in standard housing

and 4, $ps < 0.01$), it was later in training for the Adol-E (Blocks 7 & 9, $ps < 0.001$) and Adol-S (Blocks 5 & 8, $ps < 0.05$) groups. Thus, although all groups acquired goal tacking at the same rate (by Block 2), the adolescent rats tended to engage in more goal tracking than the adult rats later in training. A Groups x Block mixed ANOVA on the CS data yield a significant main effect of Group, $F(2, 21) = 6.16, p = 0.008, \eta_p^2 = 0.370$, with the Adol-S group engaged in greater headpoking than the Adult-E group ($p = 0.006$) but not the Adol-E group ($p = 0.12$). The Group x Block interaction, $F(6.9, 72.4) = 3.44, p = 0.003, \eta_p^2 = 0.247$, partly reflected the greater variability in headpoking performance between blocks in the adolescent rats than the adult rats. Nevertheless, the adolescent rats demonstrated more headpoking than adults during most blocks.

Table 1 shows the Pearson correlations between the licks and headpokes during training. Correlations including all 3 groups combined ($N = 24$) were negative for all training blocks, but statistical significance was achieved only later in training beginning at Block 4. Correlations are also shown for the Adolescent rats only ($n = 16$) and for each group ($n = 8$) separately, but all except Block 4 for the adult-trained group were not statistically significant. Fig. 3 shows the scatterplots, and Fig. 4 plots histograms of the mean total licks and headpokes on the last block of training (mean of Days 17 & 18) for the three groups. Except for two adolescent rats raised in standard housing, all rats engaged in sign tracking and goal tracking behavior.

A cursory inspection of the rats during training sessions suggested that sign tracking declined within the session in the enriched-housed groups. To better characterize these within-session decrements in sign-tracking performance, the licks during the last five training blocks were computed as 5-trial blocks (25 trials/5 trials = 5-trial blocks). Although the Group (3) x Training Block (5) x Trial Block (5) interaction was not significant, $F(14.1, 148.0) = 1.41, p = 0.16, \eta_p^2 =$

0.118, there was a reliable Group x Trial Block interaction, $F(2.6, 27.6) = 5.89, p = 0.004, \eta_p^2 = 0.358$. As shown in Fig. 5 (top panel), the two enriched-housed age groups showed within-session declines in sign tracking, whereas the adolescent group raised in standard cages did not. This impression was confirmed by significant simple main effects of trial blocks for the Adol-E ($p < 0.001$) and Adult-E ($p < 0.001$) groups but not the Adol-S group ($p = 0.56$).

The bottom panel of Fig. 5 shows the same plot for headpokes. The main effect of Trial Block, $F(1.5, 31.0) = 1.64, p = 0.30, \eta_p^2 = 0.072$, the Group x Trial Block interaction, $F(3.0, 31.0) = 1.27, p = 0.30, \eta_p^2 = 0.108$, and the Group x Session Block x Trial Block interaction, $F(9.4, 97.9) < 1.0, p = 0.45, \eta_p^2 = 0.086$, were not significant. Thus, the within-session declines in sign tracking seen in the enriched-housed age groups were not seen in goal tracking.

Phase 2: Stressor Manipulations in the enriched-housed groups

Sign tracking under the two stressor and two recovery conditions were calculated by averaging the licks from the three sign tracking test days during each condition. Licks from the last three blocks of Phase 1 training also were averaged to calculate asymptotic baseline sign-tracking performance. For goal tracking, the same averages were calculated using the headpoking responses. An initial analysis revealed no systematic effect of the type of stressor (isolation stress alone or paired with a cage mate) on the dependent measures during the stressor and recovery conditions (data not shown), thus stressor type was excluded as a factor in the following analyses. Sign-tracking and goal-tracking performance were analyzed with a 2 (Age: Adolescent, Adult) x 5 (Stressor Condition: Baseline, Stressor 1, Recovery 1, Stressor 2, Recovery 2) x 2 (Stressor Type Order: alone-pairs, pairs-

Table 1 Summary of Correlations During Acquisition: Total Licks and Headpokes During the Bottle CS

Subjects	Training Block								
	1	2	3	4	5	6	7	8	9
All rats									
All	-0.40	-0.29	-0.27	-0.43*	-0.48*	-0.41*	-0.55**	-0.50*	-0.61**
Adolescent rats only									
Adolescent	-0.43	-0.36	-0.39	-0.19	-0.32	-0.38	-0.23	-0.45	-0.48
0.48 Individual Groups									
Adol-E	-0.44	-0.44	-0.15	-0.02	0.22	-0.28	-0.54	-0.25	-0.64
Adol-S	-0.45	-0.03	0.26	0.57	-0.08	-0.32	0.09	-0.41	-0.47
Adult-E	0.09	0.24	-0.37	-0.78*	-0.43	-0.36	-0.69	-0.36	-0.59

Note. $N = 24, n = 8, * p < .05, ** p < .01$

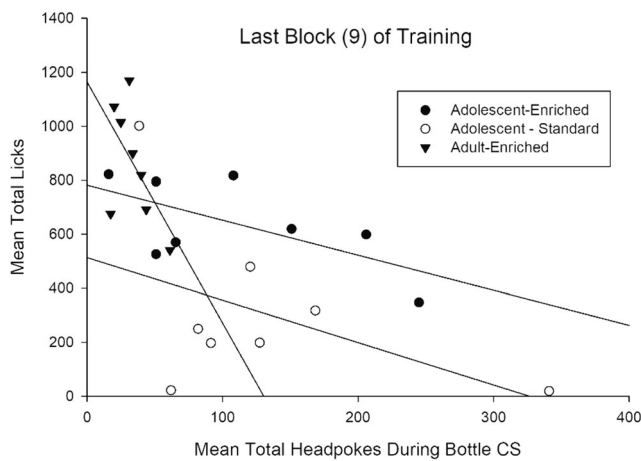


Fig. 3 Scatterplot of mean total licks and headpokes during the bottle CS presentations on the last block of training (Days 17 & 18)

alone) ANOVA, followed by additional ANOVAs and planned comparisons when necessary.

Sign Tracking The 3-way interaction, $F(4, 48) = 1.22, p = 0.31, \eta_p^2 = 0.093$, and the Stressor Condition x Order interaction, $F(4, 48) = 1.90, p = 0.13, \eta_p^2 = 0.136$, were not statistically significant. However, there was a reliable Age x Stressor Condition interaction, $F(4, 48) = 5.1, p = 0.002, \eta_p^2 = 0.298$. Simple main effects confirmed significant effects of stressor condition in the adult ($p < 0.001$) and adolescent groups ($p < 0.01$). Sign tracking performance during the stressor conditions for the adults are shown in the top panel of Fig. 6a. Planned comparisons indicated that licks on the bottle CS increased during the first stressor experience (B vs. S1: $p = 0.003$) but returned to baseline levels during the following week of recovery (B vs. R1: $p = 0.11$). Although licks did not increase during the second exposure to the restricted housing (B vs. S2: $p = 0.38$), they were elevated the following

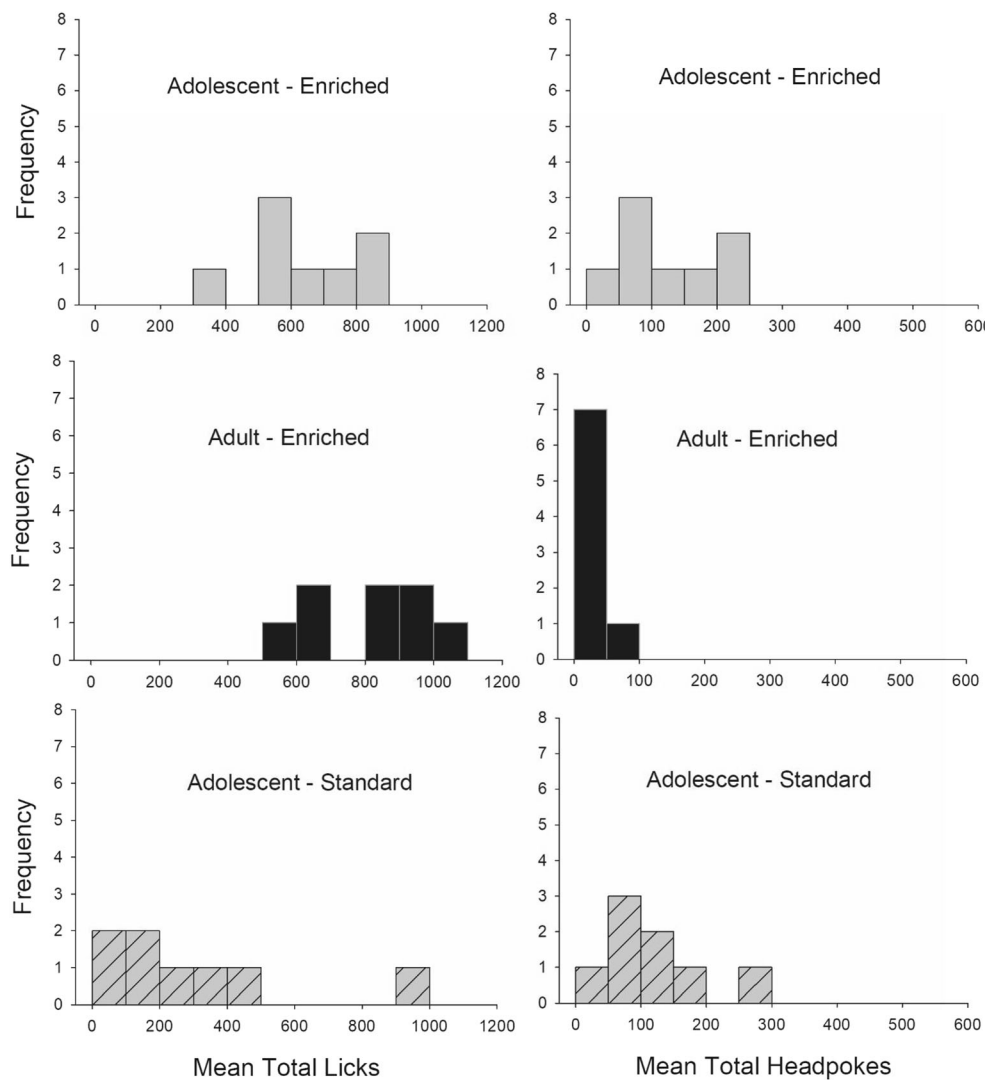


Fig. 4 Histograms of mean total licks (left panels) and headpokes (right panels) during the bottle CS presentations on the last block of training (Days 17 & 18)

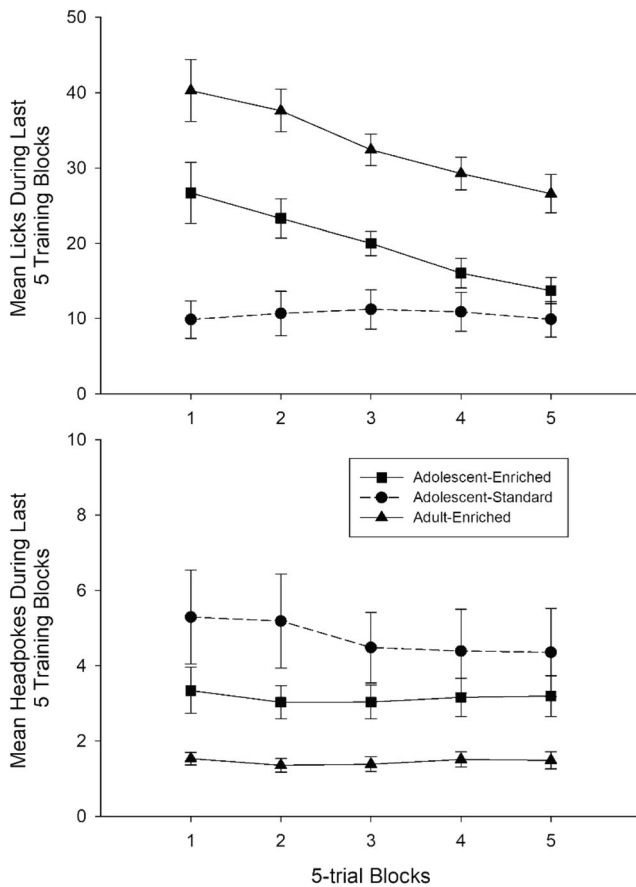


Fig. 5 Mean licks (top panel) and headpokes (bottom panel) averaged across the last 5 blocks of training and plotted as 5-trial blocks to show within-session performance of sign tracking (licks) and goal tracking (headpokes)

week when the adults were back in their enriched home environment ($p < 0.001$). In the adolescent group (Fig. 6b), licks were elevated significantly during the recovery from the second stressor (B vs. R2: $p = 0.006$), but not during the stressor manipulations (B vs. S1: $p = 0.83$; B vs. S2: $p = 0.35$) or during the recovery from the first stressor manipulation (B vs. R1: $p = 0.11$).

Goal Tracking As with the sign-tracking measure, the Age x Stressor Condition interaction was the only significant interaction for the headpoking measure, $F(2.0, 23.5) = 10.4$, $p < 0.001$, $\eta_p^2 = 0.464$. Simple main effects confirmed that while headpokes increased during the second stressor and recovery conditions ($p < 0.001$) for the adult rats (Fig. 6c), headpokes decreased ($p = 0.002$) for the adolescent rats (Fig. 6d). Simple planned comparisons yielded significant changes from baseline responding during the second stressor (S2) and subsequent recovery (R2) condition in the adult (B vs. S2: $p < 0.001$; B vs. R2: $p = 0.002$) and adolescent (B vs. S2: $p = 0.003$; B vs. R2: $p = 0.001$) groups. No significant changes in headpoke responses occurred during the first stressor and recovery conditions of the adults (B vs. S1: $p = 0.08$; B vs. R1: p

$= 0.45$) and adolescents (B vs. S1: $p = 0.24$; B vs. R1: $p = 0.76$).

Ethanol Intake The mean ethanol consumed from the bottle CS during the sign tracking sessions of Phase 2 are displayed in Fig. 7 as grams per kilogram (top panel). The main effect of Age, $F(1, 14) = 6.9$, $p = 0.02$, $\eta_p^2 = 0.330$, confirmed that adult group overall continued to consume more ethanol during sign tracking than the adolescent group. Simple main effects following a significant Age x Stressor Condition interaction, $F(4, 56) = 3.63$, $p = 0.01$, $\eta_p^2 = 0.206$, revealed that while there were no changes in ethanol consumption across conditions for the adolescent rats ($p = 0.73$), the adults' ($p < 0.001$) significantly reduced intake of ethanol during recovery 1 ($p = 0.02$) and stressor 2 ($p = 0.001$). Figure 7 (bottom panel) plots the results of the two-bottle tests conducted in a separate test chamber outside of the home environment. Although the preference is in the direction of ethanol for both groups, Age x Stressor Condition x Stressor Order mixed ANOVA yielded no statistically significant effects (all $F_s \leq 1$).

Free Range Access to the Home Cage During Sign Tracking Sessions

Equipment difficulties with the home cage lickometers occurred during home range testing. As a result, the average of the second and third day of testing were calculated for both groups. For two of the Adol-E rats and four of the Adult-E rats, the total licks from the first day were used in the mean calculations due to missing scores on the second day. Mean total licks when 5% ethanol was in the bottle CS (as in Phase 1 and 2) for the adult group ($M = 760.6$, $S.E. = 109.3$) and adolescent group ($M = 565.3$, $S.E. = 50.0$) were comparable to the levels seen at the end of acquisition (Fig. 1, top panel). When the ethanol in the bottle CS was replaced with water in the free-range sessions, the adult group reduced their total licks ($M = 573.4$, $S.E. = 78.1$), but the adolescent rats substantially increased their total licks ($M = 912.2$, $S.E. = 59.6$). An Age (2) x Bottle Content(2) mixed ANOVA revealed a significant Age x Bottle Content interaction, $F(1, 14) = 37.4$, $p < 0.001$, $\eta_p^2 = 0.727$. Simple main effects confirmed that while the Adol-E group increased sign tracking when water replaced ethanol ($p < 0.001$), the adult rats decreased their sign tracking behavior ($p = 0.02$). Moreover, although the age group difference did not reach statistical significance ($p = 0.13$) when sign tracking ethanol, the Adol-E group sign tracked significantly more with the water bottle CS than the Adult-E group ($p = 0.004$).

When no longer confined to the upper platform of the home cage where the sign-tracking equipment was located and having access to all levels of the home cage, the rats primarily remained on the platform or quickly returned after visiting other levels of the cage. The ethograms of one example rat

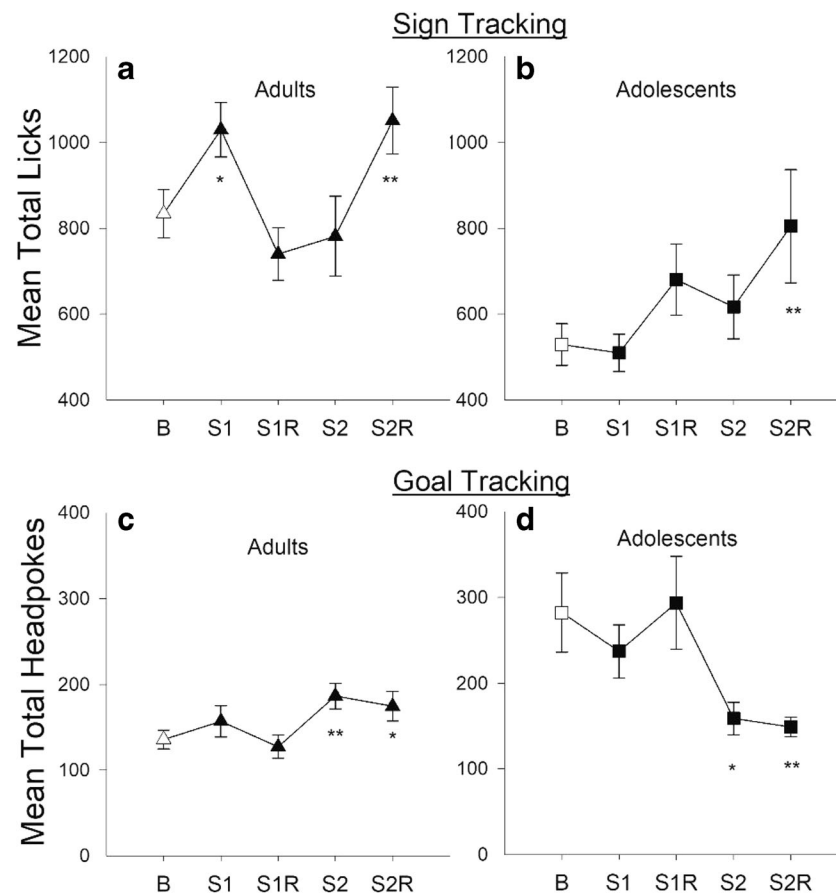


Fig. 6 Mean total licks (A & B) and mean total headpokes (C & D) during Phase 1 baseline (open symbols) and Phase 2 Stressor conditions (solid symbols) of the adult and adolescent enriched-housed groups. * $p \leq 0.01$; ** $p \leq 0.001$.

from each age group showing their location during five trials in the beginning, middle, and end of a sign-tracking session is shown in Fig. 8. The rats remained on the platform for all or most of the scored trials. During the first five trials, both rats visited the lower levels frequently but briefly, quickly returning to the sign-tracking platform before the start of the next trial. The adolescent rat (C2) did not miss the opportunity to lick the ethanol bottle CS on any of the five trials and the adult rat (C15) missed the bottle CS only on one trial.

Discussion

Adolescent Sprague-Dawley rats raised in enriched housing from 21 days of age acquired higher levels of sign-tracking behavior directed towards an ethanol-containing bottle than comparably aged rats reared in paired standard housing. Thus, sign tracking is readily observed in environmentally enriched rats and at levels greater than standard housed rats. This result conflicts with a prior study reporting that enriched housing reduces sign tracking (Beckmann & Bardo, 2012). There are several procedural differences in the two study designs that are worth noting. First, in the present study, we gave

the rats 18 days of training (acquisition) rather than 5 days more typically used in sign tracking studies investigating individual differences in attribution of incentive salience. Large increases in the sign-tracking performance of the enriched group did not become apparent until Block 3 (Days 5 and 6). This result is consistent with other studies demonstrating that with extended training rats initially characterized as goal trackers can shift performance toward sign tracking of a lever CS (Keefer et al., 2020; Srey, Maddux, & Chaudhri, 2015). Second, the comparison group in the present study consisted of pair-housed rats in standard cages rather than socially isolated rats (i.e., housed alone) used in the Beckman and Bardo study. It is possible that the main group difference in the latter study was due to the chronic social isolation rather than to enrichment and that with further training the enriched group's performance would have increased to levels that matched or exceeded the isolated group's performance. Isolated housing has been reported to increase sign tracking behavior in adolescent rats (Anderson et al., 2013; DeAngeli et al., 2017). Third, in the present study a fixed 60-sec ITI was used rather than a variable ITI that has become "standard" in sign tracking research. An examination of the long history of sign tracking research reveals that a variable ITI is not a necessary condition

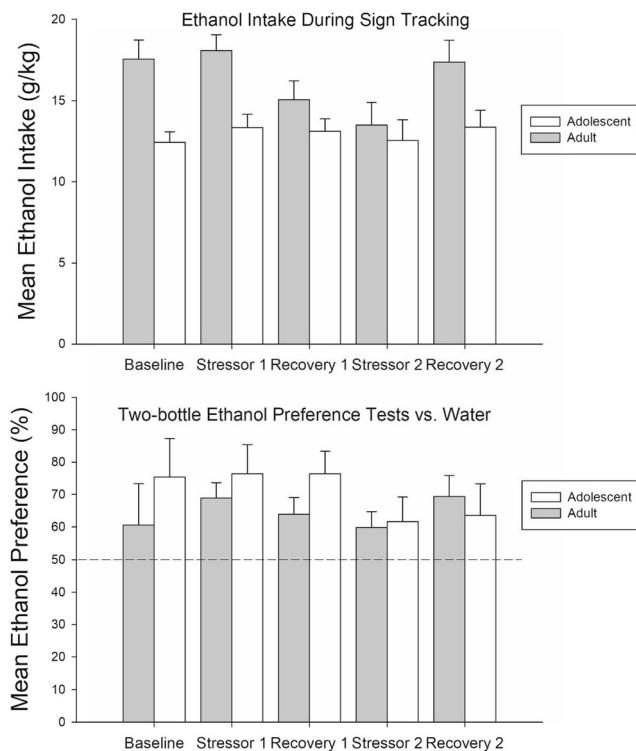


Fig. 7 Mean ethanol intakes (grams/kg) during the sign tracking sessions (top panel) and ethanol preference in two-bottle test chambers outside of the home cage (lower panel) during stressor manipulations in phase 2. Baseline values are means for the last 3 days of acquisition in phase 1

for sign tracking and that the form and timing of conditioned behavior is primarily guided by the temporal relationship of motivating events, such as the inter-food (inter-US) interval (Silva & Timberlake, 1997; Gallistel & Balsam, 2014). Although a fixed ITI can provide a reliable temporal cue for the US that may encourage goal tracking over sign tracking, this does not appear to be the case since robust sign tracking performance was observed. Finally, two procedural differences were introduced in the present study informed by a research goal that diverges from the more typical studies of sign tracking—the use of a bottle CS containing 5% alcohol as the object CS rather than a retractable lever and rats trained in their home environment rather than in a separate test chamber. Paradigmatically, both types of studies are Pavlovian, because the object CS is paired noncontingently with the food US. Nevertheless, it cannot be ruled out that the disparity in the effects of environmental enrichment on sign tracking are due to the choice of object CS and training directly in the home-enriched environment. Clearly more research is needed to determine how enriched housing influences sign tracking.

The additional procedural differences in the present study were included to increase the ecological relevance of the sign tracking procedure, an objective that is less relevant to studies using sign tracking of a lever CS as an indicator of individual differences in the attribution of incentive salience. Previous studies have increased ecological relevance of sign-tracking

procedures, for example, by using a rolling ball bearing CS to provide additional features of natural prey for rats (Timberlake, Wahl, & King, 1982) and a terrycloth object with some stimulus features of a female quail to induce sexually conditioned responding in male quail (Domjan, Cusato & Krause, 2004). In the present study, a bottle CS containing alcohol was used to increase ecological relevance that translates to human alcohol use and abuse as proposed by Tomie and associates. A limitation of the present study is that we cannot determine the extent to which the acquisition of sign tracking performance was influenced by the alcohol content, because we did not include groups sign tracking water. Prior research, however, clearly indicate that sign tracking of a bottle CS is not dependent on the motivating properties of the bottle content. Nonhungry, nonthirsty rats will sign track and drink from a bottle CS containing water and at a rate greater than a control group receiving random presentations of the CS and US (Tomie et al., 2004, 2005). Our laboratory has found that nondeprived rats raised in enriched housing and trained in separate test chambers sign track a water bottle CS substantially more than pair-housed reared rats (Casachahua, 2011 unpublished master's thesis <https://scholarship.shu.edu/dissertations/9/>). Nevertheless, the addition of alcohol to the bottle causes elevated sign-tracking performance and solution consumption compared with water, suggesting that the pharmacological effects of alcohol increase sign-tracking performance (Tomie et al., 2004; Tomie & Sharma, 2013). The use of nondeprived rats lessens the possibility that rats are consuming the ethanol primarily for calories to relieve hunger. We used alcohol bottle CS rather than water to model alcohol use behavior and to allow for potential effects of alcohol (e.g., possible self-medication; Manzo et al., 2015) during the isolation stressor manipulations. Research that includes rats sign tracking water is needed to determine how the addition of ethanol impacts on performance of rats sign tracking a bottle CS in an enriched home environment.

Despite the differences between our procedure (i.e., a bottle CS and enriched housing) and sign-tracking procedures using a retractable lever CS that have become “standard,” we observed similar age differences in sign tracking that have been reported in studies using retractable lever CSs. Prior studies found that adolescent rats sign track a retractable lever CS less than adult rats (Doremus-Fitzwater & Spear, 2011; Rode et al., 2019). In the present study, rats raised in enriched housing from 21 days of age but trained in the sign-tracking procedure as adults showed more robust sign tracking than enriched-housed rats trained as adolescents, whereas the adolescent-trained rats tended to goal track more than the adult-trained rats. Thus, we extend the finding that adolescent rats show reduced sign tracking relative to adults to a more natural situation where rats track a bottle CS in their enriched home environment. It has been proposed that adolescent rats sign track less and goal track more than adults because

adolescent rats are more sensitive to primary rewards than adult rats (Doremus-Fitzwater & Spear, 2011; Rode et al., 2019). Although young rats are thought to display greater novelty seeking than adults (Spear, 2004; Stansfield & Kirstein, 2006), and therefore more likely than adults to respond to a novel retractable object CS, their greater sensitivity to the primary reward at the food tray may reduce motivation to incorporate an object with acquired incentive value into their ongoing behavioral strategy. Consistent with the possibility that sign tracking is increased by a novelty-seeking phenotype is our finding that the enriched-housed adolescent group contacted the bottle sipper more than the adolescent group confined to standard but impoverished housing during development.

If adolescent rats are more sensitive to rewards than adults, then it is not clear why the availability of ethanol in the bottle CS, a potentially rewarding substance, did not enhance sign tracking and ethanol consumption in adolescents more than adults. Adolescent rats consume more ethanol than adult rats under a variety of continuous-access standard housing circumstances (Doremus, Brunell, Rajendran, & Spear, 2005). An ethanol solution US supports sign tracking of an object CS (Tomie & Sharma, 2013), indicating that ethanol in a sign tracking procedure is an effective reward, although a more

complex ethanol chocolate reward has been reported to shift performance from sign tracking to goal tracking (Fiorenza et al., 2018). Forced, intermittent ethanol consumption by intragastric administration has been shown to shift behavior of adolescent rats from goal tracking to sign tracking suggesting that binge-like ethanol exposure can increase compulsive-like sign tracking (Madayag et al., 2017). Our results suggest that at least with our procedure, the adolescent rats valued the sugar pellet food reward more than the 5% ethanol. Our finding that ethanol was not strongly preferred over water in repeated two-bottle choice tests suggests that the ethanol was not a very strong reward.

Adolescents often is characterized as a time of increased vulnerability to addiction (Crews, Jun, & Clyde, 2007), yet sign tracking (hypothesized to reflect increased addiction vulnerability) is seen less in adolescent rats than in adult rats (Doremus-Fitzwater & Spear, 2011; Rode et al., 2019; present study). Environmental enrichment is characterized as protective of addiction (Galaj et al., 2020; Stairs & Bardo, 2009), yet the environmentally enriched rats in the present study sign tracked more than rats housed in standard, but impoverished, housing. Because the bottle CS in the present study contained 5% ethanol, intake of alcohol while sign tracking was greater in the enriched adult group compared with the adolescent

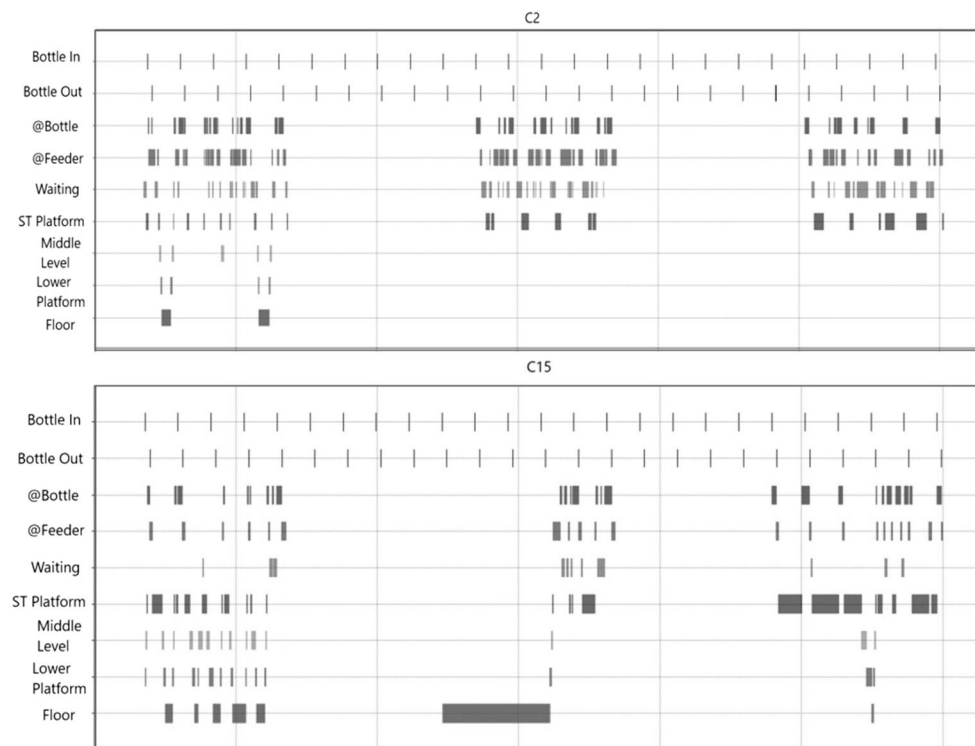


Fig. 8 Ethograms of two representative rats from the adolescent-trained group (C2) and adult-trained group (C15) during five trials in the beginning, middle, and end of a free roaming sign-tracking session. The top two lines show when the bottle CS enters and exits during a trial, and the remaining lines show the location of the rats over time. Rats were at the bottle access hole (@ Bottle), at the feeder (@Feeder), or immobile

(Waiting) near but not actively searching at the bottle or feeder location. The lower four lines mark when the rats were on the sign tracking platform but away from the bottle access hole or feeder (ST Platform) and when the rats left the sign tracking platform to visit the middle level, lower platform (where the water bottles were located) and the floor level (where the food pellets were located).

group in terms of total intake and grams per kilogram body weight, potentially making adults more vulnerable to alcohol abuse. Yet neither group showed a strong preference for alcohol over water in two-bottle tests outside the home testing environment. Although increased sign tracking of a bottle CS with a concentration of ethanol as high as 29% successfully models excessive, poorly controlled alcohol use and abuse (Tomie & Sharma, 2013), this elevated drinking does not generate sustained drinking or increased ethanol preference outside of the experimental context (Tomie et al., 2004; present study). Thus, in terms of the incentive salience hypothesis rats sign tracking an ethanol bottle demonstrate a strong “wanting” of ethanol, but to the extent that limited-access, two-choice preference tests are an indicator of “liking” (Meyerolbersleben, Winter, & Bernhardt, 2020), increased “wanting” is not accompanied with an increase in the “liking” of ethanol. Interestingly, rats given random presentations of the ethanol bottle CS and food US in a sign tracking procedure also demonstrate increased consumption with repeated experience. Apparently, intermittent access to a bottle alone is enough to induce ethanol drinking in several experimental protocols including the sign tracking procedure (Tomie, Miller, Dranoff, & Pohorecky, 2006). A limitation of the present study is that a random control group was not included for comparison. It has been established that paired CS-US presentations significantly increase sign tracking and ethanol consumption compared to the random controls (Tomie, et al., 2004), supporting the hypothesis that CS-triggered incentive salience sensitization (i.e., sign tracking) may contribute to the development of alcohol use disorder (Cofresí, Bartholow, & Piasecki, 2019; Tomie & Sharma, 2013). Nevertheless, sign tracking behavior does include associative and nonassociative components (e.g., influence of the intermittency of rewards) and the purpose of the present study was not to study associative learning per se but to investigate group differences in performance and changes in performance driven by the constraints of a sign tracking procedure.

Our emphasis on the acquisition of sign and goal tracking behavior in an enriched environment and on the experimental constraints on learning puts greater focus on issues that are less relevant to the current prominence in the sign tracking literature on identifying individual differences in incentive salience attribution. For instance, sign tracking took several days to emerge reliably, even in the enriched-housed groups. This delay may reflect learned behavioral changes that are not measured directly by the experimental design but impact on sign-tracking and goal-tracking performance. For example, during ad libitum feeding in the laboratory and in experimental procedures that simulate foraging in a closed-economy rats take meals in bouts (Collier & Johnson, 2004). The availability of only one small food pellet spaced by a fixed or variable interval does not sustain feeding for very long resulting in an environmental constraint on a rat’s natural tendency to eat a

meal in bouts. Continued headpoking during the 10 sec after the US delivery (Post US, Fig. 2) may reflect this preorganized behavioral tendency. Rats biologically prepared to feed in bouts may need several days of experience to learn that a feeding bout is not under their own control (Lucas, Timberlake, & Gawley, 1988). Thus, sign tracking may increase as rats adjust their goal-directed actions and begin to include the object CS as it gains incentive value. Although goal trackers may be more responsive to the incentive motivating properties of the food US than sign trackers (Friemel, Spanagel, & Schneider, 2010; Patitucci, Nelson, Dwyer, & Honey, 2016), and therefore are less likely to attend to the object CS, enrichment may reduce the primacy of the motivating properties of food reward by increasing motivation to interact with other objects in the environment when available, such as a retractable object CS. The hypothesis that enriched rats are likely to sign track an object CS more than nonenriched rats is suggested by the observation that enrichment increases object exploration (Einson & Morgan, 1976; Modlinska, Chrzanoska, & Pisula, 2019). Thus, the acquisition of higher levels of sign tracking in enriched rats may reflect greater flexibility and more efficient reorganization of behaviors supported by the affordances available in an environment, a characteristic that often is reported in studies investigating the effects of enriched housing on learning (Renner & Rosenzweig, 1987; van Praag, Kempermann, & Gage, 2000). It has been reported that intermittent ethanol exposure impairs adolescent behavioral flexibility as indexed by the need for longer training to achieve reversal learning (Sey et al., 2019). Ethanol exposure was not manipulated in the present study, but it would be of interest to determine whether ethanol exposure directly from the bottle object CS or supplemental long-term access protocols increases sign tracking performance relative to nonalcohol controls or if an enriched home environment precludes impaired behavioral flexibility effects of ethanol.

Sign tracking and goal tracking in the enriched housed rats allowed us to investigate the impact of an acute isolation stress on these behaviors during the stressor and during a period of recovery. We assumed on face value that isolation in a very restricted environment would be stressful for rats raised in an enriched home environment. Moreover, rats are intrinsically social animals, and a large body of research has shown that social isolation in animals has neurochemical and behavioral consequences at all levels of development but particularly during adolescence (Cacioppo, Cacioppo, Capitanio, & Cole, 2015). There also are acute social isolation effects. For example, acute isolation causes socially housed rats to increase their motivation to seek out opportunities to play (Panksepp & Beatty, 1980) and impairs performance in social memory tasks (Leser & Wagner, 2015). Our purpose was to determine whether sign tracking and goal tracking are altered by exposure to acute isolation stress. Psychosocial stress

exacerbates compulsive responding in psychiatric populations, such as obsessive-compulsive disorder and substance abuse and addiction (Adams et al., 2018). If sign tracking is a compulsive-like behavior and stress effects exaggerate this behavior, then sign tracking may increase independently of goal tracking or at the expense of goal tracking. Conversely, if stress impacts more on goal-directed action than compulsive behavior, then goal tracking may decrease while sign tracking increases as behavior is reorganized. We did not make any directional predictions but simply asked the question: does acute isolation impact on the expression of sign tracking and goal tracking during the isolation stressor and during recovery from the stressor?

We observed stress effects that differed for the adolescent-trained and adult-trained groups. The first of the two stressor manipulations increased sign tracking in the adult-trained rats but not in the adolescent-trained rats. However, this stress effect on sign tracking did not repeat in the adults during the second stressor manipulation. The adult and adolescent groups both increased sign tracking during recovery period after the second stressor, but while this increase was accompanied by decreased goal tracking in the adolescent rats, it was accompanied by increased goal tracking in the adults. These results are preliminary and need to be studied further along with the use of chronic stressors and independent measures of the stressor manipulations. What appears to be clear, however, is that sign tracking behavior is more flexible than had been typically assumed and stressor manipulations may affect adolescent and adult rats differentially (Anderson et al., 2013).

Sign tracking performance is typically described in the literature as distinct from goal-directed instrumental action and association-based expectancies. Empirical evidence appears to show that sign tracking is “automatic,” “reflexive,” and “compulsive-like.” For example, sign tracking is more resistant to extinction than goal tracking (Ahrens et al., 2016; Beckmann & Chow, 2015). When the food US is devalued with a conditioned taste aversion procedure goal tracking decreases substantially but not sign tracking of a retractable lever CS (Morrison et al., 2015; Smedley & Smith, 2018) or of a more complex object CS in the form of a rolling ball bearing (Timberlake & Mercer, 1988). More recently, changes in sign tracking following US devaluations have been reported. Sign tracking sometimes increases following devaluation of the food US via taste aversion conditioning (Morrison et al., 2015; Rode et al., 2019). When two lever CSs are presented concurrently, each paired with separate discriminable USs, sign tracking decreases to the object CS paired with the US devalued by illness (Cleland & Davey, 1982; Derman et al., 2018) or by prefeeding with the food US (Cleland & Davey, 1982). Satiety- and illness-induced devaluations also reduce responding to a lever CS+ when alternated with a second lever (CS−) that is not paired with the US (Cleland & Davey, 1982). Moreover, when a US associated with one of the two lever

CSs is shifted to a lower value, rats will switch responding to the unshifted lever (Conrad & Papini, 2018). These studies suggest that sign-tracking behavior is not as reflexive or compulsive-like as has been stated often in the literature.

Yet, studies suggest that although sign-tracking can be flexible, it is not quite as flexible as goal-directed behaviors. The addition of an omission contingency known as negative automaintenance (i.e., cancellation of the US when a response occurs to the object CS; Williams & Williams, 1969) reduces sign tracking of a lever CS but, as implied by the name, some level of lever-press responding is maintained (Locruoto, Terrace, & Gibbon, 1976). Moreover, when a more complex topography of responding to the lever CS is measured during an omission contingency, it becomes apparent that overall responding to the object CS is well maintained but shifts from mostly lever pressing (which leads to reward cancellation) to other object-directed responses, such as orientation and sniffs (Chang & Smith, 2016).

In the present study, there were additional indications that sign tracking is more flexible than typically assumed. Overall, we found that the correlation between licks and headpokes for all three groups combined were negative as expected and that although effect size increased with training, by the end of the acquisition phase the correlations were not of such a high strength to indicate that response competition was a main determinant of the asymptotic levels of sign-tracking and goal-tracking performance. That is, although sign tracking and goal tracking performance is typically negatively correlated changes can occur in each behavior independent of the other. We observed instances of increased sign tracking along with goal tracking that decreased (Adolescents stressor 2 recovery), increased (Adults stressor 2 recovery), or did not change (Adults Stressor 1).

We also found that sign-tracking performance of the enriched housed rats, but not the standard housed rats, was characterized by similar within-session declines that have been observed in standard classical and operant conditioning paradigms that present reinforcers repeatedly in a test session (McSweeney & Murphy, 2009; McSweeney & Swindell, 1999). Within-session declines in goal tracking did not occur, however, and all food pellets were always consumed, demonstrating that the within-session declines in sign tracking were not a result of satiation to the sugar pellet US. Although it is possible that within-session declines in sign tracking was due to satiation to the ethanol specifically and independent of motivation for the food US, we think that this explanation unlikely. In an unpublished master’s thesis in our laboratory (Casachahua, 2011), we see the same robust within-session declines in enriched rats tracking a water bottle CS. Our observations are consistent with Frances McSweeney’s general process sensitization-habituation account of motivated behavior which argues that responses to goal objects (i.e., reinforcers) may decrease with repeated exposure because of a

habituation process and that decrements in performance are especially likely when habituation is preceded by its companion process, sensitization (also see Lloyd et al., 2014). Our observation of within-session declines of sign tracking in enriched-housed rats may be partly due to the use of fixed ITI (rather than a variable ITI more commonly used in sign-tracking procedures), which is known to accelerate habituation (Rankin et al., 2009). Nevertheless, despite many weeks of training, the persistent recovery of sign-tracking behavior at the start of every training session seems to be well captured by the “motivational magnet” label (Berridge & Robinson, 2016) given to object CSs that induce robust sign tracking and posited to reflect elevated incentive sensitization. Within-session declines of sign-tracking in environmentally enriched rats may reflect effective adaptive regulation of evoked sign tracking behavior or shifts in ongoing behavioral strategies, at least in the short term (McDiarmid, Yu & Rankin, 2019).

In a recent review, Heather (2017) analyzed many definitions of compulsion as used in the addiction literature and distinguished between two major versions. The strong version of compulsion refers to a force-like process that compels an animal or person to behave automatically and uncontrollably and is generally independent of the agent’s motivational state (Everitt & Robbins, 2005; Koob & Le Moal, 2001). The weak version refers to a motivational state or “want” that powers drug-seeking and drug-taking behavior, but although difficult to resist, the behavior can be modified (Robinson & Berridge, 2008). Current conceptualizations of sign-tracking performance appear to favor the strong version (Tomie et al., 2018). We suggest that the weak version of compulsion may be a more appropriate definitional framework.

The use of an alcohol bottle in the sign-tracking and scheduled-induced-polydipsia procedures are less likely to be suitable to researchers developing animal models that view drug addiction and alcoholism as an advancing disease process that results not only in excessive drinking but also preference, physical dependence, and adversity. Nonetheless, excessive behavior caused by scheduled-induced constraints on preorganized (natural) behavior (Timberlake, 1984; Timberlake & Lucas, 1989; Falk & Tang, 1988) is an alternative conception of alcohol use, abuse, and addiction that is worth pursuing (Ramsden, 2015). Evolutionary explanations of psychopathology as a product of the mismatch between humans and modern environments suggests that the cultural and modern technological constraints on human behavior may not be all that different from the environmental constraints that experimentalists impose on animals in the laboratory. For humans and laboratory animals, poorly controlled compulsive behavior (in the weak sense) and well-controlled, goal-directed actions may reflect attempts to adapt preorganized species-typical behaviors to the constraints imposed by the encountered environments. Sign tracking and schedule-induced polydipsia are remarkably reliable and effective

procedures to generate animals with compulsive-like drinking and chronic intake that can vary along a continuum of compulsive responding and goal-directed action. As noted by Falk and Tang, “The animals [in scheduled-induced procedures] are exposed chronically to ethanol, because they are induced to drink it voluntarily; it is not forced upon them” (Falk & Tang, 1988, p. 577). Bringing the environmental constraints of the sign-tracking procedure into a more ecologically relevant enriched housing environment will help to determine whether compulsive responding is largely an artifact of impoverished animals confined to a test chamber or a more general phenomenon with translational value. Our results suggest that compulsive-like sign tracking is not an artifact of impoverishment and isolation.

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References

- Adams, T. G., Kelmendi, B., Brake, C. A., Gruner, P., Badour, C. L., & Pittenger, C. (2018). The role of stress in the pathogenesis and maintenance of obsessive-compulsive disorder. *Chronic Stress*, 2, 1–11. <https://doi.org/10.1177/2470547018758043>
- Ahrens, A. M., Singer, B. F., Fitzpatrick, C. J., Morrow, J. D., & Robinson, T. E. (2016). Rats that sign-track are resistant to Pavlovian but not instrumental extinction. *Behavioural Brain Research*, 296, 418–430. <https://doi.org/10.1016/j.bbr.2015.07.055>
- Amaya, K. A., Stott, J. J., & Smith, K. S. (2020). Sign-tracking behavior is sensitive to outcome devaluation in a devaluation context-dependent manner: implications for analyzing habitual behavior. *Learning & Memory*, 27(4), 136–149. <https://doi.org/10.1101/lm.051144.119>
- Anderson, R. I., Bush, P. C., & Spear, L. P. (2013). Environmental manipulations alter age differences in attribution of incentive salience to reward-paired cues. *Behavioural Brain Research*, 257, 83–89. <https://doi.org/10.1016/j.bbr.2013.09.021>
- Beckmann, J. S., & Bardo, M. T. (2012). Environmental enrichment reduces attribution of incentive salience to a food-associated stimulus. *Behavioural Brain Research*, 226(1), 331–334. <https://doi.org/10.1016/j.bbr.2011.09.021>
- Beckmann, J. S., & Chow, J. J. (2015). Isolating the incentive salience of reward-associated stimuli: value, choice, and persistence. *Learning & Memory*, 22(2), 116–127. <https://doi.org/10.1101/lm.037382.114>
- Berridge, K. C., & Robinson, T. E. (2016). Liking, wanting and the incentive-sensitization theory of addiction. *The American Psychologist*, 71(8), 670–679. <https://doi.org/10.1037/amp0000059>
- Boakes, R. A. (1977). Performance on learning to associate a stimulus with positive reinforcement. In H. Davis & H.M.B. Hurwitz (Eds.) *Operant-Pavlovian interactions* (pp. 67–97). Lawrence Erlbaum Associates.
- Breland, K., & Breland, M. (1961). The misbehavior of organisms. *American Psychologist*, 16(11), 681–684. <https://doi.org/10.1037/h0040090>
- Cabrera, F., Jiménez, Á. A., & Covarrubias, P. (2019). Timberlake’s behavior systems: A paradigm shift toward an ecological approach. *Behavioural Processes*, 167, 103892. <https://doi.org/10.1016/j.beproc.2019.103892>

- Cacioppo, J. T., Cacioppo, S., Capitanio, J. P., & Cole, S. W. (2015). The neuroendocrinology of social isolation. *Annual review of psychology*, *66*, 733–767. <https://doi.org/10.1146/annurev-psych-010814-015240>
- Casachahua, J. (2011). Environmental influences on the sign-tracking of ethanol: a rodent model of alcohol addiction. (Unpublished master's thesis), Seton Hall University, South Orange, New Jersey. <https://scholarship.shu.edu/dissertations/9/>
- Chang, S. E., & Smith, K. S. (2016). An omission procedure reorganizes the microstructure of sign-tracking while preserving incentive salience. *Learning & Memory*, *23*(4), 151–155. <https://doi.org/10.1101/lm.041574.115>
- Cleland, G. G., & Davey, G. C. L. (1982). The effects of satiation and reinforcer devaluation on signal-centered behavior in the rat. *Learning and Motivation*, *13*(3), 343–360. [https://doi.org/10.1016/0023-9690\(82\)90014-5](https://doi.org/10.1016/0023-9690(82)90014-5)
- Colaizzi, J. M., Flagel, S. B., Joyner, M. A., Gearhardt, A. N., Stewart, J. L., & Paulus, M. P. (2020). Mapping sign-tracking and goal-tracking onto human behaviors. *Neuroscience & Biobehavioral Reviews*, *111*, 84–94. <https://doi.org/10.1016/j.neubiorev.2020.01.018>
- Collier, G., & Johnson, D. F. (2004). The paradox of satiation. *Physiology & Behavior*, *82*(1), 149–153. <https://doi.org/10.1016/j.physbeh.2004.04.041>
- Cofresí, R. U., Bartholow, B. D., & Piasecki, T. M. (2019). Evidence for incentive salience sensitization as a pathway to alcohol use disorder. *Neuroscience & Biobehavioral Reviews*, *107*, 897–926. <https://doi.org/10.1016/j.neubiorev.2019.10.009>
- Conrad, S. E., & Papini, M. R. (2018). Reward shifts in forced-choice and free-choice autoshaping with rats. *Journal of Experimental Psychology: Animal Learning and Cognition*, *44*(4), 422–440. <https://doi.org/10.1037/xan0000187>
- Crews F, He J, & Hodge C. (2007). Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacology Biochemistry & Behavior*. *86*(2), 189–199. <https://doi.org/10.1016/j.pbb.2006.12.001>
- DeAngeli, N. E., Miller, S. B., Meyer, H. C., & Bucci, D. J. (2017). Increased sign-tracking behavior in adolescent rats. *Developmental Psychobiology*, *59*(7), 840–847. <https://doi.org/10.1002/dev.21548>
- Derman, R. C., Schneider, K., Juarez, S., & Delamater, A. R. (2018). Sign-tracking is an expectancy-mediated behavior that relies on prediction error mechanisms. *Learning & Memory*, *25*(10), 550–563. <https://doi.org/10.1101/lm.047365.118>
- Domjan, M. (2016). Elicited versus emitted behavior: Time to abandon the distinction. *Journal of the Experimental Analysis of Behavior*, *105*(2), 231–245. <https://doi.org/10.1002/jeab.197>
- Domjan, M., Cusato, B., & Krause, M. (2004). Learning with arbitrary versus ecological conditioned stimuli: Evidence from sexual conditioning. *Psychonomic Bulletin & Review*, *11*(2), 232–246. <https://doi.org/10.3758/BF03196565>
- Doremus, T. L., Brunell, S. C., Rajendran, P., & Spear, L. P. (2005). Factors influencing elevated ethanol consumption in adolescent relative to adult rats. *Alcoholism, Clinical and Experimental Research*, *29*(10), 1796–1808. <https://doi.org/10.1097/01.alc.0000183007.65998.aa>
- Doremus-Fitzwater, T. L., & Spear, L. P. (2011). Amphetamine-induced incentive sensitization of sign-tracking behavior in adolescent and adult female rats. *Behavioral Neuroscience*, *125*(4), 661–667. <https://doi.org/10.1037/a0023763>
- Einson, D., & Morgan, M. (1976). Habituation of object contact in socially-reared and isolated rats (*Rattus norvegicus*). *Animal Behaviour*, *24*(2), 415–420. [https://doi.org/10.1016/S0003-3472\(76\)80051-6](https://doi.org/10.1016/S0003-3472(76)80051-6)
- Everitt, B. J., & Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: from actions to habits to compulsion. *Nature Neuroscience*, *8*(11), 1481–1489. <https://doi.org/10.1038/nn1579>
- Falk, J. L., & Tang, M. (1988). What schedule-induced polydipsia can tell us about alcoholism. *Alcoholism, Clinical and Experimental Research*, *12*(5), 577–585. <https://doi.org/10.1111/j.1530-0277.1988.tb00246.x>
- Field, M., & Kersbergen, I. (2019). Are animal models of addiction useful? *Addiction*, *115*, 6–12. <https://doi.org/10.1111/add.14764>
- Fiorenza, A. M., Shnitko, T. A., Sullivan, K. M., Vemuru, S. R., Gomez-A, A., Esaki, J. Y., Boettiger, C. A., Da Cunha, C., & Robinson, D. L. (2018). Ethanol exposure history and alcoholic reward differentially alter dopamine release in the nucleus accumbens to a reward-predictive cue. *Alcoholism, Clinical and Experimental Research*, *42*(6), 1051–1061. <https://doi.org/10.1111/acer.13636>
- Flagel, S. B., Akil, H., & Robinson, T. E. (2009). Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. *Neuropharmacology*, *56*, 139–148. <https://doi.org/10.1016/j.neuropharm.2008.06.027>
- Friard, O., & Gamba, M. (2016). BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. <https://doi.org/10.1111/2041-210X.12584>
- Friemel, C. M., Spanagel, R., & Schneider, M. (2010). Reward Sensitivity for a Palatable Food Reward Peaks During Pubertal Developmental in Rats. *Frontiers in Behavioral Neuroscience*, *4*. <https://doi.org/10.3389/fnbeh.2010.00039>
- Galaj, E., Barrera, E. D., & Ranaldi, R. (2020). Therapeutic efficacy of environmental enrichment for substance use disorders. *Pharmacology, Biochemistry, and Behavior*, *188*, 172829. <https://doi.org/10.1016/j.pbb.2019.172829>
- Gallistel, C. R., & Balsam, P. D. (2014). Time to rethink the neural mechanisms of learning and memory. *Neurobiology of Learning and Memory*, *108*, 136–144. <https://doi.org/10.1016/j.nlm.2013.11.019>
- Hearst, E., & Jenkins, H. M. (1974). *Sign tracking: The stimulus-reinforcer relation and directed action*. Psychonomic Society.
- Heather, N. (2017). Is the concept of compulsion useful in the explanation or description of addictive behaviour and experience? *Addictive Behaviors Reports*, *6*, 15–38. <https://doi.org/10.1016/j.abrep.2017.05.002>
- Kawa, A. B., Bentzley, B. S., & Robinson, T. E. (2016). Less is more: prolonged intermittent access cocaine self-administration produces incentive-sensitization and addiction-like behavior. *Psychopharmacology*, *233*(19–20), 3587–3602. <https://doi.org/10.1007/s00213-016-4393-8>
- Keefer, S. E., Bacharach, S. Z., Kochli, D. E., Chabot, J. M., & Calu, D. J. (2020). Effects of limited and extended Pavlovian training on devaluation sensitivity of sign- and goal-tracking rats. *Frontiers in Behavioral Neuroscience*, *14*, 3. <https://doi.org/10.3389/fnbeh.2020.00003>
- Koob, G. F., & Le Moal, M. (2001). Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology*, *24*(2), 97–129. [https://doi.org/10.1016/S0893-133X\(00\)00195-0](https://doi.org/10.1016/S0893-133X(00)00195-0)
- Leser N, & Wagner S. (2015) The effects of acute social isolation on long-term social recognition memory. *Neurobiology of Learning and Memory*, *124*, 97–103. <https://doi.org/10.1016/j.nlm.2015.07.002>
- Lloyd, D. R., Medina, D. J., Hawk, L. W., Fosco, W. D., & Richards, J. B. (2014). Habituation of reinforcer effectiveness. *Frontiers in Integrative Neuroscience*, *7*. <https://doi.org/10.3389/fnint.2013.00107>
- Locurto, C., Terrace, H. S., & Gibbon, J. (1976). Autoshaping, random control, and omission training in the rat. *Journal of the Experimental Analysis of Behavior*, *26*(3), 451–462. <https://doi.org/10.1901/jeab.1976.26-451>
- Lucas, G. A., Timberlake, W., & Gawley, D. J. (1988). Adjunctive behavior of the rat under periodic food delivery in a 24-hour

- environment. *Animal Learning & Behavior*, 16(1):19–30. <https://doi.org/10.3758/BF03209039>
- Madayag, A. C., Stringfield, S. J., Reissner, K. J., Boettiger, C. A., & Robinson, D. L. (2017). Sex and adolescent ethanol exposure influence Pavlovian conditioned approach. *Alcoholism, Clinical and Experimental Research*, 41(4), 846–856. <https://doi.org/10.1111/acer.13354>
- Manzo, L., Donaire, R., Sabariego, M., Papini, M. R., & Torres, C. (2015). Anti-anxiety self-medication in rats: Oral consumption of chlordiazepoxide and ethanol after reward devaluation. *Behavioural Brain Research*, 278, 90–97. <https://doi.org/10.1016/j.bbr.2014.09.017>
- McDiarmid, T. A., Yu, A. J., & Rankin, C. H. (2019). Habituation is more than learning to ignore: Multiple mechanisms serve to facilitate shifts in behavioral strategy. *BioEssays*, 41(9), 1900077. <https://doi.org/10.1002/bies.201900077>
- McSweeney, F. K., & Murphy, E. S. (2009). Sensitization and habituation regulate reinforcer effectiveness. *Neurobiology of Learning and Memory*, 92(2), 189–198. <https://doi.org/10.1016/j.nlm.2008.07.002>
- McSweeney, F. K., & Swindell, S. (1999). General-process theories of motivation revisited: The role of habituation. *Psychological Bulletin*, 125(4), 437–457. <https://doi.org/10.1037/0033-2909.125.4.437>
- Meyer, P. J., Lovic, V., Saunders, B. T., Yager, L. M., Flagel, S. B., Morrow, J. D., & Robinson, T. E. (2012). Quantifying Individual Variation in the Propensity to Attribute Incentive Salience to Reward Cues. *PLOS ONE*, 7(6), e38987. <https://doi.org/10.1371/journal.pone.0038987>
- Meyer-rolbersleben, L., Winter, C., & Bernhardt, N. (2020). Dissociation of wanting and liking in the sucrose preference test in dopamine transporter overexpressing rats. *Behavioural Brain Research*, 378, 112244. <https://doi.org/10.1016/j.bbr.2019.112244>
- Modlinska, K., Chrzanowska, A., & Pisula, W. (2019). The impact of changeability of enriched environment on exploration in rats. *Behavioural Processes*, 164, 78–85. <https://doi.org/10.1016/j.beproc.2019.04.015>
- Morrison, S. E., Bamkole, M. A., & Nicola, S. M. (2015). Sign tracking, but not goal tracking, is resistant to outcome devaluation. *Frontiers in Neuroscience*, 9, 468. <https://doi.org/10.3389/fnins.2015.00468>
- Morrow, J. D. (2018). Relevance of sign-tracking to co-occurring psychiatric disorders. In A. Tomie & J. D. Morrow (Eds.), *Sign-Tracking and Drug Addiction*. Ann Arbor, MI: Maize Books. <https://quod.lib.umich.edu/m/maize/mpub10215070/1:8/%2D%2Dsign-tracking-and-drug-addiction?rgn=div1;view=fulltext>
- Panksepp, J., & Beatty, W. (1980). Social deprivation and play in rats. *Behavioral and Neural Biology*, 30 (2), 197–206. [https://doi.org/10.1016/s0163-1047\(80\)91077-8](https://doi.org/10.1016/s0163-1047(80)91077-8)
- Patitucci, E., Nelson, A. J. D., Dwyer, D. M., & Honey, R. C. (2016). The origins of individual differences in how learning is expressed in rats: A general-process perspective. *Journal of Experimental Psychology: Animal Learning and Cognition*, 42(4), 313–324. <https://doi.org/10.1037/xan0000116>
- Ramsden, E. (2015). Making animals alcoholic: Shifting laboratory models of addiction. *Journal of the History of the Behavioral Sciences*, 51(2), 164–194. <https://doi.org/10.1002/jhbs.21715>
- Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C.-F., & Thompson, R. F. (2009). Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiology of Learning and Memory*, 92(2), 135–138. <https://doi.org/10.1016/j.nlm.2008.09.012>
- Renner, M. J., & Rosenzweig, M. R. (1987). *Enriched and Impoverished Environments: Effects on Brain and Behavior*. Springer-Verlag. <https://doi.org/10.1007/978-1-4612-4766-1>
- Robinson, T. E., & Berridge, K. C. (2008). The incentive sensitization theory of addiction: some current issues. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 363(1507), 3137–3146. <https://doi.org/10.1098/rstb.2008.0093>
- Robinson, T. E., & Flagel, S. B. (2009). Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. *Biological Psychiatry*, 65(10), 869–873. <https://doi.org/10.1016/j.biopsych.2008.09.006>
- Rode, A. N., Moghaddam, B., & Morrison, S. E. (2019). Increased goal tracking in adolescent rats is goal-directed and not habit-like. *Frontiers in Behavioral Neuroscience*, 13, 291. <https://doi.org/10.3389/fnbeh.2019.00291>
- Saunders, B. T., & Robinson, T. E. (2011). Individual variation in the motivational properties of cocaine. *Neuropsychopharmacology*, 36(8), 1668–1676. <https://doi.org/10.1038/npp.2011.48>
- Sey, N., Gómez-A, A., Madayag, A. C., Boettiger, C. A., & Robinson, D. L. (2019). Adolescent intermittent ethanol impairs behavioral flexibility in a rat foraging task in adulthood. *Behavioural brain research*, 373, 112085. <https://doi.org/10.1016/j.bbr.2019.112085>
- Silva, K. M., & Timberlake, W. (1997). A behavior systems view of conditioned states during long and short CS–US intervals. *Learning and Motivation*, 28(4), 465–490. <https://doi.org/10.1006/lmot.1997.0986>
- Smedley, E. B., & Smith, K. S. (2018). Evidence of structure and persistence in motivational attraction to serial Pavlovian cues. *Learning & Memory*, 25(2), 78–89. <https://doi.org/10.1101/lm.046599.117>
- Spear, L. P. (2004). Adolescent brain development and animal models. *Annals of the New York Academy of Sciences*, 1022(1), 23–26. <https://doi.org/10.1196/annals.1308.002>
- Srey, C. S., Maddux, J.-M. N., & Chaudhri, N. (2015). The attribution of incentive salience to Pavlovian alcohol cues: a shift from goal-tracking to sign-tracking. *Frontiers in Behavioral Neuroscience*, 9, 54. <https://doi.org/10.3389/fnbeh.2015.00054>
- Stairs, D. J., & Bardo, M. T. (2009). Neurobehavioral effects of environmental enrichment and drug abuse vulnerability. *Pharmacology, Biochemistry, and Behavior*, 92(3), 377–382. <https://doi.org/10.1016/j.pbb.2009.01.016>
- Stansfield, K. H., & Kirstein, C. L. (2006). Effects of novelty on behavior in the adolescent and adult rat. *Developmental Psychobiology*, 48(1), 10–15. <https://doi.org/10.1002/dev.20127>
- Timberlake, W. (1993). Behavior systems and reinforcement: An integrative approach. *Journal of the Experimental Analysis of Behavior*, 60(1):105–128. <https://doi.org/10.1901/jeab.1993.60-105>
- Timberlake, W. (1994). Behavior systems, associationism, and Pavlovian conditioning. *Psychonomic Bulletin & Review*, 1(4), 405–420. <https://doi.org/10.3758/BF03210945>
- Timberlake, W. (1984). The functional organization of appetitive behavior: Behavior systems and learning. In M. D. Zeiler & P. Harzem (Eds.), *Advances in the analysis of behavior: Vol. 3. Biological factors in learning*, (pp. 177–221). Chichester: Wiley.
- Timberlake, W., & Lucas, G.A. (1989). Behavior systems and learning: From misbehavior to general principles. In: S. B. Klein, & R. R. Mowrer (Eds.), *Contemporary Learning Theories: Instrumental Conditioning Theory and the Impact of Constraints on Learning* (pp. 237–275). Erlbaum.
- Timberlake, W., & Melcer, T. (1988). Effects of poisoning on predatory and ingestive behavior toward artificial prey in rats (*Rattus norvegicus*). *Journal of Comparative Psychology*, 102(2), 182–187. <https://doi.org/10.1037/0735-7036.102.2.182>
- Timberlake, W., Wahl, G., & King, D. A. (1982). Stimulus and response contingencies in the misbehavior of rats. *Journal of Experimental Psychology: Animal Behavior Processes*, 8(1), 62–85. <https://doi.org/10.1037/0097-7403.8.1.62>
- Timberlake, W., & Washburne, D. L. (1989). Feeding ecology and laboratory predatory behavior toward live and artificial moving prey

- in seven rodent species. *Animal Learning & Behavior*, 17(1):2-11. <https://doi.org/10.3758/BF03205206>
- Tomie, A., di Poce, J., Derenzo, C. C., & Pohorecky, L. A. (2002). Autoshaping of ethanol drinking: an animal model of binge drinking. *Alcohol and Alcoholism (Oxford, Oxfordshire)*, 37(2), 138–146. <https://doi.org/10.1093/alcalc/37.2.138>
- Tomie, A., Kuo, T., Apor, K. R., Salomon, K. E., & Pohorecky, L. A. (2004). Autoshaping induces ethanol drinking in nondeprived rats: evidence of long-term retention but no induction of ethanol preference. *Pharmacology, Biochemistry, & Behavior*, 77(4), 797–804. <https://doi.org/10.1016/j.pbb.2004.02.005>
- Tomie, A., Miller, W. C., Dranoff, E., & Pohorecky, L. A. (2006). Intermittent presentations of ethanol sipper tube induce ethanol drinking in rats. *Alcohol and Alcoholism (Oxford, Oxfordshire)*, 41(3), 225–230. <https://doi.org/10.1093/alcalc/agl002>
- Tomie, A., Mohamed, W. M., & Pohorecky, L. A. (2005). Effects of age on Pavlovian autoshaping of ethanol drinking in non-deprived rats. *International Journal of Comparative Psychology*, 18(2). <https://escholarship.org/uc/item/7m92q4k9>
- Tomie, A., Jeffers, P., & Zito, B. (2018). Sign-tracking model of the addiction blind spot. In A. Tomie & J. D. Morrow (Eds.) *Sign tracking and drug addiction* (pp. 8 – 34). Michigan Publishing, University of Michigan Library. <https://doi.org/10.3998/mpub.10215070>
- Tomie, A., & Sharma, N. (2013). Pavlovian sign-tracking model of alcohol abuse. *Current Drug Abuse Reviews*, 6(3), 201–19. <https://doi.org/10.2174/18744737113069990023>
- van Praag, H., Kempermann, G., & Gage, F. H. (2000). Neural consequences of environmental enrichment. *Nature Reviews Neuroscience*, 1(3), 191–198. <https://doi.org/10.1038/35044558>
- Williams, D. R., & Williams, H. (1969). Auto-maintenance in the pigeon: Sustained pecking despite contingent non-reinforcement. *Journal of the Experimental Analysis of Behavior*, 12(4), 511–520. <https://doi.org/10.1901/jeab.1969.12-511>
- Yager, L. M., Pitchers, K. K., Flagel, S. B., & Robinson, T. E. (2015). Individual variation in the motivational and neurobiological effects of an opioid cue. *Neuropsychopharmacology*, 40(5), 1269–1277. <https://doi.org/10.1038/npp.2014.314>

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