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ORIGINAL INVESTIGATION

Individual differences in the propensity to approach signals vs goals promote different adaptations in the dopamine system of rats

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Abstract
Rationale The way an individual responds to cues associated with rewards may be a key determinant of vulnerability to compulsive behavioral disorders.
Objectives We studied individual differences in Pavlovian conditioned approach behavior and examined the expression of neurobiological markers associated with the dopaminergic system, the same neural system implicated in incentive motivational processes.
Methods Pavlovian autoshaping procedures consisted of the brief presentation of an illuminated retractable lever (conditioned stimulus) followed by the *response-independent* delivery of a food pellet (unconditioned stimulus), which lead to a Pavlovian conditioned response. In situ hybridization was performed on brains obtained either following the first or last (fifth) day of training.
Results Two phenotypes emerged. Sign-trackers (ST) exhibited behavior that seemed to be largely controlled by the cue that signaled impending reward delivery; whereas goal-trackers (GT) preferentially approached the location where the reward was delivered. Following a single training session, ST showed greater expression of dopamine D1 receptor mRNA relative to GT. After 5 days of training, GT exhibited greater expression levels of tyrosine hydroxylase, dopamine transporter, and dopamine D2 receptor mRNA relative to ST.
Conclusions These findings suggest that the development of approach behavior towards signals vs goal leads to distinct adaptations in the dopamine system. The sign-tracker vs goal-tracker phenotype may prove to be a valuable animal model to investigate individual differences in the way incentive salience is attributed to environmental stimuli, which may contribute to the development of addiction and other compulsive behavioral disorders.

Keywords Autoshaping · Conditioned stimuli · Dopamine · Goal-tracking · Incentive salience · Motivation · Pavlovian conditioning · Sign-tracking

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Introduction

Stimuli associated with reward—either natural rewards (i.e., food, sex, water) or drugs of abuse—become imbued with incentive salience, gaining the power to control behavior (Berridge and Robinson 2003). Incentive salience refers to a motivational component of reward, one that "transforms mere sensory information about rewards and their cues (sights, sounds, and smells) into attractive, desired, riveting incentives" (p. 510, Berridge and Robinson 2003). That is, incentive stimuli become motivational magnets, eliciting approach towards them, as seen in Pavlovian conditioned approach (PCA) behavior towards rewards and their

signals. However, reward-related cues in the environment not only guide normal behavior, but can also lead to uncontrollable behavior (Falk and Feingold 1987), and the way an individual responds to signals associated with rewards may be a key determinant of vulnerability to psychopathology, such as substance abuse.

One way to study PCA behavior involves the use of procedures that lead to a phenomenon called "autoshaping," or more appropriately, sign-tracking (Hearst and Jenkins 1974). In this situation, multiple presentations of a discrete appetitive cue (conditioned stimulus, CS) paired with a reward (unconditioned stimulus, US) elicits a conditioned response (CR). The typical CR is one of approach to the CS and often includes a repertoire of consummatory behaviors similar to those involved in consuming the US (Davey et al. 1984; Jenkins and Moore 1973). Thus, if presentation of a lever is immediately followed by the *response-independent* delivery of a food pellet, the animal will approach and often grasp and gnaw the lever as if it were itself food (Hearst and Jenkins 1974; Tomie 1996). This CS-directed response develops even though no response is required for the animal to obtain the reward, and remarkably, this behavior persists even if approach delays the receipt of the reward or leads to reward omission (Breland and Breland 1961; Hearst and Jenkins 1974; Williams and Williams 1969). The alternative, and less studied, approach response that can emerge from autoshaping training is that of goal-tracking (Boakes 1977). Goal-tracking is directed towards the location of US delivery rather than the CS. Whether an animal develops a sign-tracking or goal-tracking response seems to depend on a number of variables including the species (Kemenes and Benjamin 1989; Purdy et al. 1999), the nature of the CS or US (Burns and Domjan 1996; Uslaner et al. 2006), and the temporal and spatial contingencies between the CS and US (Brown et al. 1993; Holland 1980; Silva et al. 1992). However, little work has addressed whether or not there are individual differences in the propensity to approach signals vs goals.

In the present study, we found that some animals sign-tracked and others goal-tracked despite the fact that it was the same species and the same paradigm being utilized. Thus, two phenotypes emerged, defining the extremes of the population: sign-trackers (ST) were those animals that responded to the CS by approaching the CS and attempting to "consume" it, whereas goal-trackers (GT) responded to the CS by approaching the location where the US would be delivered (i.e., the food receptacle). In situ hybridization was used to determine whether differences in gene expression (mRNA levels) on day 1 may have contributed to the behavioral patterns that emerged during the conditioning process and to assess the effects of the conditioning process on the dopaminergic system, the same neurobiological system implicated in incentive motivational processes.

Materials and methods

Animals The present studies followed the *Principles of Laboratory Animal Care* http://www.nap.edu/readingroom/books/labrats/) and the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Research Council 2003). Forty-four adult male Sprague–Dawley rats from Charles River (Wilmington, MA, USA) weighing 250–300 g upon arrival were used. Rats were housed in pairs and kept on a 12-h light/dark cycle (lights on 0600 hours) with controlled temperature and humidity and maintained in accordance with the University Committee Use and Care of Animals. Food and water were available ad libitum. For 2 days prior to the start of the autoshaping paradigm, 45-mg banana-flavored food pellets (BioServe, #F0059, Frenchtown, NJ, USA) were placed in their home cage to familiarize the animals with this food, which was used later in training.

Operant conditioning chambers Fifteen standard MED Associates "operant" conditioning chambers (20.5× 24.1 cm floor area, 29.2 cm high; MED Associates, St. Albans, VT, USA) were used for Pavlovian training. Each chamber was equipped with an illuminated retractable lever (MED Associates) located 6 cm above the stainless steel grid floor and placed on one side of a food receptacle, which was located on the centerline of one of the 24-cm-wide sides and placed 3 cm above the floor (Fig. 1). A red house light was located on the wall opposite the food

Fig. 1 Close-up photograph of retractable lever next to the food receptacle in Med Associates operant conditioning chamber

receptacle and remained on throughout each training session. Two nose-holes were located approximately 6 cm above the grid floor on either side of the house light. Responses in the nose-holes were without consequence and served as an index of general exploratory behavior. A 0.635-cm-high output white LED was flush mounted on the inside of the retractable lever and used to illuminate the lever. The lever required a 10-g force to operate, such that mere contact with the lever triggered recording of a "lever press." The side of the lever with respect to the food receptacle was counterbalanced across boxes to eliminate side bias. Operation of a pellet dispenser (Med Associates) delivered 45-mg banana-flavored food pellets into the food receptacle. Each operant conditioning chamber was located in a sound-attenuating enclosure, and white noise was supplied using a ventilating fan to mask outside noise.

Pavlovian conditioned approach (PCA) procedures All Pavlovian training sessions were conducted between the hours of 1300 and 1700. Three waves of rats (14–15 animals per wave) were tested per day. After 1 week of acclimation to the colony room, rats were placed in the operant chambers for pretraining sessions during which the red house light remained on but the lever was retracted. Fifty food pellets were delivered on a variable interval (VI) 90-s schedule, and it was determined whether the rats were reliably retrieving the pellets. The pretraining sessions lasted approximately 25 min. Typically, by the end of the second pretraining session, all of the rats consumed all of the food pellets. After 2 days of pretraining, the development of PCA behavior was assessed using standard autoshaping procedures (adapted from Meneses 2003; Meneses et al. 2004). Each trial during a test session consisted of presentation of the illuminated lever (CS) into the chamber for 8 s. Retraction of the lever was immediately followed by the response-independent operation of the pellet dispenser resulting in the delivery of one 45-mg food pellet (US). The beginning of the next intertrial interval (ITI) commenced immediately after pellet delivery. The CS was presented on a random interval 60-s schedule (i.e., one presentation of the CS occurred on average every 60 s, but the actual time between CS presentations varied randomly between 30 and 90 s). Each test session consisted of 25 trials, wherein the lever (CS) and the food (US) were presented in a paired fashion, resulting in a 35- to 40-min test session each day. We have demonstrated in previous studies that animals receiving pseudorandom pairings of the CS and US do not develop a CR (unpublished data). This set of control animals was not examined in the present study.

Repeated CS–US pairings led to the acquisition of a Pavlovian CR. The topography of the Pavlovian CR included approach followed by grasping and gnawing of the lever, recorded as lever presses, or approach to the food receptacle, recorded as magazine entries. The dependent variable used to characterize the animals (as ST or GT) was the number of lever presses (i.e., contact with the lever) since it is a robust measure of sign-tracking in our paradigm. The total number of lever presses, the latency to the first lever press per trial, the number of magazine entries during ITI and during presentation of the CS, the latency to magazine entry during the CS presentation, and the number of nose-pokes were recorded for data analysis using Med Associates software. The number of food pellets consumed was also recorded following each session.

Tissue collection Immediately following the first day of training, the top and bottom lever pressers (i.e., sign-trackers and goal-trackers) within each wave were killed by rapid decapitation and brains were obtained. Accordingly, three ST and 3 GT were killed from wave 1, two ST and three GT from wave 2, and three ST and two GT from wave 3, for a total of eight GT and eight ST. The remaining 22 rats continued with the Pavlovian autoshaping paradigm for 4 more days. These remaining animals were killed immediately following the fifth day of training. Only brains from the top and bottom eight lever pressers (based on their average number of lever presses across 5 days) were processed for further analysis. Thus, the six animals that ranked in the intermediate group of lever pressers were not included in any of the analyses to follow. A control group of animals (n=6) was killed prior to the first day of autoshaping training (i.e., basal time point). All animals were killed by rapid decapitation. Brains were immediately removed, frozen in isopentane (−30 to −40°C), and stored at −80°C. Coronal brain sections (10 μm) were cut on a cryostat (at 100-μm intervals) and thaw mounted onto Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA, USA). Regions of interest were identified with cresyl violet staining. Slides were stored at −80°C until processing for in situ hybridization.

In situ hybridization For detailed methodology of the in situ hybridization techniques used in our laboratory, see Kabbaj et al. (2000). Post-fixed sections were hybridized with ^{35}S-labeled cRNA probes produced using standard in vitro transcription methodology. The tyrosine hydroxylase (TH) probe was a 274-base-pair fragment directed against the rat TH mRNA. The dopamine transporter (DAT) probe was a 532-base-pair fragment directed against the rat DAT mRNA. The D1 receptor probe was a 480-base-pair fragment directed against the rat D1 mRNA. The D2 receptor probe was a 495-base-pair fragment directed against the rat D2 mRNA. The probes were diluted in hybridization buffer, and brain sections were coverslipped and incubated overnight at 55°C. Following posthybridization rinses and dehydration, slides were apposed to Kodak

Biomax MR film (Eastman Kodak, Rochester, NY, USA). Sections were exposed for approximately 1 day. The specificity of the hybridization was confirmed by control experiments using sense probes.

Quantification of the radioactive signal The autoradiograms were digitized and captured using Micro Computer Imaging Device (Ontario, Canada), and the magnitude of the signal from the hybridized ^{35}S-cRNA probe was determined using National Institutes of Health Image software. A macro was used (Dr. Serge Campeau, University of Colorado, Boulder, CO, USA) which enabled signal above background to be automatically determined. The "net" optical density (OD) of these signal pixels was obtained by multiplying the size of the area quantified by the signal intensity. The person quantifying was blind to group assignments. DAT and TH mRNA were quantified in the ventral tegmental area (VTA), and dopamine D1 and D2 receptor mRNA were quantified in the nucleus accumbens (NAcc). Optical density measurements were taken from the left and right side of at least four brain sections per animal for each probe and region of interest. A mean value was then generated for each probe and region of interest to yield one data point per animal for statistical analysis.

Statistical analysis

Group assignments Animals were categorized based on their lever press behavior on day 1 (for analysis of neurobiological data) or across the 5 days of training (for behavioral analysis). From previous studies, we know that we can predict with approximately 86% accuracy what an animal's classification would be on day 5 based on its day 1 behavior as long as we include only those animals that fall into the extremes of the population (i.e., top and bottom 33% based on day 1 lever-pressing behavior).

Statistical analyses for behavior Of the 22 animals remaining on day 5 of training, ST were the eight animals with the highest mean number of lever presses (range of 39–73) across the 5 days of training, whereas GT were the eight animals with the lowest mean number of lever presses (range of 1–22) across the 5 days of training. Linear mixed-effects models (Verbeke and Molenberghs 2000) were used to assess longitudinal trends in PCA behavior. The covariance structure for the longitudinal data was explored and modeled appropriately for each dependent variable (i.e., lever press, latency to lever press, magazine entry, latency to magazine entry, and nosepokes). When significant main effects or group × day interactions were revealed, Bonferonni post hoc comparisons were conducted. For all analyses, significance was set at $P \leq 0.05$.

Statistical analyses for neurobiology Nonparametric tests were used to detect significant differences in gene expression since examination of these data revealed that the dependent variables were not normally distributed. The Kruskal–Wallis test (for three or more samples) was used to compare basal mRNA levels to those obtained following the first and fifth training session (i.e., effect of time with groups collapsed). The Wilcoxon Mann–Whitney test was used to examine group differences in molecular expression on day 1 and day 5. In addition, the effect of time was analyzed for each group separately to determine if mRNA levels changed significantly from day 1 to day 5. The relationship between PCA behavior and neurobiological markers was also examined using correlation *Z* tests with a 95% confidence interval.

Results

Pavlovian conditioned approach behavior

Lever press As expected, based on the defining criterion, ST exhibited increased lever-pressing behavior relative to GT (effect of group; $F_{(1,\ 18.46)}=111.10$; $P<0.0001$; Fig. 2a) across the five training sessions. Mixed-effects model analysis also revealed a significant effect of day ($F_{(4,\ 26.71)}=14.71$, $P<0.0001$) and a group × day interaction ($F_{(4,\ 26.71)}=7.74$; $P<0.0001$). Post hoc comparisons confirmed a significant effect of group for each of the 5 days of training ($P<0.0001$).

When lever press behavior was analyzed separately for each group, there was a significant effect of day for ST ($F_{(4,\ 7.00)}=P<0.001$), but not for GT. Specifically, lever press behavior on days 1 and 2 was significantly lower than that on days 3, 4, and 5 ($P<0.005$; Fig. 2a). These data suggest that the CS (i.e., lever) gains significant value over time for the ST. In agreement with this interpretation, ST show a decreased latency to lever press relative to GT (effect of group; $F_{(1,\ 15.86)}=75.01$; $P<0.0001$) on all 5 days of training (post hoc comparisons, $P \leq 0.001$; Fig. 2b). Moreover, the latency to lever press significantly decreased across time for the ST (effect of day for ST; $F_{(4,\ 7.00)}=110.94$; $P<0.0001$), but not for GT (Fig. 2b). Specifically, for ST, latency to lever press on days 3, 4, and 5 was significantly less than that on days 1 and 2. Taken together, these data suggest that the behavior ST exhibited towards the lever (i.e., cue or CS) was acquired over time.

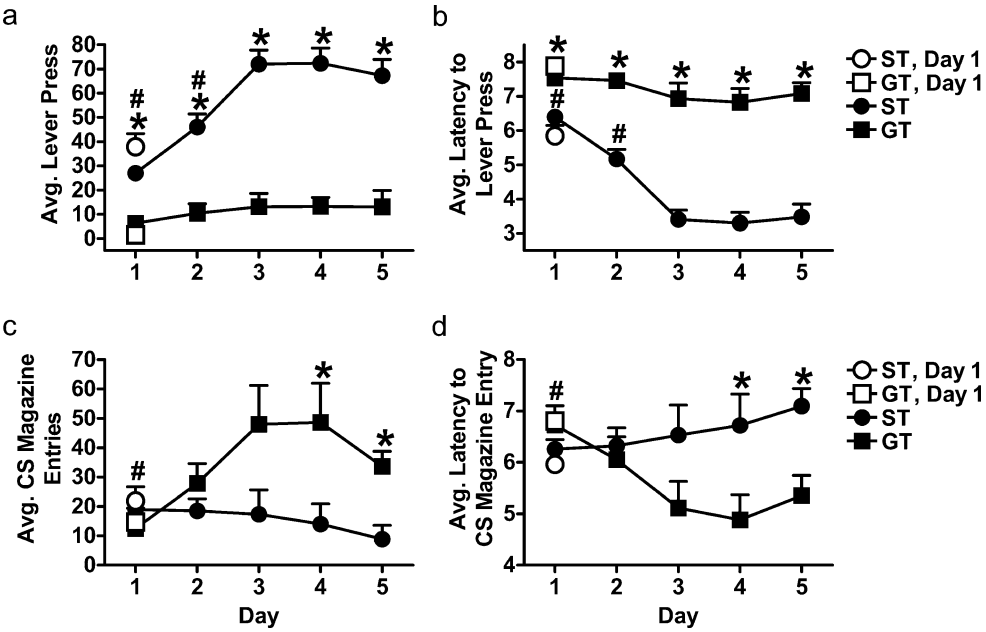

Fig. 2 Lever press behavior and magazine entries during CS presentation. *Open symbols* represent behavior of those animals that were killed following the first day of training (n=8 per group). *Circles* represent sign-trackers and *squares* represent goal-trackers. **a** Mean lever press±SEM on each of the 5 days of training. **b** Mean latency to lever press±SEM (in seconds). **c** Mean number of CS magazine entries±SEM on each of the 5 days of training. **d** Mean latency to magazine entry during CS presentation±SEM (in seconds). (*P<0.05, effect of group for specified day; #P<0.05, effect of day for specified group)

Magazine entries during CS presentation Although GT did not approach the cue, or CS, they did approach the goal, or location of the US. That is, GT showed increased magazine entries during CS presentation relative to ST (effect of group; $F_{(1,\ 14.00)}$=5.30; P=0.04; Fig. 2c). There was a significant group × day interaction ($F_{(1,\ 14.00)}$=7.02; P=0.003), and a trend level effect of day for magazine entries during CS presentation ($F_{(1,\ 14.00)}$=2.57; P=0.08). Post hoc comparisons revealed that GT showed greater CS magazine entries relative to ST on days 4 and 5 (P<0.04; Fig. 2c). There was also a significant effect of day for GT (effect of day for GT; $F_{(4,\ 7.00)}$=8.92; P=0.007; Fig. 2c), but not for ST. Specifically, the number of magazine entries during CS presentation on day 1 for GT was significantly less than the number of CS magazine entries during days 3, 4, and 5 (post hoc comparisons, P<0.03).

There was a significant group × day interaction for the latency to enter the food receptacle during CS presentation (group × day interaction; $F_{(4,\ 35.69)}$=4.48; P=0.005; Fig. 2d). GT showed a decreased latency to enter the magazine receptacle during CS presentation relative to ST on days 4 and 5 (post hoc comparisons, P<0.03). Moreover, the latency to enter the food receptacle during CS presentation decreased across training sessions for GT (effect of day for GT; $F_{(4,\ 7.00)}$=11.86; P=0.003; Fig. 2d), but not for ST. The latency to enter the food receptacle during CS presentation was significantly less on days 3,4, and 5 than that on day 1 for GT (post hoc comparisons, P<0.01). Taken together, these data suggest that the behavior GT exhibited towards the food receptacle (i.e., goal or US) was also acquired over time.

Behavior during ITI The behavior towards the food receptacle during the ITI (i.e., not during CS presentation) was also examined (data not shown). There was a significant effect of day ($F_{(4,\ 14.00)}$=3.75; P=0.03) and a group × day interaction ($F_{(4,\ 14.00)}$=5.47; P=0.007) for intertrial magazine entries, but no effect of group. GT showed an increased number of intertrial magazine entries relative to ST only on day 3 (P≤0.05). When each group was analyzed separately, there was a significant effect of day for ST ($F_{(4,\ 7.00)}$=76.21; P=<0.0001) and a trend for a significant effect for GT ($F_{(4,\ 7.00)}$=3.59; P=0.07) for the number of intertrial magazine entries. Both groups of animals showed a decrease in the number of magazine entries during the ITI across time; however, post hoc comparisons did not reveal any significant differences between days for either group. Nonetheless, it seems that both groups eventually learn that there was little or no benefit in repeatedly entering the receptacle once they obtained the food pellet that was delivered from the previous trial.

General exploratory behavior As an index of general exploratory behavior, we examined the number of nose-pokes each group made across the 5 days of training (data not shown). There was not a significant effect of group or a group × day interaction for the number of nose-pokes. However, there was a significant effect of day ($F_{(4,\ 34.69)}$= 5.981; P=0.001). Interestingly, ST showed a significant decrease in the number of nose-pokes across days ($F_{(4,\ 24.23)}$=4.2; P=0.01) such that day 5 was significantly different from days 2, 3, and 4 (post hoc comparisons,

$P \leq 0.01$). This decrease in general exploratory behavior was only evident in ST and could be a reflection of the escalating anticipation, or increased attractiveness, of the lever across time. These data suggest that the different behavioral patterns that emerge for ST and GT are not due to differences in general exploratory behavior.

In situ hybridization

Examination of the mesolimbic dopamine system When groups were collapsed, we found no significant differences between the basal time point, day 1, or day 5 mRNA levels for any of the molecules examined (i.e., effect of time for TH, DAT, D1, or D2 receptor; $P > 0.10$). To compare brains obtained on day 1 with those obtained on day 5, all animals were classified based solely on their day 1 behavior. Thus, only those animals that had less than 5 (i.e., bottom 33%) lever presses or greater than 25 (i.e., top 33%) lever presses on day 1 were included in the following analyses.

There was a significant effect of group for dopamine D1 receptor mRNA in the NAcc ($U=3$, $P=0.03$) on day 1. ST exhibited higher levels of D1 mRNA relative to GT on the first day of training (Fig. 4b). Dopamine D1 receptor mRNA was the only molecule examined that showed significant differences between groups on day 1. After 5 days of training, there were no significant group differences in D1 mRNA levels, but GT showed a slight increase in D1 mRNA levels on day 5 relative to day 1 ($U=5$, $P=0.07$). There were significant group differences on day 5 for TH ($U=0$, $P=0.02$) and DAT ($U=0$, $P=0.02$) mRNA in the VTA (Fig. 3b,d) and for D2 receptor mRNA in the NAcc ($U=1$, $P=0.03$; Fig. 4d). For each of these molecules, GT showed greater expression relative to ST on day 5, but there were no significant differences in mRNA levels between day 1 and day 5 for either group.

Brain/behavior correlations

Mean behavior score and neurobiological correlates To further examine the relationship between PCA behavior and the expression of dopaminergic molecules, we performed correlational analyses. PCA behavior (i.e., lever press, latency to lever press, magazine entries) was averaged across the 5 days of testing and correlated with mRNA levels examined after day 5. We found a negative correlation between lever press behavior and DAT, TH, and D2 receptor mRNA and a positive correlation between latency to lever press and DAT, TH, and D2 mRNA (see Table 1). There was also a positive, but less robust, correlation between magazine entries during CS presentation and D2 mRNA in the NAcc. These findings complement the data described above, suggesting that those animals that spend more time attending to the lever (i.e., ST) have blunted dopaminergic expression patterns following 5 days of training relative to those animals that spend more time at the food receptacle (i.e., GT).

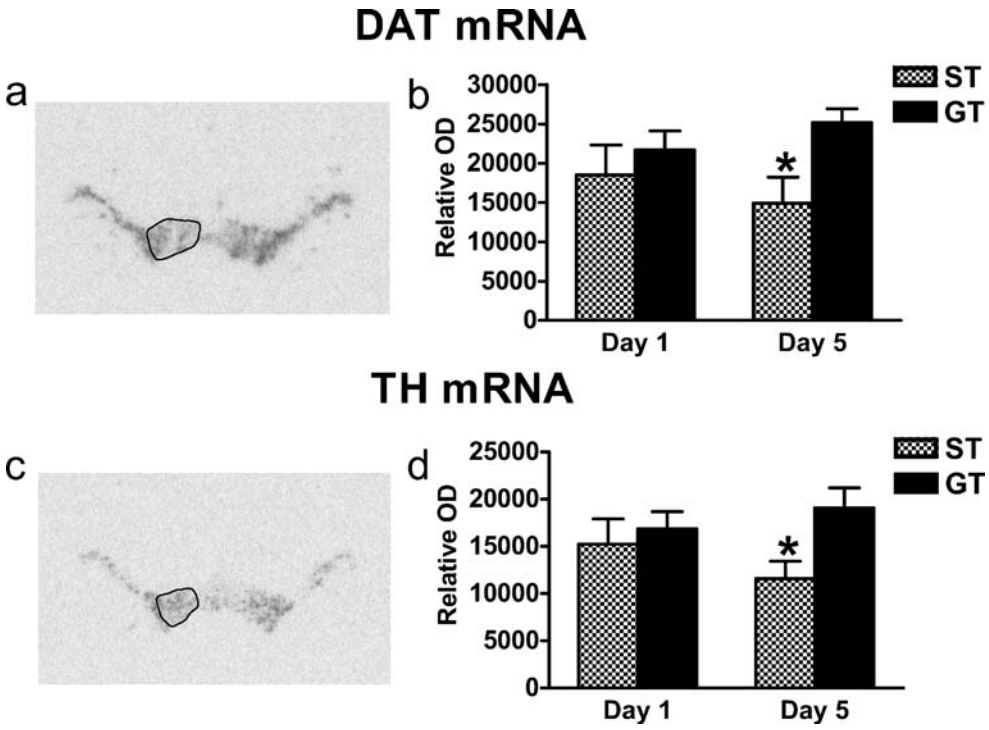

Fig. 3 In situ hybridization results for DAT and TH mRNA in the VTA. *Left panels* contain representative images from in situ hybridization of **a** DAT and **c** TH mRNA in VTA. *Outlined region* represents area quantified for analysis. *Right panels* depict mean±SEM relative optical density for sign-trackers (*checkered bars*) and goal-trackers (*solid black bars*) ($n=4$–6 per group). **b** DAT mRNA in VTA in brains obtained on day 1 and day 5. **d** TH mRNA in VTA in brains obtained on day 1 and day 5. (*$P<0.05$, effect of group for specified day)

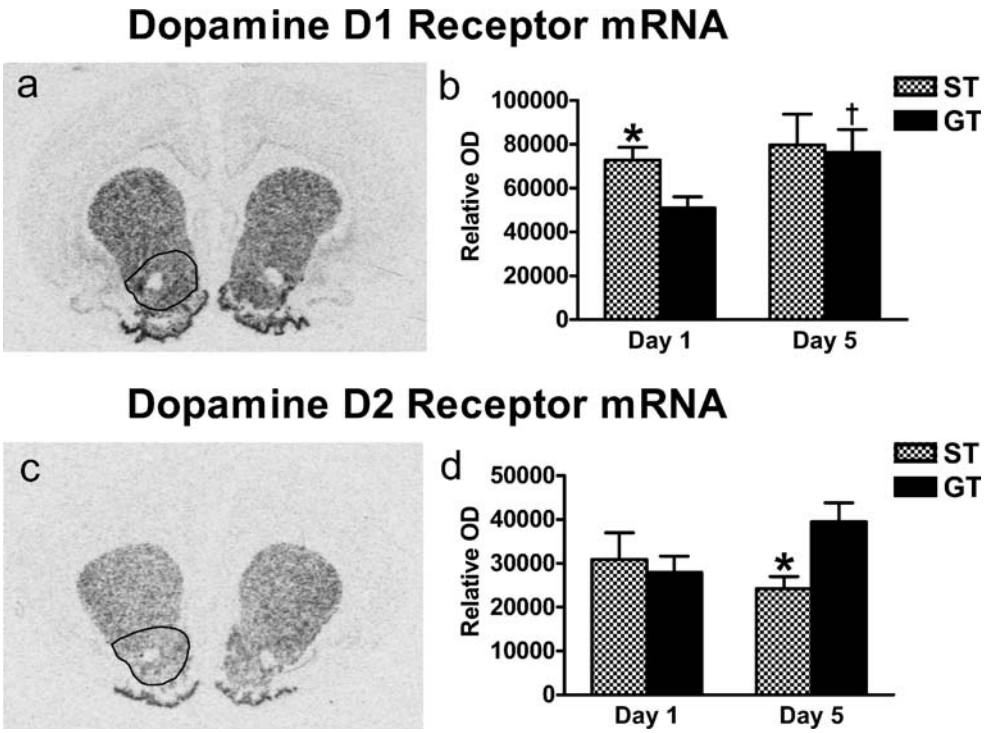

Fig. 4 In situ hybridization results for dopamine receptors D1 and D2 in the NAcc. *Left panels* contain representative images from in situ hybridization of **a** D1 and **c** D2 mRNA in NAcc. *Outlined region* represents area quantified for analysis. *Right panels* depict mean± SEM relative optical density for sign-trackers (*checkered bars*) and goal-trackers (*solid black bars*) (n=4–6 per group). **b** D1 receptor mRNA in brains obtained on day 1 and day 5. **d** D2 receptor mRNA in brains obtained on day 1 and day 5. (*P<0.05, effect of group for specified day; †P=0.07, effect of day for GT)

Neurobiological correlates of "learning" To investigate whether the differences in dopaminergic expression patterns were related to learning per se, we examined the relationship between mRNA levels and a learning score which was generated by subtracting day 1 behavior scores from day 5 behavior scores (e.g., lever press score on day 5 - lever press score on day 1 = lever press learning score). Using this measure, we found a positive correlation between magazine entries during CS presentation and D2 receptor mRNA in the nucleus accumbens after day 5 (r=0.6, P=0.01). This analysis did not reveal any significant correlations between lever press behavior and dopaminergic molecules. These results suggest that learning of the goal-seeking response (i.e., magazine entries during the CS presentation) may be more tightly regulated by D2 receptors than learning of the sign-tracking response.

Table 1 Neurobiological correlates and Pavlovian conditioned approach behavior

Behavior, mRNA	Correlation coefficient (r)	P value
Lever press, DAT	−0.64	0.01
Lever press, TH	−0.59	0.02
Lever press, D2 receptor	−0.59	0.02
Latency to lever press, DAT	0.67	0.01
Latency to lever press, TH	0.63	0.01
Latency to lever press, D2 receptor	0.62	0.01
CS magazine entries, D2 receptor	0.51	0.05

Correlational analyses were performed for behavioral measures and mRNA levels obtained after 5 days of training. The dependent variables are depicted in the far left column followed by the correlation coefficient (r) and the level of significance (P value).

Discussion

We used Pavlovian training procedures to examine individual differences in the process by which signals associated with reward come to elicit approach towards them. One group of animals (sign-trackers) came to approach a sign (CS, lever) that signaled reward delivery as if it were a surrogate for the reward (US, food), not only approaching it, but as described earlier, attempting to consume it (i.e., biting and gnawing it) (Hearst and Jenkins 1974). The other group of animals (goal-trackers) simply treated the presentation of the lever as a signal for impending reward delivery and, during CS presentation, came to approach the food receptacle (location of the US) rather than the CS (Boakes 1977). Thus, ST exhibit behavior that seems to be largely controlled by signals for rewards, and GT appear to be more concerned with the reward itself. Although these differences are apparent on day 1, our data suggest that these approach behaviors, be it sign-tracking or goal-tracking, are learned over time. Furthermore, our findings suggest that these two behavioral patterns cannot be attributed to differences in general exploratory behavior.

Upon examination of the neurobiological correlates underlying these behaviors, we found that ST exhibit higher

levels of dopamine D1 receptor mRNA in the NAcc relative to GT on day 1. These findings are in agreement with a recent report describing the role of D1 receptors in learning a PCA response to a CS predictive of food reward (i.e., sign-tracking). Dalley et al. (2005) elegantly demonstrated that D1 receptors in the nucleus accumbens contribute to, and are necessary for, the early consolidation and acquisition of appetitive Pavlovian conditioning (i.e., approach to a CS predictive of food reward). These findings support our results which suggest that the increased levels of D1 receptor mRNA in ST relative to GT may contribute to the initial acquisition of the CR and subsequently to the emergence of different behavioral patterns. Unfortunately, we do not know whether the group differences in D1 mRNA levels on day 1 are preexisting or a consequence of the first training session. Additional studies are required to investigate the time-course of mRNA induction following a Pavlovian training session. Moreover, we are currently utilizing a selectively bred line of animals that may allow us to predetermine the ST/GT phenotype, providing the opportunity to examine the "basal" state of these brains.

Following 5 days of training, GT exhibited greater levels of TH, DAT, and D2 mRNA relative to ST. Interestingly, however, there were no significant differences between day 1 and day 5 mRNA levels for any of the molecules examined for either group. Nonetheless, the experience of 5 days of training seems to be altering dopaminergic gene expression such that significant group differences emerge on day 5 that are not apparent on day 1. Thus, we assume that the achieved differences in behavior on day 5 are associated with differences in the dopamine system. In agreement, we found a positive correlation between the rate at which magazine entries during CS presentation were learned and D2 receptor mRNA levels in the NAcc on day 5. Our measure of learning was generated by subtracting day 1 behavior scores from day 5 behavior scores and may therefore not be a pure assessment of learning per se. Despite this caveat, these findings suggest that learning of the goal-seeking response (i.e., magazine entries during CS presentation) may be regulated by the D2 receptor.

Although we found differences in mRNA levels between ST and GT, it remains to be determined whether there are differences in dopaminergic activity or neurotransmission between these phenotypes. To our knowledge, immediate dopamine release in response to CS presentation and to delivery of the US has not been examined. However, differences in mesolimbic levels of monoamines using similar Pavlovian autoshaping procedures have been reported. Tomie et al. (2000) found increased levels of dopamine and DOPAC in the NAcc of animals with high CR frequency (i.e., ST). In addition, Tomie et al. (2000) report a positive correlation between NAcc dopamine levels and lever-pressing behavior. These findings indicate that ST may have increased dopaminergic tone relative to GT. Although the data of Tomie et al. may seem discrepant to the in situ hybridization data presented here, differences in experimental design and outcome measures make this a problematic comparison. Additional studies are in progress to further investigate the neurobiological correlates of sign-tracking and goal-tracking and to address the neurochemical and anatomical heterogeneity of the nucleus accumbens.

It has been suggested that autoshaping, or sign-tracking, provides a conceptual and empirical link between drug conditioning and craving in both animals and humans (Newlin 1992, 1999; Tomie 1996). The underlying neural circuitry implicated in addiction is the same neural circuitry shown here to be altered as a consequence of behavior towards goals vs signals. In fact, lower levels of D2 receptor have been associated with increased craving (Heinz et al. 2004) and increased reports of "drug-liking" in humans (Volkow et al. 2002), and these findings nicely correspond to the lower levels of D2 mRNA in ST relative to GT. Based on the reported findings, one would expect ST that seem to be highly reactive to cues in the environment to also exhibit increased drug-taking behavior and perhaps increased susceptibility to addiction. Further studies are currently under way to assess the differences in drug-taking behavior between ST and GT.

In conclusion, we believe that the ST/GT phenotype provides an excellent animal model to investigate individual differences in the degree to which incentive salience is attributed to environmental stimuli associated with rewards, which may contribute to the development of addiction. Moreover, further investigation of these phenotypes will allow us to begin parsing out the psychological and neurobiological components of motivational reward processes that underlie normal behavior as well as uncontrollable behavior associated with a number of psychopathologies.

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References

Berridge KC, Robinson TE (2003) Parsing reward. Trends Neurosci 26:507–513

Boakes R (1977) Performance on learning to associate a stimulus with positive reinforcement. In: Davis H, HMB H (eds) Operant-pavlovian interactions. Erlbaum, Hillsdale, NJ, pp 67–97

Breland K, Breland M (1961) The misbehavior of organisms. Am Psychol 16:681–683

Brown B, Hemmes N, Vaca SCd, Pagano C (1993) Sign and goal tracking during delay and trace autoshaping in pigeons. Anim Learn Behav 21:360–368

Burns M, Domjan M (1996) Sign tracking versus goal tracking in the sexual conditioning of male Japanese quail (*Coturnix japonica*). J Exp Psychol Anim Behav Processes 22:297–306

Dalley JW, Laane K, Theobald DE, Armstrong HC, Corlett PR, Chudasama Y, Robbins TW (2005) Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. Proc Natl Acad Sci U S A 102:6189–6194

Davey GC, Cleland GG, Oakley DA, Jacobs JL (1984) The effect of early feeding experience on signal-directed response topography in the rat. Physiol Behav 32:11–15

Falk J, Feingold D (1987) Environmental and cultural factors in the behavioral actions of drugs. In: HY M (ed) Psychopharmacology: the third generation of progress. Raven, New York, pp 1503–1510

Hearst E, Jenkins H (1974) Sign-tracking: the stimulus–reinforcer relation and directed action. Monograph of the Psychonomic Society, Austin

Heinz A, Siessmeier T, Wrase J, Hermann D, Klein S, Grusser SM, Flor H, Braus DF, Buchholz HG, Grunder G, Schreckenberger M, Smolka MN, Rosch F, Mann K, Bartenstein P (2004) Correlation between dopamine D(2) receptors in the ventral striatum and central processing of alcohol cues and craving. Am J Psychiatry 161:1783–1789

Holland PC (1980) CS–US interval as a determinant of the form of Pavlovian appetitive conditioned responses. J Exp Psychol Anim Behav Processes 6:155–174

Jenkins HM, Moore BR (1973) The form of the auto-shaped response with food or water reinforcers. J Exp Anal Behav 20:163–181

Kabbaj M, Devine DP, Savage VR, Akil H (2000) Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. J Neurosci 20:6983–6988

Kemenes G, Benjamin PR (1989) Goal-tracking behavior in the pond snail, *Lymnaea stagnalis*. Behav Neural Biol 52:260–270

Meneses A (2003) A pharmacological analysis of an associative learning task: 5-HT(1) to 5-HT(7) receptor subtypes function on a pavlovian/instrumental autoshaped memory. Learn Mem 10:363–372

Meneses A, Manuel-Apolinar L, Rocha L, Castillo E, Castillo C (2004) Expression of the 5-HT receptors in rat brain during memory consolidation. Behav Brain Res 152:425–436

National Research Council (2003) Guidelines for the care and use of mammals in neuroscience and behavioral research. National Academy Press, Washington, DC

Newlin DB (1992) A comparison of drug conditioning and craving for alcohol and cocaine. Recent Dev Alcohol 10:147–164

Newlin DB (1999) Evolutionary game theory and multiple chemical sensitivity. Toxicol Ind Health 15:313–322

Purdy JE, Roberts AC, Garcia CA (1999) Sign tracking in cuttlefish (*Sepia officinalis*). J Comp Psychol 113:443–449

Silva FJ, Silva KM, Pear JJ (1992) Sign- versus goal-tracking: effects of conditioned-stimulus-to-unconditioned-stimulus distance. J Exp Anal Behav 57:17–31

Tomie A (1996) Locating reward cue at response manipulandum (CAM) induces symptoms of drug abuse. Neurosci Biobehav Rev 20:505–535

Tomie A, Aguado AS, Pohorecky LA, Benjamin D (2000) Individual differences in pavlovian autoshaping of lever pressing in rats predict stress-induced corticosterone release and mesolimbic levels of monoamines. Pharmacol Biochem Behav 65:509–517

Uslaner JM, Acerbo MJ, Jones SA, Robinson TE (2006) The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine. Behav Brain Res 169: 320–324

Verbeke G, Molenberghs G (2000) Linear mixed models for longitudinal data. Springer, Berlin Heidelberg New York

Volkow ND, Wang GJ, Fowler JS, Thanos PP, Logan J, Gatley SJ, Gifford A, Ding YS, Wong C, Pappas N (2002) Brain DA D2 receptors predict reinforcing effects of stimulants in humans: replication study. Synapse 46:79–82

Williams D, Williams H (1969) Automaintenance in the pigeon: sustained pecking despite contingent non-reinforcement. J Exp Anal Behav 12:511–520