

A selective role for dopamine in stimulus–reward learning

Shelly B. Fligel^{1*}, Jeremy J. Clark^{2*}, Terry E. Robinson³, Leah Mayo¹, Alayna Czuj³, Ingo Willuhn², Christina A. Akers², Sarah M. Clinton¹, Paul E. M. Phillips² & Huda Akil¹

Individuals make choices and prioritize goals using complex processes that assign value to rewards and associated stimuli. During Pavlovian learning, previously neutral stimuli that predict rewards can acquire motivational properties, becoming attractive and desirable incentive stimuli. However, whether a cue acts solely as a predictor of reward, or also serves as an incentive stimulus, differs between individuals. Thus, individuals vary in the degree to which cues bias choice and potentially promote maladaptive behaviour. Here we use rats that differ in the incentive motivational properties they attribute to food cues to probe the role of the neurotransmitter dopamine in stimulus–reward learning. We show that intact dopamine transmission is not required for all forms of learning in which reward cues become effective predictors. Rather, dopamine acts selectively in a form of stimulus–reward learning in which incentive salience is assigned to reward cues. In individuals with a propensity for this form of learning, reward cues come to powerfully motivate and control behaviour. This work provides insight into the neurobiology of a form of stimulus–reward learning that confers increased susceptibility to disorders of impulse control.

Dopamine is central for reward-related processes^{1,2}, but the exact nature of its role remains controversial. Phasic neurotransmission in the mesolimbic dopamine system is initially triggered by the receipt of reward (unconditional stimulus, US), but shifts to a cue that predicts a reward (conditional stimulus, CS) after associative learning^{3,4}. Dopamine responsiveness appears to encode discrepancies between rewards received and those predicted, consistent with a ‘prediction error’ teaching signal used in formal models of reinforcement learning^{5,6}. Therefore, a popular hypothesis is that dopamine is used to update the predictive value of stimuli during associative learning⁷. In contrast, others have argued that the role of dopamine in reward is in attributing Pavlovian incentive value to cues that signal reward, rendering them desirable in their own right^{8–11}, and thereby increasing the pool of positive stimuli that have motivational control over behaviour. Until now it has been difficult to determine whether dopamine mediates the predictive or the motivational properties of reward-associated cues, because these two features are often acquired together. However, the extent to which a predictor of reward acquires incentive value differs between individuals, providing the opportunity to parse the role of dopamine in stimulus–reward learning.

Individual variation in behavioural responses to reward-associated stimuli can be seen using one of the simplest reward paradigms, Pavlovian conditioning. If a CS is presented immediately before US delivery at a separate location, some animals approach and engage the CS itself and go to the location of food delivery only upon CS termination. This conditional response (CR), which is maintained by Pavlovian contingency¹², is called ‘sign-tracking’ because animals are attracted to the cue or sign that indicates impending reward delivery. However, other individuals do not approach the CS, but during its presentation engage the location of US delivery, even though the US is not available until CS termination. This CR is called ‘goal-tracking’¹³. The CS is an effective predictor in animals that learn either a sign-tracking or a goal-tracking response; it acts as an excitor, evoking a CR

in both. However, only in sign-trackers is the CS an attractive incentive stimulus, and only in sign-trackers is it strongly desired (that is, ‘wanted’), in the sense that animals will work avidly to get it¹⁴. In rats selectively bred for differences in locomotor responses to a novel environment¹⁵, high responders to novelty (bHR rats) consistently learn a sign-tracking CR but low responders to novelty (bLR rats) consistently learn a goal-tracking CR¹⁶. Here, we exploit these predictable phenotypes in the selectively bred rats, as well as normal variation in outbred rats, to probe the role of dopamine transmission in stimulus–reward learning in individuals that vary in the incentive value they assign to reward cues.

Stimulus–reward learning

bHR and bLR rats from the twentieth generation of selective breeding (S20) were used for behavioural analysis of Pavlovian conditional approach behaviour¹⁶ (Fig. 1a–e). When presentation of a lever-CS was paired with food delivery both bHR and bLR rats developed a Pavlovian CR, but as we have described previously¹⁶, the topography of the CR was different in the two groups. With training, bHR rats came to rapidly approach and engage the lever-CS (Fig. 1a, b), whereas upon CS presentation bLR rats came to rapidly approach and engage the location where food would be delivered (Fig. 1c and d; see detailed statistics in Supplementary Information). Both bHR and bLR rats acquired their respective CRs as a function of training, given that there was a significant effect of number of sessions for all measures of sign-tracking behaviour for bHR rats (Fig. 1a, b; $P \leq 0.0001$), and of goal-tracking behaviour for bLR rats (Fig. 1c, d; $P \leq 0.0001$). Furthermore, bHR and bLR rats learned their respective CRs at the same rate, as indicated by analyses of variance in which session was treated as a continuous variable and the phenotypes were directly compared. There were non-significant phenotype \times session interactions for (1) the number of contacts with the lever-CS for bHR rats versus the food-tray for bLR rats ($F_{(1, 236)} = 3.02$, $P = 0.08$) and (2) the latency to approach the lever-CS for bHR rats versus the

¹Molecular and Behavioral Neuroscience Institute, University of Michigan, Michigan, USA. ²Department of Psychiatry and Behavioral Sciences and Department of Pharmacology, University of Washington, Washington, USA. ³Department of Psychology, University of Michigan, Michigan, USA.

*These authors contributed equally to this manuscript.

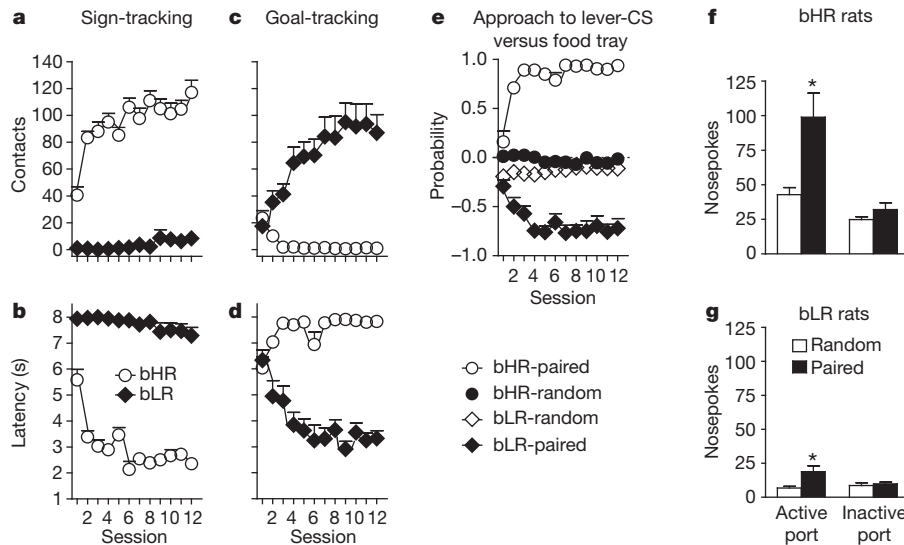


Figure 1 | Development of sign-tracking versus goal-tracking CRs in bHR and bLR rats. Behaviour directed towards the lever-CS (sign-tracking) is shown in **a** and **b** and behaviour directed towards the food-tray (goal-tracking) is shown in **c** and **d** ($n = 10$ per group). Data are shown as mean + s.e.m. **a**, Number of lever-CS contacts made during the 8-s CS period. **b**, Latency to the first lever-CS contact. **c**, Number of food-tray beam breaks during lever-CS presentation. **d**, Latency to the first beam break in the food-tray during lever-CS presentation. For all of these measures (**a–d**) there was a significant effect of phenotype, session, and a phenotype \times session interaction ($P \leq 0.0001$). **e**, Probability of

food-tray for bLR rats ($F_{(1, 236)} = 0.93$, $P = 0.34$). Importantly, rats that received non-contiguous (pseudorandom) presentations of the CS and the US did not learn either a sign-tracking or a goal-tracking CR (Fig. 1e).

These data indicate that the CS acquired one defining property of an incentive stimulus in bHR rats but not bLR rats: the ability to attract. Another feature of an incentive stimulus is to be ‘wanted’ and as such animals should work to obtain it^{10,17}. Therefore, we quantified the ability of the lever-CS to serve as a conditioned reinforcer in the two groups (Fig. 1f, g) in the absence of the food-US. Following Pavlovian training, rats were given the opportunity to perform an instrumental response (a nosepoke) for presentation of the lever-CS. Responses into a port designated ‘active’ resulted in the brief presentation of the lever-CS and responses into an ‘inactive’ port were without consequence. Both conditioned bHR and bLR rats made more active than inactive nose pokes, and more active nose pokes than control groups that received pseudorandom presentations of the CS and the US (Fig. 1f, g; detailed statistics in Supplementary Information). However, the lever-CS was a more effective conditioned reinforcer in bHR rats than in bLR rats, as indicated by a significant phenotype \times group interaction for active nose pokes ($F_{(1, 33)} = 4.82$, $P = 0.04$), which controls for basal differences in nosepoke responding. Moreover, in outbred rats in which this baseline difference in responding does not exist, we have found similar results, indicating that the lever-CS is a more effective conditioned reinforcer for sign-trackers than goal-trackers¹⁴. In summary, the lever-CS was equally predictive, evoking a CR in both groups, but it acquired two properties of an incentive stimulus to a greater degree in bHR rats than bLR rats: it was more attractive, as indicated by approach behaviour (Fig. 1a) and more desirable, as indicated by its ability to serve as a conditioned reinforcer (Fig. 1f, g).

Dopamine signalling during stimulus–reward learning

The core of the nucleus accumbens is an important anatomical substrate for motivated behaviour^{18,19} and has been specifically implicated as a site where dopamine acts to mediate the acquisition and/or performance of Pavlovian conditional approach behaviour^{20–23}. Therefore,

we used fast-scan cyclic voltammetry (FSCV) at carbon-fibre microelectrodes²⁴ to characterize the pattern of phasic dopamine signalling in this region during Pavlovian conditioning (see Supplementary Fig. 1 for recording locations). Similarly to surgically naive animals, bHR rats learned a sign-tracking CR (session effect on lever contacts: $F_{(5, 20)} = 5.76$, $P = 0.002$) and bLR rats learned a goal-tracking CR (session effect on food-receptacle contacts: $F_{(5, 20)} = 5.18$, $P = 0.003$) during neurochemical data collection (Supplementary Fig. 2). Changes in latency during learning were very similar in each group for their respective CRs (main effect of session: $F_{(5, 40)} = 10.5$, $P < 0.0001$; main effect of phenotype: $F_{(1, 8)} = 0.13$, $P = 0.73$; session \times phenotype interaction: $F_{(5, 40)} = 1.16$, $P = 0.35$), indicating that the CS acts as an equivalent predictor of reward in both groups. Therefore, if CS-evoked dopamine release encodes the strength of the reward prediction, as previously postulated^{5–7}, it should increase to a similar degree in both groups during learning; however, if it encodes the attribution of incentive value to the CS, then it should increase to a greater degree in sign-trackers than in goal-trackers. During the acquisition of conditional approach, CS-evoked dopamine release (Fig. 2 and Supplementary Fig. 3) increased in bHR rats relative to unpaired controls (pairing \times session interaction: $F_{(5, 35)} = 4.58$, $P = 0.003$), but there was no such effect in bLR rats (Fig. 2 and Supplementary Fig. 3; pairing \times session interaction: $F_{(5, 35)} = 0.94$, $P = 0.46$). Indeed, the trial-by-trial correlation between CS-evoked dopamine release and trial number was significant for bHR rats ($r^2 = 0.14$, $P < 0.0001$) but not bLR rats ($r^2 = 0.003$, $P = 0.54$), producing significantly different slopes ($P = 0.005$) and higher CS-evoked dopamine release in bHR rats after acquisition (Supplementary Fig. 4, session 6; $P = 0.04$). US-evoked dopamine release also differed between bHR and bLR rats during training (session \times phenotype interaction: $F_{(5, 40)} = 6.09$, $P = 0.0003$), but for this stimulus dopamine release was lower after acquisition in bHR rats (session 6; $P = 0.002$; Supplementary Fig. 4). Collectively, these data highlight that bHR and bLR rats produce fundamentally different patterns of dopamine release in response to reward-related stimuli during learning (see Supplementary Videos 1 and 2). The CS and US signals diverge in bHR rats (stimulus \times session interaction: $F_{(5, 40)} = 5.47$,

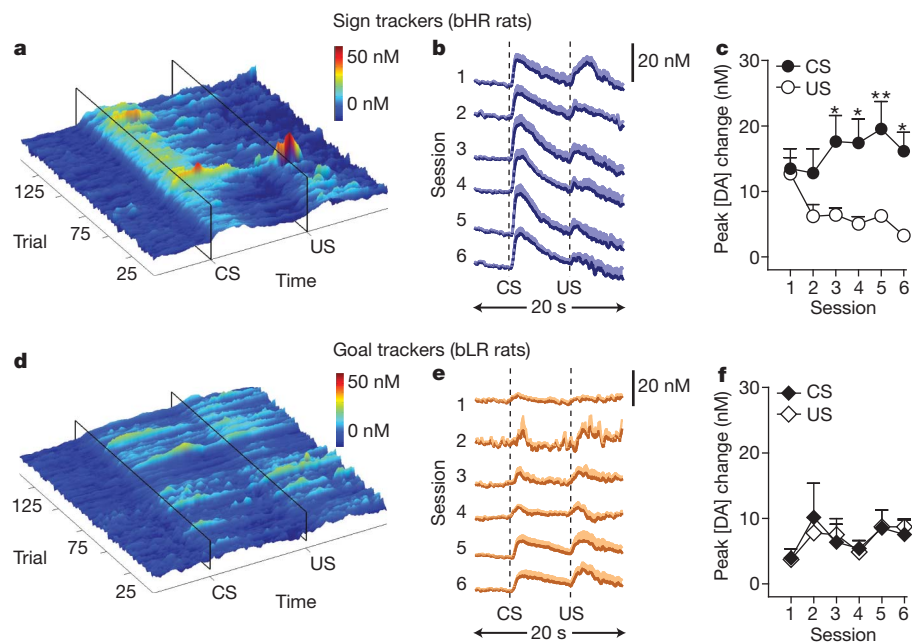


Figure 2 | Phasic dopamine signalling in response to CS and US presentation during the acquisition of Pavlovian conditional approach behaviour in bHR and bLR rats. Phasic dopamine release was recorded in the core of the nucleus accumbens using FSCV over six days of training. **a, d**, Representative surface plots depict trial-by-trial fluctuations in dopamine concentration [DA] during the twenty-second period around CS and US presentation in individual animals throughout training. **b, e**, Change in dopamine concentration (mean + s.e.m.) in response to CS and US presentation for each session of conditioning. **c, f**, Change in peak amplitude (mean + s.e.m.) of the dopamine signal observed in response to CS and US presentation for each session of conditioning ($n = 5$ per group; Bonferroni post-hoc comparison between CS- and US-evoked dopamine release: * $P < 0.05$; ** $P < 0.01$). Panels **a–c** demonstrate that bHR rats, which developed a sign-tracking CR, show increasing phasic dopamine responses to CS presentation and decreasing responses to US presentation across the six sessions of training. In contrast, panels **d–f** demonstrate that bLR rats, which developed a goal-tracking CR, maintain phasic responses to US presentation throughout training.

$P = 0.0006$; Fig. 2c) but not bLR rats (stimulus \times session interaction: $F_{(5, 40)} = 0.28$, $P = 0.92$; Fig. 2f).

Importantly, experiments conducted in commercially obtained outbred rats reproduced the pattern of dopamine release observed in the selectively bred rats (Fig. 3 and Supplementary Fig. 4). Specifically, there was an increase in CS-evoked and a decrease in US-evoked dopamine release during learning in outbred rats that learned a sign-tracking CR (stimulus \times session interaction: $F_{(5, 50)} = 4.43$, $P = 0.002$; Fig. 3d), but not in those that learned a goal-tracking CR (stimulus \times session interaction: $F_{(5, 40)} = 0.48$, $P = 0.72$; Fig. 3f). To test the robustness of these patterns of dopamine release, a subset of outbred rats received extended training. During four additional sessions, the profound differences in dopamine release between sign- and goal-trackers were stable (Supplementary Fig. 5), demonstrating that these differences are not limited to the initial stages of learning. The consistency of these dopamine patterns in selectively bred and outbred rats indicates that they are neurochemical signatures for sign- and goal-trackers rather than an artefact of selective breeding.

Stimulus–reward learning under dopamine blockade

Given the disparate patterns of dopamine signalling observed during learning a sign- versus goal-tracking CR, we tested whether the acquisition and performance of these CRs were differentially dependent on dopamine transmission. Systemic administration of flupenthixol, a nonspecific dopamine receptor antagonist, attenuated performance of the CR for both bHR and bLR rats. This effect was clearly evident when the antagonist was administered during training (Fig. 4, sessions 1–7). It was also observed after the rats had already acquired their respective CR (Supplementary Fig. 6), but this latter finding needs to be interpreted cautiously because of a non-specific effect on activity (Supplementary Fig. 6e). More importantly, when examined off flupenthixol during the eighth test session, bHR rats still failed to demonstrate a sign-tracking CR ($P \leq 0.01$ versus saline, session 8; Fig. 4a–c), indicating that dopamine is necessary for both the performance and the learning of a sign-tracking CR, consistent with previous findings²¹. In contrast, flupenthixol had no effect on learning the CS–US association that lead to a goal-tracking CR ($P \geq 0.6$ versus saline, session 8; Fig. 4d–f), because on the drug-free session bLR rats showed a fully developed goal-tracking CR—their session 8 performance differed significantly from their session 1 performance ($P \leq 0.0002$). Further, they differed from the bLR saline group on session 1 ($P \leq 0.0001$), but did not differ from the bLR saline group on session 8. Thus, whereas dopamine may be

necessary for the performance of both sign-tracking and goal-tracking CRs, it is only necessary for acquisition of a sign-tracking CR, indicating that these forms of learning are mediated by distinct neural systems.

Collectively, these data provide several lines of evidence demonstrating that dopamine does not act as a universal teaching signal in stimulus–reward learning, but selectively participates in a form of stimulus–reward learning whereby Pavlovian incentive value is attributed to a CS. First, US-evoked dopamine release in the nucleus accumbens decreased during training in sign-trackers, but not in goal-trackers. Thus, during the acquisition of a goal-tracking CR, there is not a dopamine-mediated prediction-error teaching signal because, by definition, prediction errors become smaller as delivered rewards become better predicted. Second, the CS evoked dopamine release in both sign- and goal-tracking rats, but this signal increased to a greater degree in sign-trackers, which attributed incentive salience to the CS. These data indicate that the strength of the CS–US association is reflected by dopamine release to the CS only in some forms of stimulus–reward learning. Third, bHR rats that underwent Pavlovian training in the presence of a dopamine receptor antagonist did not acquire a sign-tracking CR, consistent with previous reports⁸; however, dopamine antagonism had no effect on learning a goal-tracking CR in bLR rats. Thus, learning a goal-tracking CR does not require intact dopamine transmission, whereas learning a sign-tracking CR does.

The attribution of incentive salience is the product of previous experience (that is, learned associations) interacting with an individual's genetic propensity and neurobiological state^{8,17,25–27}. The selectively bred rats used in this study have distinctive behavioural phenotypes, including greater behavioural disinhibition and reduced impulse control in bHR rats¹⁶. Moreover, in these lines, unlike in outbred rats^{14,28}, there is a strong correlation between locomotor response to novelty and the tendency to sign-track¹⁶. These behavioural phenotypes are accompanied by baseline differences in dopamine transmission, with bHR rats showing elevated sensitivity to dopamine agonists, increased proportion of striatal D2 receptors in a high-affinity state, greater frequency of spontaneous dopamine transients¹⁶, and higher reward-related dopamine release before conditioning, all of which could enhance their attribution of incentive salience to reward cues^{29,30}. However, basal differences in dopaminergic tone do not provide the full explanation for differences in learning styles and associated dopamine responsiveness. Outbred rats with similar baseline locomotor activity¹⁴ and similar baseline levels of reward-related

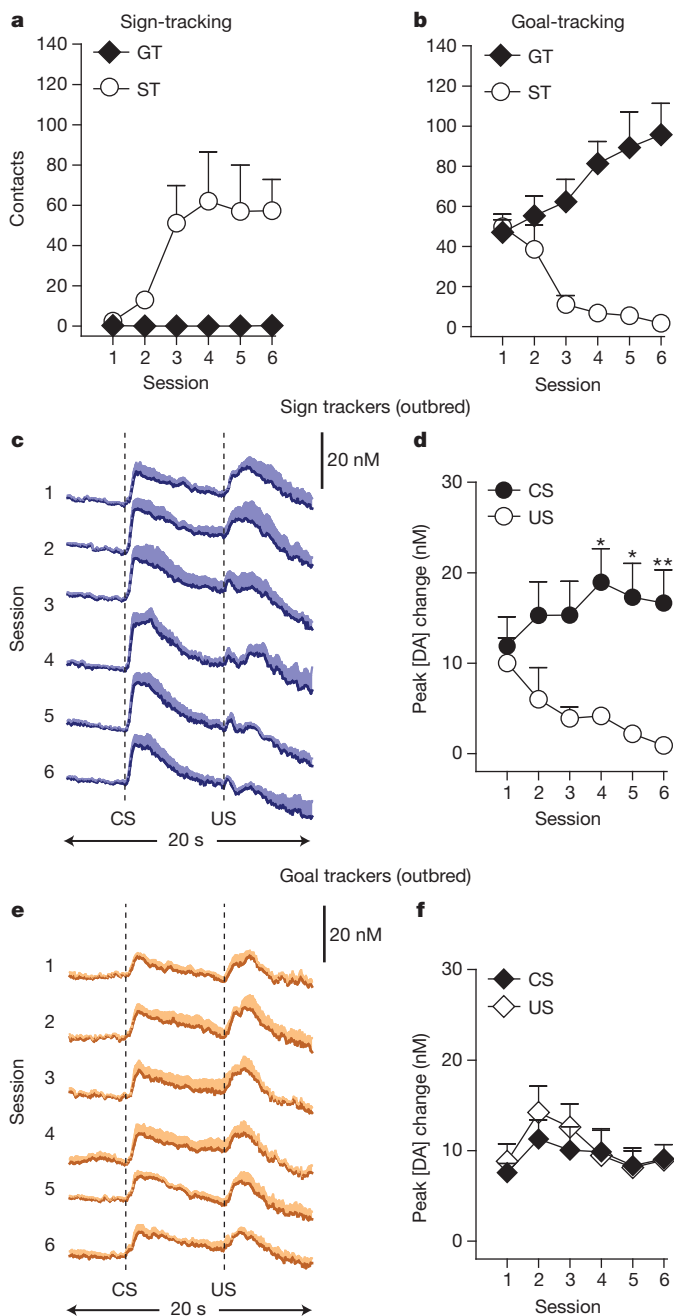


Figure 3 | Conditional responses and phasic dopamine signalling in response to CS and US presentation in outbred rats. Phasic dopamine release was recorded in the core of the nucleus accumbens using FSCV across six days of training. **a, b**, Behaviour directed towards the lever-CS (sign-tracking) (**a**) and behaviour directed towards the food-tray (goal-tracking) (**b**) during conditioning. Learning was evident in both groups because there was a significant effect of session both for rats that learned a sign-tracking response ($n = 6$; session effect on lever contacts: $F_{(5,25)} = 11.85$, $P = 0.0001$) and for rats that learned a goal-tracking response ($n = 5$; session effect on food-receptacle contacts: $F_{(5,20)} = 3.09$, $P = 0.03$). **c, e**, Change in dopamine concentration (mean + s.e.m.) in response to CS and US presentation for each session of conditioning. **d, f**, Change in peak amplitude (mean + s.e.m.) of the dopamine signal observed in response to CS and US presentation for each session of conditioning. (Bonferroni post-hoc comparison between CS- and US-evoked dopamine release: $*P < 0.05$; $**P < 0.01$). Panels **c** and **d** demonstrate that animals developing a sign-tracking CR ($n = 6$) show increasing phasic dopamine responses to CS presentation and decreasing responses to US presentation consistent with bHR rats. Panels **e–f** demonstrate that animals developing a goal-tracking CR ($n = 5$) maintain phasic responses to US presentation consistent with bLR rats.

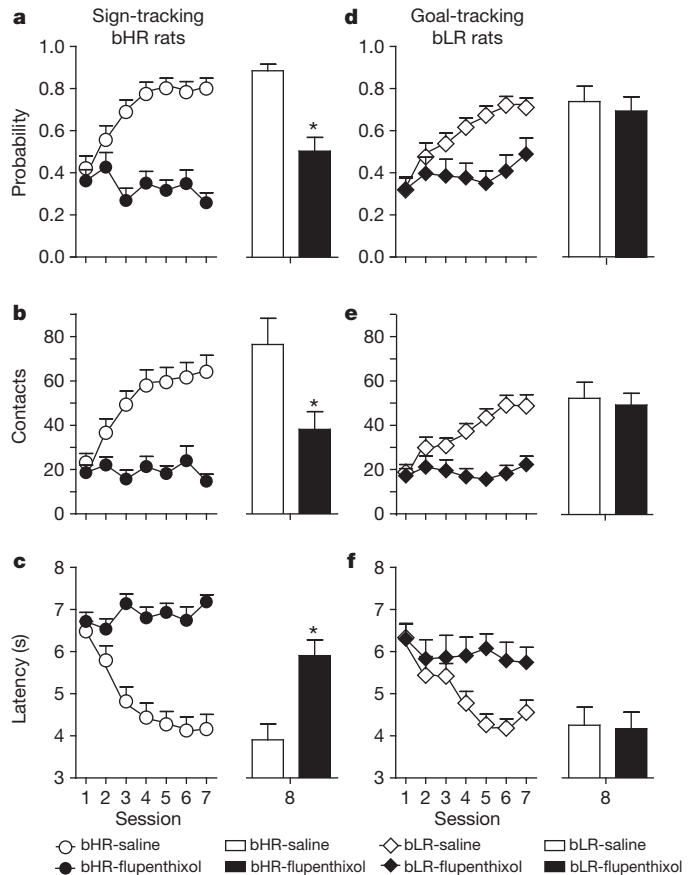


Figure 4 | Dopamine is necessary for learning CS-US associations that lead to sign-tracking, but not goal-tracking. **a–c**, The effects of flupentixol on sign-tracking. **a**, Probability of approaching the lever-CS. **b**, Number of contacts with the lever-CS. **c**, Latency to contact the lever-CS. **d–f**, The effects of flupentixol on goal-tracking. **d**, Probability of approaching the food-tray during lever-CS presentation. **e**, Number of contacts with the food-tray during lever-CS presentation. **f**, Latency to contact the food-tray during lever-CS presentation. Data are expressed as mean + s.e.m. Flupentixol (sessions 1–7) blocked the performance of both sign-tracking and goal-tracking CRs. To determine whether flupentixol influenced performance or learning of a CR, behaviour was examined following a saline injection on session 8 for all rats. bLR rats that were treated with flupentixol before sessions 1–7 ($n = 16$) responded similarly to the bLR saline group ($n = 10$) on all measures of goal-tracking behaviour on session 8, whereas bHR rats treated with flupentixol ($n = 22$) differed significantly from the bHR saline group ($n = 10$) on session 8 ($*P < 0.01$, saline versus flupentixol). Thus, bLR rats learned the CS-US association that produced a goal-tracking CR even though the drug prevented the expression of this behaviour during training. Parenthetically, bHR rats treated with flupentixol did not develop a goal-tracking CR.

dopamine release in the nucleus accumbens (see Fig. 3), differ in whether they are prone to learn a sign-tracking or goal-tracking CR, but they still develop patterns of dopamine release specific to that CR. Therefore, it appears that different mechanisms control basal dopamine neurotransmission versus the unique pattern of dopamine responsiveness to a reward cue.

The neural mechanisms underlying sign- and goal-tracking behaviour remain to be elucidated. Here we have shown that stimulus-reward associations that produce different CRs are mediated by different neural circuitry. Previous research using site-specific dopamine antagonism²¹ and dopamine-specific lesions²² indicated that dopamine acts in the nucleus accumbens core to support the learning and performance of sign-tracking behaviour. This work demonstrates that dopamine-encoded prediction-error signals are indeed present in the nucleus accumbens of sign-trackers, but not in the nucleus accumbens of goal-trackers. Although these neurochemical data alone do not rule out the possibility that prediction-error signals are present in other

dopamine terminal regions, the results from systemic dopamine antagonism demonstrate that intact dopamine transmission is generally not required for learning of a goal-tracking CR.

We thus show that dopamine is an integral part of stimulus–reward learning that is specifically associated with the attribution of incentive salience to reward cues. Individuals who attribute reward cues with incentive salience find it more difficult to resist such cues, a feature associated with reduced impulse control^{16,31}. Human motivated behaviour is subject to a wide span of individual differences ranging from highly deliberative to highly impulsive actions directed towards the acquisition of rewards³². This work provides insight into the biological basis of these individual differences, and may provide an important step for understanding and treating impulse-control problems that are prevalent across several psychiatric disorders.

METHODS SUMMARY

The majority of these studies were conducted with adult male Sprague–Dawley rats from a selective-breeding colony which has been previously described¹⁵. The data presented here were obtained from bHR and bLR rats from generations S18–S22. Equipment and procedures for Pavlovian conditioning have been described in detail elsewhere^{14,16}. Selectively bred rats from generations S18, S20 and S21 were transported from the University of Michigan to the University of Washington for the FSCV experiments. During each behaviour session, chronically implanted microsenors, placed in the core of the nucleus accumbens, were connected to a head-mounted voltammetric amplifier for detection of dopamine by FSCV²⁴. Voltammetric scans were repeated every 100 ms to obtain a sampling rate of 10 Hz. Voltammetric analysis was carried out using software written in LabVIEW (National Instruments). On completion of the FSCV experiments, recording sites were verified using standard histological procedures. To examine the effects of flupenthixol (Sigma; dissolved in 0.9% NaCl) on the performance of sign-tracking and goal-tracking behaviour, rats received an injection (intraperitoneal, i.p.) of 150, 300 or 600 $\mu\text{g kg}^{-1}$ of the drug one hour before Pavlovian conditioning sessions 9, 11 and 13. Doses of the drug were counterbalanced between groups and interspersed with saline injections (i.p., 0.9% NaCl; before sessions 8, 10, 12 and 14) to prevent any cumulative drug effects. To examine the effects of flupenthixol on the acquisition of sign-tracking and goal-tracking behaviour, rats received an injection (i.p.) of either saline or 225 $\mu\text{g kg}^{-1}$ of the drug one hour before Pavlovian conditioning sessions 1–7.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 3 May; accepted 15 October 2010.

Published online 8 December 2010.

- Schultz, W. Behavioral theories and the neurophysiology of reward. *Annu. Rev. Psychol.* **57**, 87–115 (2006).
- Wise, R. A. Dopamine, learning and motivation. *Nature Rev. Neurosci.* **5**, 483–494 (2004).
- Day, J. J., Roitman, M. F., Wightman, R. M. & Carelli, R. M. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nature Neurosci.* **10**, 1020–1028 (2007).
- Schultz, W., Dayan, P. & Montague, P. R. A neural substrate of prediction and reward. *Science* **275**, 1593–1599 (1997).
- Montague, P. R., Dayan, P. & Sejnowski, T. J. A framework for mesencephalic dopamine systems based on predictive Hebbian learning. *J. Neurosci.* **16**, 1936–1947 (1996).
- Waelti, P., Dickinson, A. & Schultz, W. Dopamine responses comply with basic assumptions of formal learning theory. *Nature* **412**, 43–48 (2001).
- Balleine, B. W., Daw, N. D. & O'Doherty, J. P. in *Neuroeconomics: Decision Making and the Brain* (eds Glimcher, P. W., Camerer, C. F., Fehr, E. & Poldrack, R. A.) 367–389 (Academic Press, 2008).
- Berridge, K. C. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology* **191**, 391–431 (2007).
- Berridge, K. C. & Robinson, T. E. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Brain Res. Rev.* **28**, 309–369 (1998).
- Berridge, K. C., Robinson, T. E. & Aldridge, J. W. Dissecting components of reward: 'liking', 'wanting', and learning. *Curr. Opin. Pharmacol.* **9**, 65–73 (2009).
- Panksepp, J. Affective consciousness: core emotional feelings in animals and humans. *Conscious. Cogn.* **14**, 30–80 (2005).

- Hearst, E. & Jenkins, H. *Sign-Tracking: The Stimulus-Reinforcer Relation and Directed Action* (Monograph of the Psychonomic Society, 1974).
- Boakes, R. in *Operant-Pavlovian Interactions* (eds Davis, H. & Hurwitz, H. M. B.) 67–97 (Erlbaum, 1977).
- Robinson, T. E. & Flagel, S. B. Dissociating the predictive and incentive motivational properties of reward-related cues through the study of individual differences. *Biol. Psychiatry* **65**, 869–873 (2009).
- Stead, J. D. et al. Selective breeding for divergence in novelty-seeking traits: heritability and enrichment in spontaneous anxiety-related behaviors. *Behav. Genet.* **36**, 697–712 (2006).
- Flagel, S. B. et al. An animal model of genetic vulnerability to behavioral disinhibition and responsiveness to reward-related cues: implications for addiction. *Neuropsychopharmacology* **35**, 388–400 (2010).
- Berridge, K. C. in *Psychology of Learning and Motivation* (ed. Medin, D. L.) 223–278 (Academic Press, 2001).
- Cardinal, R. N., Parkinson, J. A., Hall, J. & Everitt, B. J. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Neurosci. Biobehav. Rev.* **26**, 321–352 (2002).
- Kelley, A. E. Functional specificity of ventral striatal compartments in appetitive behaviors. *Ann. NY Acad. Sci.* **877**, 71–90 (1999).
- Dalley, J. W. et al. Time-limited modulation of appetitive Pavlovian memory by D1 and NMDA receptors in the nucleus accumbens. *Proc. Natl Acad. Sci. USA* **102**, 6189–6194 (2005).
- Di Ciano, P., Cardinal, R. N., Cowell, R. A., Little, S. J. & Everitt, B. J. Differential involvement of NMDA, AMPA/kainate, and dopamine receptors in the nucleus accumbens core in the acquisition and performance of pavlovian approach behavior. *J. Neurosci.* **21**, 9471–9477 (2001).
- Parkinson, J. A. et al. Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behaviour: implications for mesoaccumbens dopamine function. *Behav. Brain Res.* **137**, 149–163 (2002).
- Parkinson, J. A., Olmstead, M. C., Burns, L. H., Robbins, T. W. & Everitt, B. J. Dissociation in effects of lesions of the nucleus accumbens core and shell on appetitive pavlovian approach behavior and the potentiation of conditioned reinforcement and locomotor activity by D-amphetamine. *J. Neurosci.* **19**, 2401–2411 (1999).
- Clark, J. J. et al. Chronic microsenors for longitudinal, subsecond dopamine detection in behaving animals. *Nature Methods* **7**, 126–129 (2010).
- Robinson, T. E. & Berridge, K. C. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res. Brain Res. Rev.* **18**, 247–291 (1993).
- Tindell, A. J., Smith, K. S., Berridge, K. C. & Aldridge, J. W. Dynamic computation of incentive salience: "wanting" what was never "liked". *J. Neurosci.* **29**, 12220–12228 (2009).
- Zhang, J., Berridge, K. C., Tindell, A. J., Smith, K. S. & Aldridge, J. W. A neural computational model of incentive salience. *PLOS Comput. Biol.* **5**, e1000437 (2009).
- Beckmann, J. S., Marusch, J. A., Gipson, C. D. & Bardo, M. T. Novelty seeking, incentive salience and acquisition of cocaine self-administration in the rat. *Behav. Brain Res.* **216**, 159–165 (2011).
- Wyvell, C. L. & Berridge, K. C. Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: enhancement of reward "wanting" without enhanced "liking" or response reinforcement. *J. Neurosci.* **20**, 8122–8130 (2000).
- Wyvell, C. L. & Berridge, K. C. Incentive sensitization by previous amphetamine exposure: increased cue-triggered "wanting" for sucrose reward. *J. Neurosci.* **21**, 7831–7840 (2001).
- Tomie, A., Aguado, A. S., Pohorecky, L. A. & Benjamin, D. Ethanol induces impulsive-like responding in a delay-of-reward operant choice procedure: impulsivity predicts autoshaping. *Psychopharmacology* **139**, 376–382 (1998).
- Kuo, W. J., Sjoström, T., Chen, Y. P., Wang, Y. H. & Huang, C. Y. Intuition and deliberation: two systems for strategizing in the brain. *Science* **324**, 519–522 (2009).

Supplementary Information. is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements This work was supported by National Institutes of Health grants: R01-MH079292 (to P.E.M.P.), R01-DA027858 (to P.E.M.P.), T32-DA07278 (to J.J.C.), F32-DA24540 (to J.J.C.), R37-DA04294 (to T.E.R.), and 5P01-DA021633-02 (to T.E.R. and H.A.). The selective breeding colony was supported by a grant from the Office of Naval Research to H.A. (N00014-02-1-0879). We thank K. Berridge and J. Morrow for comments on earlier versions of the manuscript, and S. Ng-Evans for technical support.

Author Contributions S.B.F., J.J.C., T.E.R., P.E.M.P. and H.A. designed the experiments and wrote the manuscript. S.B.F., J.J.C., L.M., A.C., I.W. and C.A.A. conducted the experiments, S.M.C. oversaw the selective breeding colony, and S.B.F. and J.J.C. analysed the data.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to P.E.M.P. (pemp@uw.edu) or H.A. (akil@umich.edu).

METHODS

Animals. Adult male Sprague-Dawley rats selectively bred for reactivity to a novel environment were used for the majority of these studies¹⁵. The data presented here were obtained from bHR and bLR rats from generations S18 to S22. The experiments followed the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioural Research (National Research Council 2003) and the procedures were approved by the University Committee on the Use and Care of Animals. Unless otherwise indicated, rats were housed in pairs and kept on a 12-h light/12-h dark cycle (lights on 06:00 h) with controlled temperature and humidity and food and water were available *ad libitum*.

Voltammetry studies were conducted at the University of Washington using bHR and bLR rats from generations S18, S20 and S21 as well as male Sprague-Dawley rats obtained from Charles River weighing between 300 g and 350 g upon arrival. These rats were housed individually and kept on a 12-h light/12-h dark cycle (lights on at 0700) with controlled temperature and humidity. Prior to behavioural training, food was restricted so that rats maintained 90% of their free-feeding body weight and water was available *ad libitum*. All animal procedures followed the University of Washington Institutional Animal Care and Use Committee guidelines.

Screening for selectively bred phenotypes. To confirm the selectively bred phenotypes, each generation of rats were screened for locomotor activity in novel test chambers at around 60 days of age, as previously described^{15,33}.

Pavlovian conditioning procedures. Equipment and procedures for Pavlovian conditioning have been described in detail elsewhere^{14,16}. Briefly, standard Med Associates test chambers were equipped with a food-tray located in the middle of the front wall and a retractable lever located to the left or right of the food-tray (counterbalanced). The lever required only a 10-g force to operate, such that most contacts with the lever were detected and recorded as a 'lever press'. Operation of the pellet dispenser (Med Associates) delivered one 45-mg banana-flavoured food pellet (Bio-Serv) into the food-tray. Head entries into the food-tray were recorded each time the rat broke a photobeam located inside the receptacle.

All Pavlovian training sessions were conducted between 13:00 h and 18:00 h. Banana-flavoured food pellets were placed into the rats' home cages for 2 days before training to familiarize the animals with this food (the unconditioned stimulus, US). Two pre-training sessions were conducted that consisted of the delivery of 50 food pellets, which were randomly delivered on a variable-interval 30-s schedule (25-min session), during which it was determined whether the rats were reliably retrieving the food pellets. Following pre-training sessions, Pavlovian training sessions consisted of the presentation of the illuminated lever (conditioned stimulus, CS) in the chamber for 8 s, and then immediately upon its retraction a 45-mg food pellet (US) was delivered into the food-tray (the 'goal'). The CS was presented on a random-interval 90-s schedule and each Pavlovian training session consisted of 25 trials (or CS-US pairings). Training continued for 6–12 sessions. Rats in the 'random' groups received presentations of the CS and US, each on a variable-interval 90-s schedule.

The following events were recorded using Med Associates software: (1) the number of lever-CS contacts, (2) the latency to the first lever-CS contact, (3) the number of food-tray entries during lever-CS presentation, and (4) the latency to the first food-tray entry during lever-CS presentation. It is important to note that no response is required for the rat to receive the reward (US), yet distinct CRs emerge as a result of Pavlovian conditioning. The outcome measures listed above allow us to examine CS-directed (sign-tracking) versus goal-directed (goal-tracking) responses. Using these measures we calculated the probability that a rat would approach the lever-CS or the food-tray as well as the difference in its probability of approaching the lever-CS versus the food-tray.

Statistical analysis of Pavlovian conditional responses. Differences in the conditional response that emerged across training sessions were analysed using linear mixed effects models (SPSS 17.0; see also ref. 34), in which phenotype and session were treated as independent variables. In addition, the effect of session for each phenotype was analysed separately. For all analyses, the covariance structure was explored and modelled appropriately. When significant main effects or interactions were detected, Bonferroni post-hoc comparisons were made. The differences in the probability of approaching the lever-CS versus the food-tray (Fig. 1e) were further examined using one-sample *t*-tests (with hypothesized value of 0) to determine whether either phenotype exhibited a preference for the lever-CS or the food-tray.

Conditioned reinforcement test. The conditioned reinforcement test occurred one day after the last of 12 Pavlovian training sessions. The conditioned reinforcement test was conducted in the same standard Med Associates chambers as described above. However, for the purposes of this test the chambers were rearranged such that the retractable lever was placed in the centre of the front wall in between two nosepoke ports. The 'active' port was placed on the side of the wall opposite to the location of the lever-CS during Pavlovian training. During the

40-min conditioned reinforcement test nosepokes into the port designated 'active' resulted in the 2-s presentation of the illuminated lever, whereas pokes into the other 'inactive' port were without consequence. The number of nosepokes into the active and inactive ports and the number of contacts with the lever were recorded throughout the test session.

Statistical analysis of conditioned reinforcement. Performance on the conditioned reinforcement test was analysed using a three-way analysis of variance (ANOVA) in which phenotype, group (paired versus unpaired) and port (active versus inactive) were treated as independent variables and the number of pokes as the dependent variable. Further analyses were then conducted to determine the effect of group or port for each phenotype and the effect of phenotype or group for each port. **FSCV.** The following procedures were in accordance with the University of Washington Institutional Animal Care and Use Committee guidelines. Surgical preparation for *in vivo* voltammetry used an aseptic technique. Rats were anaesthetized with isoflurane and placed in a stereotaxic frame. The scalp was swabbed with 10% povidone iodine, bathed with a mixture of lidocaine (0.5 mg kg⁻¹) and bupivacaine (0.5 mg kg⁻¹), and incised to expose the cranium. Holes were drilled and cleared of dura mater above the nucleus accumbens core (1.3-mm lateral and 1.3-mm rostral from the bregma), and at convenient locations for a reference electrode and three anchor screws. The reference electrode and anchor screws were positioned and secured with cranioplastic cement, leaving the working electrode holes exposed. Once the cement cured, the microsensors were attached to the voltammetric amplifier and lowered into the target recording regions (the core of the nucleus accumbens, 7.0-mm ventral of dura mater). Finally, cranioplastic cement was applied to the part of the cranium still exposed to secure the working electrode.

Voltammetric measurement. During all experimental sessions, chronically implanted microsensors were connected to a head-mounted voltammetric amplifier for dopamine detection by FSCV²⁴. Voltammetric scans were repeated every 100 ms to obtain a sampling rate of 10 Hz. When dopamine is present at the surface of the electrode during a voltammetric scan, it is oxidized during the anodic sweep to form dopamine-*o*-quinone (peak reaction at approximately +0.7 V), which is reduced back to dopamine in the cathodic sweep (peak reaction at approximately -0.3 V). The ensuing flux of electrons is measured as current and is directly proportional to the number of molecules that undergo the electrolysis. The redox current obtained from each scan provides a chemical signature that is characteristic of the analyte, allowing resolution of dopamine from other substances. For quantification of changes in dopamine concentration over time, the current at its peak oxidation potential can be plotted for successive voltammetric scans. Waveform generation, data acquisition and analysis were carried out on a PC-based system using two PCI multifunction data acquisition cards and software written in LabVIEW (National Instruments).

Statistical analysis of voltammetry data. Voltammetric data analysis was carried out using software written in LabVIEW (National Instruments) and low-pass filtered at 2,000 Hz. Dopamine was isolated from the voltammetric signal with chemometric analysis³⁵ using a standard training set based upon stimulated dopamine release detected by chronically implanted electrodes. Dopamine concentration was estimated on the basis of the average post-implantation sensitivity of electrodes²⁴. Before the generation of surface plots and analysis of peak values, all data were smoothed with a 5-point within-trial running average. Peak dopamine values in response to the US and CS were obtained by taking the largest value in the 3-s period after stimulus presentation. Peak values were then compared using mixed models ANOVA with training session as the repeated measure and stimulus (CS and US) or phenotype (bHR and bLR) as the between-group measure. Peak CS-evoked dopamine signalling was also analysed across trials using linear regression. The slopes obtained for the regression were compared between groups using independent, two-sample *t*-tests. All post-hoc comparisons were made with the Bonferroni correction for multiple tests. All statistical analyses were carried out using Prism (GraphPad Software). Voltammetric data for dopamine responses to the CS and US were also analysed using an area-under-the-curve approach. This approach did not alter the statistical effects of any comparison reported in the paper for peak dopamine value (specific statistical results not shown).

Histological verification of recording site. On completion of experimentation, animals were anesthetized with intraperitoneal ketamine (100 mg kg⁻¹) and xylazine (20 mg kg⁻¹) and then transcardially perfused with saline followed by 4% paraformaldehyde. Brains were removed and post-fixed in paraformaldehyde for 24 h and then rapidly frozen in an isopentane bath (~5 min), sliced on a cryostat (50- μ m coronal sections, 20 °C) and stained with cresyl violet to aid in visualization of anatomical structures.

Effects of flupenthixol on sign-tracking and goal-tracking performance. The effects of flupenthixol (a D1/D2 antagonist; Sigma) on the performance of sign-tracking and goal-tracking behaviour were examined after seven sessions of Pavlovian conditioning. All rats received an injection (i.p.) of 150, 300 or

600 $\mu\text{g kg}^{-1}$ of the drug one hour before Pavlovian conditioning sessions 9, 11 and 13. Doses of the drug (dissolved in 0.9% NaCl) were counterbalanced between groups and interspersed with saline injections (i.p., 0.9% NaCl; before sessions 8, 10, 12 and 14) to prevent any cumulative drug effects. The following measures were recorded to examine the effects of the drug on the CR: (1) the number of lever-CS contacts, (2) the latency to the first lever-CS contact, (3) the number of food-tray entries during lever-CS presentation, and (4) the latency to the first food-tray entry during lever-CS presentation. In addition, a nosepoke port was added to the test chamber on the wall opposite the retractable lever and responses into this port were recorded as an index of nonspecific activity. For all measures the response to saline was averaged (across sessions 8, 10, 12 and 14) and compared to the response following each of the three doses of flupenthixol.

Statistical analysis of effects of flupenthixol on performance of the CRs. The effects of flupenthixol on the performance of sign-tracking and goal-tracking behaviour (Supplementary Fig. 6) were analysed using linear mixed effects models with phenotype and dose treated as independent variables. Each phenotype was also analysed separately to determine the effect of dose on a given behaviour and Bonferroni post-hoc comparisons were made to determine whether behaviour at a given dose was significantly different from that in response to saline.

Effects of flupenthixol on the learning of sign-tracking and goal-tracking. The effects of flupenthixol on the acquisition of sign-tracking and goal-tracking CRs were examined using two generations of bred rats (S21 and S22). Rats received an injection of either saline (i.p.; 0.9% NaCl) or 225 $\mu\text{g kg}^{-1}$ of flupenthixol one hour before Pavlovian conditioning sessions 1–7. This dose of drug was chosen based on the ‘performance’ study described above, because we wanted to avoid any nonspecific inhibitory effects on motor activity. Rats from both generations that received flupenthixol before sessions 1–7 then received an injection of saline before session 8. However, only rats from the S22 generation that received saline

before sessions 1–7 also received saline before session 8. Thus, the number of rats that received saline during training and were also pretreated with saline before session 8 is lower than that for the other groups (that is, on session 8, bHR saline, $n = 10$; bLR saline, $n = 10$). The following measures were recorded and analysed to examine the effects of flupenthixol on sign-tracking and goal-tracking behaviour: (1) the number of lever-CS contacts, (2) the latency to the first lever-CS contact, (3) the number of food-tray entries during lever-CS presentation, and (4) the latency to the first food-tray entry during lever-CS presentation.

Statistical analysis of effects of flupenthixol on the learning of the CRs. Linear mixed effects models were used to examine the effects of flupenthixol on the performance and learning of sign-tracking or goal-tracking behaviour (Supplementary Fig. 6). For these analyses each phenotype was analysed separately to determine the effect of dose on a given behaviour and treatment (saline versus flupenthixol) and session (1–7) were treated as independent variables. To determine whether flupenthixol prevented the expression of the conditioned response or the learning of a conditioned response we also examined behaviour following a saline injection on session 8 (drug-free test session). Behaviour on session 8 was compared between treatment groups using an unpaired *t*-test for each phenotype separately. We also compared the response on session 8 of the groups that received flupenthixol during training to that of the group that received flupenthixol on session 1 (using a paired *t*-test) and to that of the saline control group on session 1 (using an unpaired *t*-test).

33. Clinton, S. M, *et al.* Individual differences in novelty-seeking and emotional reactivity correlate with variation in maternal behavior. *Horm. Behav.* **51**, 655–664 (2007).
34. Verbeke, G. & Molenberghs, G. *Linear Mixed Models for Longitudinal Data* (Springer, 2000).
35. Heien, M. L. Johnson, M. A. & Wightman, R. M. Resolving neurotransmitters detected by fast-scan cyclic voltammetry. *Anal. Chem.* **76**, 5697–5704 (2004).