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RESULTS

Development of sign-tracking vs. goal-tracking CRs in bHR and bLR animals, respectively. To further examine the emergent CR for bHR and bLR rats, we compared the difference in the probability to approach the lever-CS vs. the food-tray during lever-CS presentation (i.e. lever-food-tray difference; Fig. 1e; see also[1,2]). If a rat came into contact with the lever-CS on all 25 trials in a session and never made an entry into the food-tray it received a score of +1. A score of zero indicates that neither approach to the lever-CS nor approach to the food-tray was dominant. bHR rats preferably approached the lever-CS and bLR rats exhibited a preference for goal-directed approach (effect of phenotype, $F_{(1,18)}$=676.47, P≤0.0001; effect of session, $F_{(1,18)}$=5.14, P=0.001; phenotype x session interaction, $F_{(11,18)}$=17.39, P≤0.0001). In support, bHR rats had a lever-tray difference score significantly >0 for sessions 2-12 (P≤0.0001) and bLR rats had a score significantly <0 for all sessions (P≤0.001). Rats that received pseudorandom presentations of the lever-CS and the US (bHR, n=10; bLR, n=10) did not exhibit a clear preference for either the lever-CS or the food-tray. bLR rats in the random group showed a slight preference for the food-tray across training sessions, but these animals did not show evidence in support of learning this response. It is possible that the preference for the food-tray for the bLR-random group is a result of "pre-training" since all of the rats were required to retrieve pellets from the food-tray during these sessions.

The lever-CS is a more effective conditioned reinforcer in bHR than bLR rats. Selectively-bred rats from the S18 generation were used to assess the conditioned reinforcing properties of the lever-CS (Fig. 1f-g). The conditioned reinforcement test was conducted following 12 Pavlovian training sessions where rats received either paired CS-US presentations (bHRs, n=10; bLRs, n=9) or pseudorandom presentations of the CS and US (bHRs, n=9; bLRs, n=9). As in Experiment 1, bHR rats developed a sign-tracking CR and bLR rats a goal-tracking CR. The data are qualitatively similar to those shown in Fig. 1a-e and therefore are not shown. Neither bHR nor bLR rats given pseudorandom CS-US pairings developed a CR.

The number of nosepokes into the active and inactive ports during the conditioned reinforcement test were analyzed. There was a significant main effect of phenotype ($F_{(1,66)}$=54.02, P<0.0001), a significant effect of group (paired vs. unpaired; $F_{(1,66)}$=13.18, P=0.0006), a significant effect of port ($F_{(1,66)}$=19.11, P<0.0001), and significant two-way interactions (i.e. phenotype x group, $F_{(1,66)}$=5.68, P=0.02; phenotype x port, $F_{(1,66)}$=13.65, P=0.0004; group x port, $F_{(1,66)}$=8.0, P=0.006). bHRs responded more on both the active and inactive nose ports relative to bLRs (P≤0.0001). bHRs in the paired group also made significantly more nose pokes into the active port than bHRs in the random group ($F_{(1,17)}$=8.60, P=0.01). Likewise, bLRs in the paired group responded more on the active port relative to bLRs in the random group ($F_{(1,16)}$=6.99, P=0.02), but the magnitude of this difference was greater for bHRs compared to bLRs as indicated by a significant phenotype x group interaction for active nose pokes ($F_{(1,33)}$=4.82, P=0.04).

Phasic dopamine signals in bHR and bLR rats that received unpaired CS-US presentations. FSCV was conducted on selectively-bred rats (n=4/phenotype) from the S20 and S21 generations that received pseudorandom CS-US presentations. As previously described (see [2] and Fig. 1e), bred rats in the "random" groups did not show evidence of learning a CR. The change in peak amplitude of phasic dopamine release in response to the unpaired presentation of the CS and US were first analyzed separately for bHR and bLR rats. The comparison of CS-evoked and US-evoked phasic dopamine release across sessions in bHR rats revealed a significant main effect of stimulus ($F_{(1,30)}$=16.79, P=0.006). Post-hoc tests confirmed that US-evoked phasic dopamine release remained elevated across sessions and was significantly higher than CS-evoked release by the fourth session for bHRs (P<0.05; Fig. S3b). The comparison of CS-evoked and US-evoked phasic dopamine release across sessions in bLR rats also revealed a significant main effect of stimulus ($F_{(1,30)}$=50.59, P=0.0004). Post-hoc tests confirmed that US-evoked phasic dopamine release remained elevated across sessions and was significantly higher than CS-evoked release for sessions 2, 5 and 6 for bLRs (P<0.05; Fig. S3d). Thus, contrary to the finding of a

differential pattern of phasic dopamine activity during learning between bHR and bLR rats, their dopamine responses to the presentation of stimuli that do not favor learning showed the same profile.

The effects of flupenthixol on the *performance* of sign-tracking and goal-tracking behaviors after acquiring the CR. The effects of flupenthixol on the *performance* of sign-tracking and goal-tracking behaviors were examined following 7 days of Pavlovian training in bHR (n=14) and bLR rats (n=14) from the S21 generation (Fig. S6). The emergent behavior during Pavlovian training was qualitatively similar to that described above (and is therefore not shown). The effects of flupenthixol on sign-tracking behavior were examined using the measures of contact with the lever-CS (Fig. S6a) and latency to contact the lever-CS (Fig. S6b) and the effects on goal-tracking behavior were examined using the measures of contact with the food-tray (Fig. S6c) and latency to contact the food-tray (Fig. S6d). For all of these measures there was a significant effect of phenotype ($P \leq 0.0001$), dose ($P \leq 0.01$) and a phenotype x dose interaction ($P \leq 0.04$). On measures of sign-tracking behavior there was a significant effect of dose for bHR rats ($P \leq 0.02$), but not bLR rats; whereas, on measures of goal-tracking behavior we found a significant effect of dose for bLR rats ($P \leq 0.01$), but not bHR rats. Flupenthixol significantly attenuated performance of both CRs at doses of 300 and 600 µg/kg relative to saline. However, there was also an effect of flupenthixol on nonspecific behavior (Fig. S6e; i.e. nosepokes into a random port; effect of phenotype ($F_{(1,26)}=24.28$, $P<0.0001$), effect of dose ($F_{(3,26)}=14.03$, $P<0.0001$), phenotype x dose interaction ($F_{(3,26)}=4.62=0.01$)), with the biggest effect at the highest dose examined (600 µg/kg). Thus, although these data suggest a dose-specific attenuation of both sign-tracking and goal-tracking behavior in response to a D1/D2 antagonist, the results should be interpreted with caution given the effects of the drug on nonspecific activity.

The effects of flupenthixol on the *learning* of sign-tracking and goal-tracking behaviors. To determine whether dopamine is necessary for *learning* a Pavlovian CR, bred rats from the S21 and S22 generations were administered flupenthixol (or saline) one hour prior to each of 7 training sessions. bHR

rats that received saline prior to each training session developed a sign-tracking CR (Fig. 4a-c) and bLR rats that received saline developed a goal-tracking CR (Fig. 4d-f). Flupenthixol (sessions 1-7) blocked the development of these CRs for both bHRs and bLRs. For bHRs there was an effect of treatment ($P \leq 0.0001$), an effect of session ($P < 0.0001$), and a treatment x session interaction ($P \leq 0.0001$) on all measures of sign-tracking behavior (Fig. 4a-c) and for bLRs there was a significant effect of treatment ($P \leq 0.003$) and session ($P \leq 0.01$) on all measures of goal-tracking behavior (Fig. 4d-f) and a significant treatment x session interaction ($P = 0.02$) for the number of food-tray contacts (Fig. 4e). To determine whether flupenthixol prevented *learning* of a CR, behavior was examined following a saline injection on session 8 for all rats. On session 8 (drug-free test session), the response of bHR rats that were treated with flupenthixol during training was similar to that of bHR rats in the same group on session 1 ($P \geq 0.06$) and to that of bHR rats that received saline on session 1 ($P > 0.10$) for all measures.

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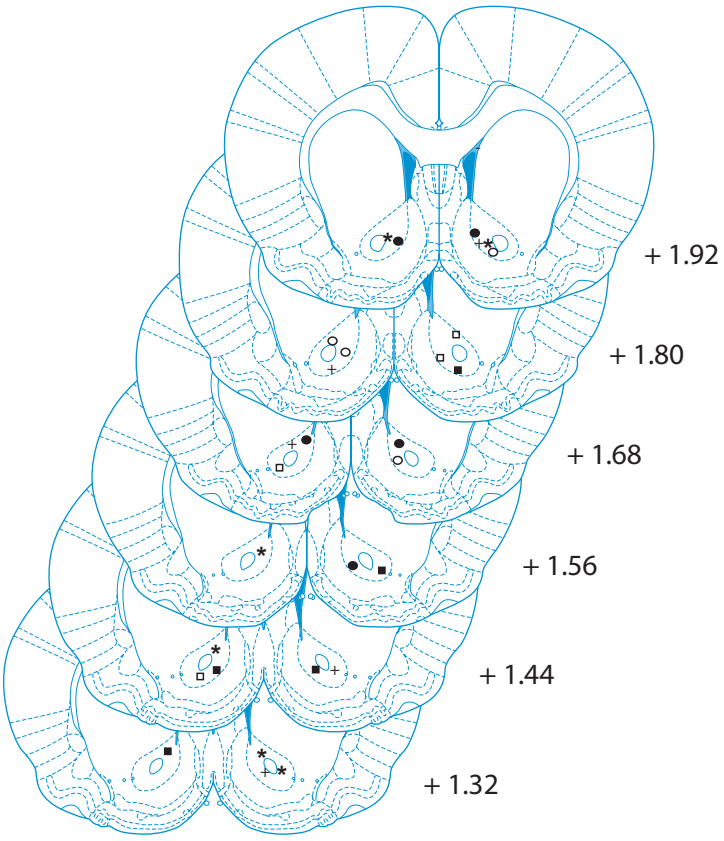

Figure S1: Histological verification of recording sites. All recording sites were confirmed to be within the nucleus accumbens core. The numbers on each plate indicate distance in millimeters anterior from bregma[3]. Each experimental group is denoted by a unique symbol: bHR paired (●); bHR unpaired (○); bLR paired (■); bLR unpaired (□); outbred sign-trackers (*); outbred goal-trackers (+).

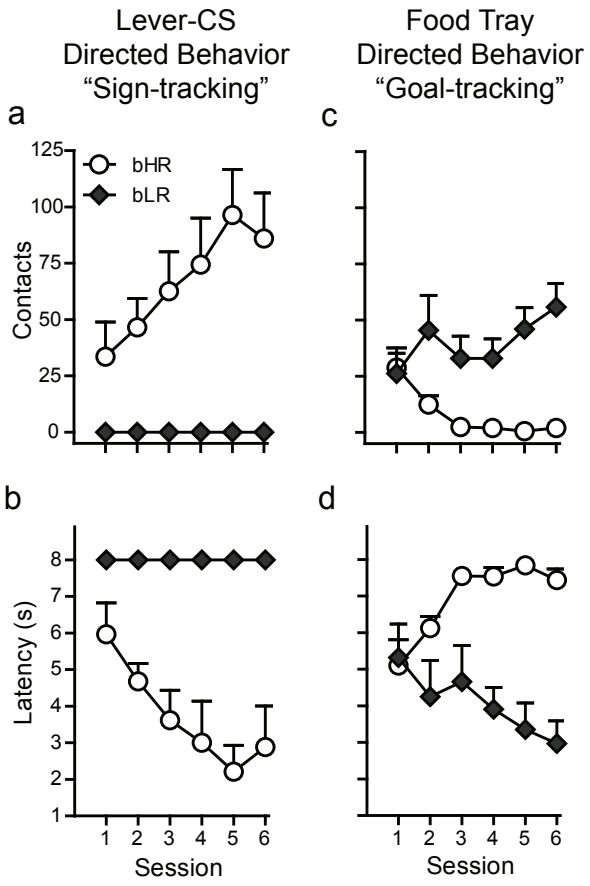

Figure S2: Development of sign-tracking vs. goal-tracking CRs in bHR and bLR animals, respectively, that were used for FSCV. Measures of sign-tracking behavior are shown in panels a-b and goal-tracking behavior in panels c-d (n=5/phenotype). Mean + SEM **(a)** number of lever-CS contacts made during 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. Learning was evident in both groups as there was a significant effect of session for all measures of sign-tracking behavior for bHRs (session effect on lever contacts: $F_{(5,20)} = 5.76$, $P = 0.002$; session effect for latency to contact: $F_{(5,20)} = 7.00$, $P = 0.0006$) and for all measures of goal-tracking behavior for bLRs (session effect on food-receptacle contacts: $F_{(5,20)} = 5.18$, $P = 0.003$; session effect for latency to contact: $F_{(5,20)} = 4.21$, $P = 0.009$.

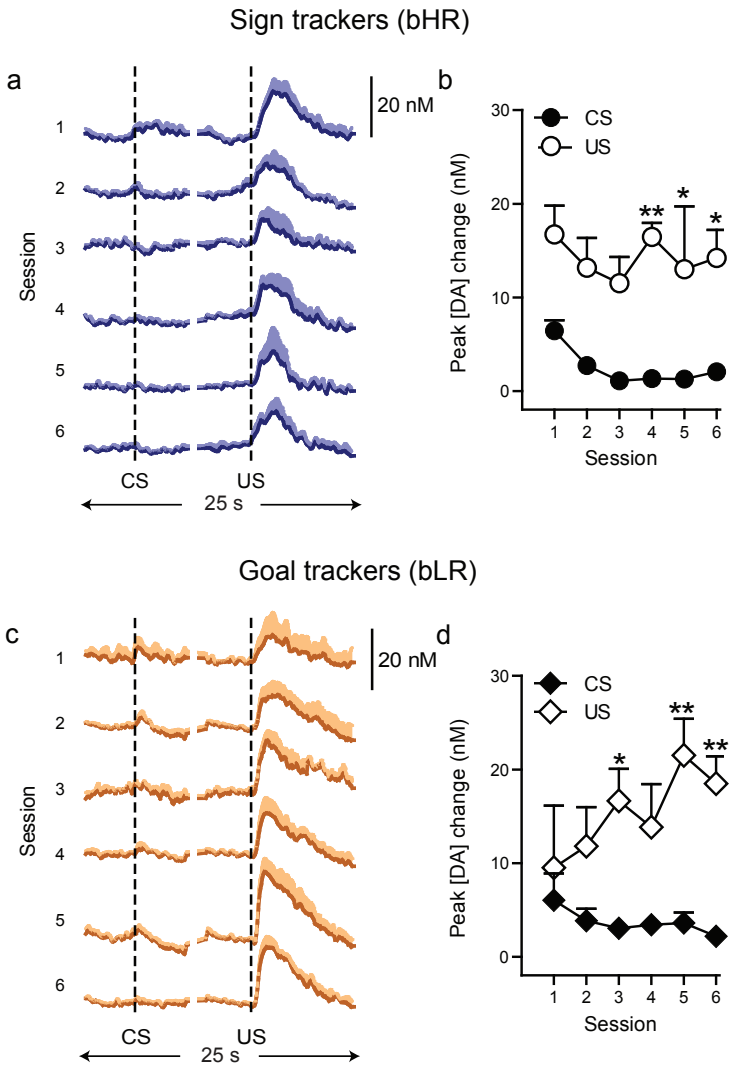

Figure S3: Phasic dopamine release in response to the CS and US when the stimuli are presented pseudorandomly during training. Phasic dopamine release was recorded from the core of the nucleus accumbens in bHR and bLR rats that received unpaired presentations of the CS and US (n=4/phenotype). **(a, c)** Mean + SEM change in dopamine concentration in response to CS and US presentation for all sessions. Dopamine response to the US was maintained across sessions when animals received unpaired CS-US presentations. **(b, d)** Mean + SEM change in peak amplitude of the dopamine signal observed in response to CS and US presentation for each session of conditioning (Bonferroni post-hoc comparisons of CS- and US-evoked dopamine release *P<0.05, **P<0.01).

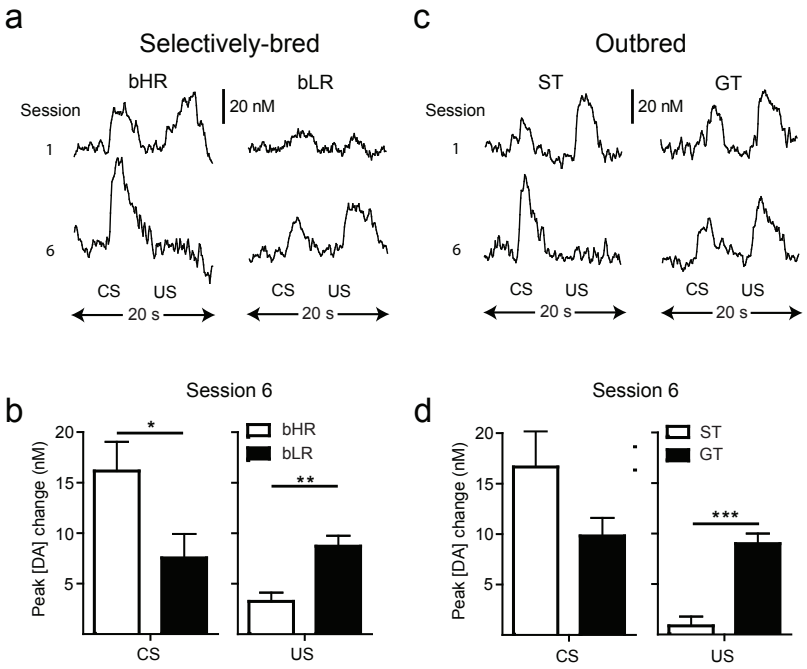

Figure S4: Phasic dopamine release in response to the CS and US before and after learning in selectively-bred and outbred rats. Phasic dopamine release was recorded from the core of the nucleus accumbens in selectively-bred and outbred rats that received paired presentations of the CS and US. **(a)** Representative traces recorded during sessions one and six from bHR and bLR rats. **(c)** Representative traces recorded during sessions one and six from outbred animals classified as sign-trackers and goal-trackers. **(b)** Mean + SEM change in peak amplitude of the dopamine signal observed in response to CS and US presentation during the last session of training in bHR and bLR rats (n=5/phenotype; *P<0.05, **P<0.01). **(d)** Mean + SEM change in peak amplitude of the dopamine signal observed in response to CS and US presentation during the last session of training in outbred rats (ST, n=6; GT, n=5; ***P<0.001).

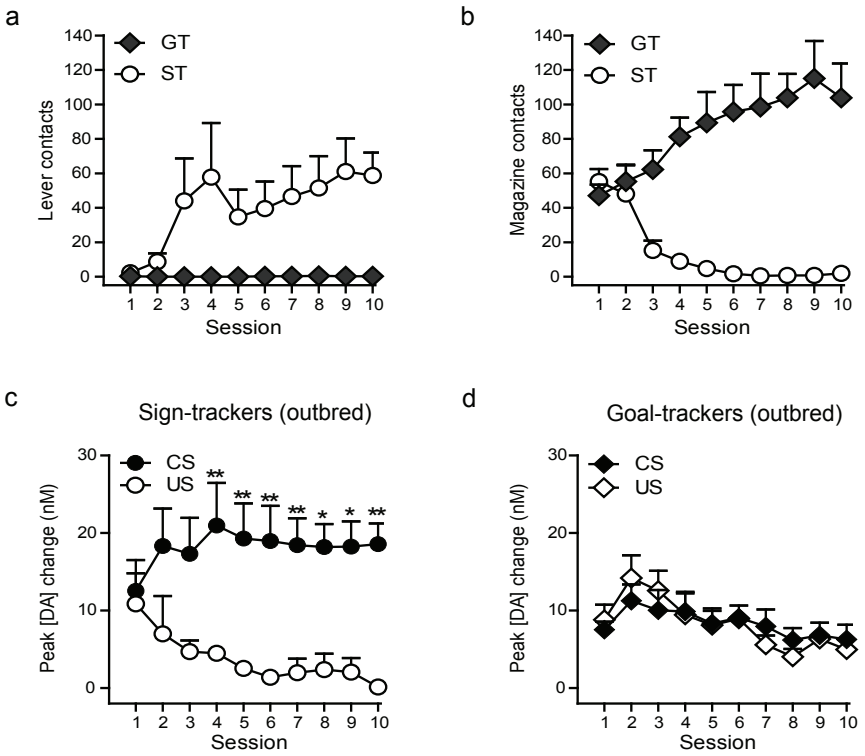

Figure S5: Conditional responses and phasic dopamine release in outbred rats after extended training. Sign-tracking behavior (n=4) is shown in panel **(a)** and goal-tracking behavior (n=4) in panel **(b)** for rats that received 10 training sessions. Mean + SEM **(a)** number of lever-CS contacts, **(b)** number food-tray contacts during lever-CS presentation. Mean + SEM change in dopamine concentration in response to CS and US presentation for 10 sessions of training in **(c)** sign-trackers, and **(d)** goal-trackers. (Bonferroni post-hoc comparisons of CS- and US-evoked dopamine release *P<0.05, **P<0.01).

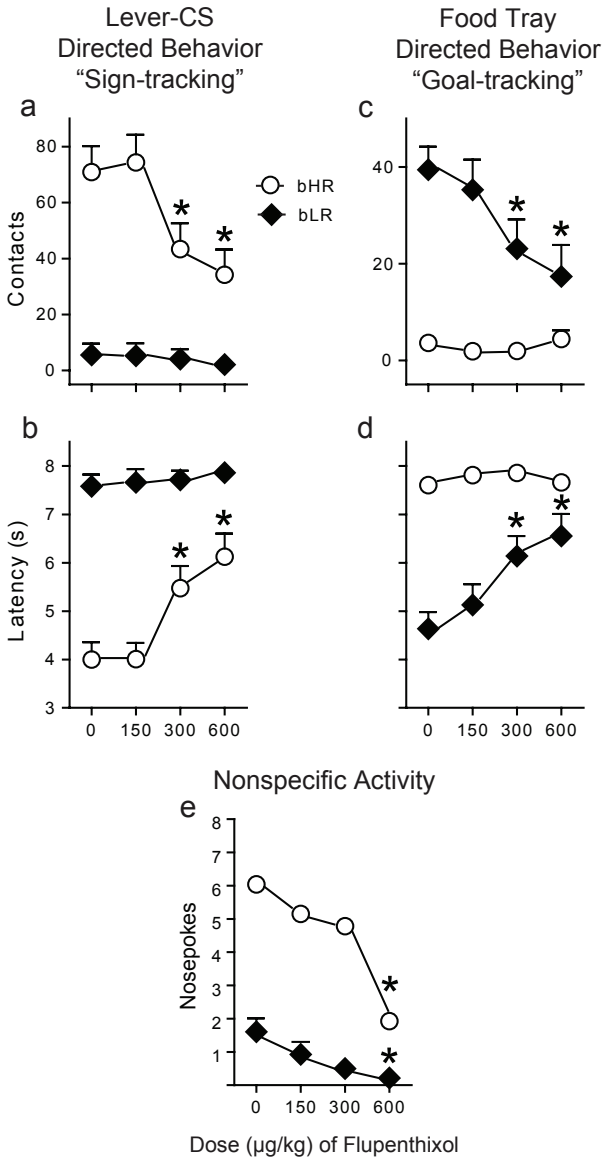

Figure S6: DA antagonist attenuates the performance of both sign-tracking and goal-tracking CRs. Following 7 days of training and acquisition of sign-tracking and goal-tracking CRs, bHR (n=14) and bLR (n=14) rats received an injection of either saline or 150, 300 or 600 µg/kg of flupenthixol (counterbalanced) one hour prior to the start of the next Pavlovian training session. **(a)** Mean + SEM number of lever contacts made during the lever-CS period; **(b)** latency to the first lever contact; **(c)** number of food-tray beam breaks during lever-CS presentation; and **(d)** latency to the first beam break in the food-tray during lever-CS presentation. Flupenthixol significantly attenuated the performance of both the sign-tracking and goal-tracking CR at doses of 300 and 600 µg/kg (Bonferroni post-hoc comparisons between saline and each dose of drug for sign-tracking behavior of bHRs (panels a and b) and goal-

tracking behavior of bLRs (panels c and d): *P<0.05). However, flupenthixol also affected nonspecific behavior, as measured by the number of nosepokes into a port that was without consequence. The effects on nonspecific behavior were most pronounced at 600 μg/kg (Bonferroni post-hoc comparisons between saline and each dose of drug for non-specific activity of bHRs and bLRs: *P<0.05).

SUPPLEMENTARY MOVIE FILES (S1-S2)

Video clips show a single trial of CS-US pairing for a **(S1)** bHR and **(S2)** bLR rat during the 6th Pavlovian conditioning session. Below each video is a real-time report of the fluctuation of dopamine concentration measured in the nucleus accumbens core. **(S1)** Upon lever-CS presentation (located to the left of the food-tray), the bHR rat immediately approaches, grasps and gnaws the lever and this behavior is accompanied by a large peak in the dopamine response. Delivery of the food-US after lever-CS retraction does not elicit a dopamine response even though the bHR rat retrieves the food pellet. **(S2)** Upon lever-CS presentation (located to the right of the food-tray), the bLR rat approaches the food-tray and a large rise in dopamine is evident after the lever-CS is retracted and the food-US is delivered.