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Short communication

Dopamine antagonism does not impair learning of Pavlovian conditioned approach to manipulable or non-manipulable cues but biases responding towards goal tracking

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HIGHLIGHTS

- FLU treatment biased conditioned responding towards goal tracking behaviour.
- Acquisition of the CS-US association was DA-independent (using a tone cue as CS).
- DA does not play a role in learning about the predictive CS-US relationship.
- DA is required for the development of sign tracking behaviour.

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ABSTRACT

Dopamine's (DA) role in reward-processing is currently discussed as either providing a teaching signal to guide learning or mediating the transfer of incentive salience (*i.e.* motivational aspects) from unconditioned stimuli (US) to conditioned stimuli (CS). We used a Pavlovian conditioned approach (PCA) procedure to further investigate DAs contribution to these processes. Experiment 1 assessed the acquisition of PCA to a manipulable lever cue for 7 days under DA-blockade with Flupenthixol (FLU; 225 μg/kg) or Saline (SAL) treatment, followed by 6-days off-drug testing. FLU decreased the number of conditioned responses (CR) during the treatment phase, but cessation of treatment resulted in an immediate increase in CR to levels comparable to SAL controls; notably, CR in FLU-treated rats were restricted to goal tracking behaviour. During continued off-drug testing, rats from the FLU group developed sign tracking with a similar temporal pattern as controls. In experiment 2, acquisition of PCA to a non-manipulable auditory cue was investigated. FLU reduced the number of CR during treatment, and removing DA antagonism resulted in a similar rapid increase of CR as seen in experiment 1.

These data complement other reports by demonstrating that, independently from the physical properties of the CS, DA is not required for learning predictive aspects of a CS-US relationship but for the development of behaviour (namely sign tracking) which is based on the motivational aspects of a CS-US relationship.

There is longstanding evidence proposing a role for dopamine (DA) in learning by providing a prediction error/teaching signal [1]. However, another stream of research suggests that DA may not be required for learning per se, but instead functions as a motivational signal attributing incentive salience to reward-related cues [2–4].

One approach to directly differentiate between a role of DA as a learning and/or motivational signal has been the use of so-called autoshaping procedures [5], in which individuals differ in the conditioned response (CR) they develop during Pavlovian conditioned approach (PCA): some animals approach the location of US delivery and thus apparently respond to the predictive properties of the CS (goal tracking, GT), while others approach the CS location, exhibiting consummatory behaviour (*e.g.* chewing, licking) and thus appear to respond to incentive properties of the CS (sign tracking, ST [6]). Importantly, these two CR types appear to be differentially dependent on DAergic activity and may therefore allow to disentangle learning, *i.e.* pure CS-US association formation, from motivation-related effects [7,8]. Evidence arguing in favour of DA functioning as a motivational signal comes from a study by Flagel et al.: they systemically blocked DA D_1/D_2 receptors during a

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7-day acquisition of PCA, which strongly reduced the number of CR made by all rats. However, when animals were then tested off-drug (allowing normal expression of CRs), rats bred to almost exclusively display GT (bLR rats) immediately responded by approaching the food-cup, while rats bred to display ST (bHR) did not show responding [9]. Flagel et al. concluded that DA was necessary for learning CS–US associations leading to ST, but was not required for GT. More general, Berridge [4] and Saunders & Robinson [10], after having shown that expression of ST, but not GT, immediately vanished after local DA antagonism within the nucleus accumbens core (NAcore), emphasized that DA might be necessary for the development of ST due to its role in transferring salience to the CS, but maybe not required to form the underlying CS-US association.

While the absence of ST in bHR rats in the study by Flagel et al. is compatible with the idea that DA is required to develop ST independently from CS-US association learning, this remains to be explicitly shown because an alternative explanation could be that rats prone to developing ST acquire the CS-US association in a different (*i.e.* DA-dependent) way than GT animals. However, if the latter were the case, one should expect that under D_1/D_2 antagonism, CRs would occur only in rats with a propensity to develop GT; alternatively, should DA blockade in fact spare CS-US learning and only affect development of ST, all rats should exhibit some kind of CR immediately after withdrawal of the DA blockade; presumably, DA blockade would bias the development of CRs towards GT. Further, once DA blockade would be removed, a fraction of animals should be expected to now develop ST. Unfortunately, Flagel et al. [9] did not report on the development of the alternative CR (*i.e.* GT) in their bHR rats and did not continue PCA training to observe the above prediction; additionally, development of such an alternative CR type would have been confounded by the selectively bred phenotype of the rats.

The main goal of the current study therefore was to decide between the two above explanations, *i.e.* to explicitly demonstrate the independency of the development of ST and GT from acquisition of the underlying CS-US association. Similar to Flagel et al., we investigated acquisition of PCA under D_1/D_2 antagonism using systemic Flupenthixol (FLU) treatment, but in a cohort of wild type rats expected to show the full spectrum of ST and GT responses, and continued training after cessation of treatment (experiment 1).

Classical autoshaping procedures commonly employ localizable CSs (*e.g.* lever or light) and are therefore suited to detect ST versus GT behaviour. Of course, similar learning occurs when auditory cues are used, but rats appear to develop only GT CRs (*i.e.* they do not approach the tone [11]), possibly due to the fact that a tone cue is much less localizable and manipulable. In a series of experiments, Meyer et al. [12] demonstrated that all rats approached the food-cup (*i.e.* GT) when an auditory CS was used, but in contrast to a lever-CS, the auditory CS became an effective conditioned reinforcer in all rats and, hence, seemed to be attributed with incentive salience by all of them. Further, using a compound lever/tone CS, the results of their study implicate that learning a GT may occur through different mechanisms, depending on the physical properties of the CS.

The goal of the second experiment therefore was, in analogy to experiment 1, to investigate whether the acquisition of the CS-US relationship underlying GT would be independent from DA when an auditory cue was used as the CS. Another goal of experiment 2 resulted directly from observations from experiment 1: rats acquired CR rapidly within 2–3 days; this raised the possibility that a 7-day acquisition phase could have masked slowing of learning due to DA-blockade in FLU-treated rats. Therefore rats were treated with FLU during acquisition of conditioned responding, but FLU was withdrawn after two days.

Male Sprague-Dawley rats (approx. 30 weeks old; food restricted to ~20 g/day/rat; water available *ad libitum*) were used in this study and all procedures were performed in accordance with national and international ethical guidelines and conducted in compliance with the German Animal Welfare Act. The general procedure followed earlier descriptions [*e.g.* 10] with minor adaptations (see supplement for procedure details). *Experiment 1* started with 20 free deliveries of the US (80 μl of a 20% sweetened condense milk solution) on three successive days. This was followed by 7 days of acquisition ("on-drug phase"): each rat was injected intraperitoneally one hour before the conditioning session with either 0.9% NaCl solution (SAL) or Flupenthixol hydrochloride (FLU; 225 μg/kg; Tocris Bioscience, Bristol, UK). Every session consisted of 20 trials: the lever (CS) was presented for 8s, and after its retraction the liquid dispenser provided the US. Trials were separated by ITIs (30–115 s); however, trial-onset was postponed by 8 s if a head entry occurred immediately prior to trial start. This avoided a confounding recording bias towards GT behaviour due to non-CS triggered ITI activity. Following a resting day to avoid drug carryover effects, on subsequent six days ("off-drug phase") the behavioural procedure was continued, but every rat was injected with SAL one hour before the start of a session. In experiment 2, the behavioural and pharmacological procedures were mainly the same as in experiment 1 with the following exceptions: i) the "on-drug phase" consisted of only two days and was immediately followed by two "off-drug phase" days; ii) the CS consisted of a sine tone presentation (5 KHz, 66 dB SPL, 8s); iii) no lever was available in the chamber.

In experiment 1, a CR was scored if at least one lever deflection or food-cup entry occurred during CS presentation. Acquisition of PCA was analysed using a RM ANOVA (day x treatment) for the "on-drug phase" and a priori planned within-subject *t*-tests (two-tailed) within SAL and FLU groups: day 1 versus day 7 (last day on-drug) and day 1 vs day 8 (first day off-drug). The pattern of responding (ST versus GT) was quantified using a PCA score, consisting of the mean of three measures: the probability of either lever deflection or food-cup entry, the response bias for lever/food-cup responses, and the latency to make lever/food-cup responses [details in: 10]. An *a priori* planned comparison between groups on day 8 was used to analyse the response pattern. In experiment 2, a CR was scored if a head entry into the food-cup occurred during CS presentation. Trials were analysed in blocks of ten, *i.e.* two blocks/day. Acquisition of PCA was analysed using a RM ANOVA (block x treatment) for the "on-drug phase" and *a priori* planned within-subject *t*-tests (two-tailed) within SAL and FLU groups: block 1 versus block 4 (last block on-drug) and block 1 vs block 5 (first block off-drug). Another RM ANOVA (block x treatment) was performed for the "off-drug phase". For all data, normal distribution was verified using Kolmogorov-Smirnov tests (all $p > 0.1$); Huynh-Feldt corrections were used in case sphericity was violated. See supplementary results for a validation of the PCA training procedure.

In experiment 1, during acquisition (unshaded area in Fig. 1A), the number of CRs was significantly lower in FLU-rats (*treatment*: $F_{1,16} = 25.310$, $p < 0.001$; *day*: ns.; *day x treatment*: ns.). The number of CR increased from day 1 to day 7 in the SAL-treated ($p = 0.013$) but not in FLU-treated animals rats ($p = 0.776$). The results from experiment 2, using a tone-CS instead of a lever-CS, were strikingly similar. During acquisition (unshaded area in Fig. 3), the number of CR was significantly lower in FLU-rats (*treatment*: $F_{1,15} = 6.71$, $p = 0.02$; *block*: $F_{2.12,54} = 3.751$, $p = 0.032$.; *block x treatment*: ns.). The significant *block* effect was attributable to the SAL-treated group, in which the number of CR increased from block 1 to 4 ($p = 0.004$), while this was not the case in FLU-treated rats ($p = 0.683$). These results from both experiments show that DA antagonism affects the performance of PCA. The lower number of CR in FLU-treated rats during the "on-drug phase" is in line with other studies reporting attenuated PCA after systemically given FLU [9,13]. However, we found that some rats exhibited considerable CRs even under DA antagonism, albeit exclusively as GT in experiment 1, while others made only very few CRs (notably, this behaviour was inde-