No evidence that post-training D2R dopaminergic drug administration affects differential fear responding in rats

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Abstract

The neurotransmitter dopamine plays an important role in the processing of emotional memories, and prior research suggests that dopaminergic manipulations immediately after fear learning can affect the retention and generalization of acquired fear. The current study focuses specifically on the role of dopamine D2 receptors (D2Rs) in differential fear responding in male Wistar rats. In a series of five experiments, D2R dopaminergic drugs were injected systemically after differential cued fear conditioning. One day later, the differential expression of acquired fear was assessed. All five experiments involved the administration of the D2R agonist quinpirole at different doses or saline ($n = 12, 16$ or 44 rats/treatment condition). Additionally, one of the studies administered the D2R antagonist raclopride ($n = 12$ rats/treatment condition). We found no indications for an effect of quinpirole or raclopride on differential fear responding during a later drug-free test. In addition, there were no effects of treatment on baseline freezing levels (i.e., before tone onset) or freezing during the conditioned tones (CS+) at test. We did observe acute behavioral effects of both drugs on a locomotor activity test.

Keywords

differential cued fear conditioning, generalization, rats, dopamine, quinpirole, raclopride

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Acknowledgements

We acknowledge the financial support of the European Research Council (ERC Consolidator Grant to T. Beckers, grant number 648176), the Research Foundation – Flanders, Belgium (FWO Doctoral Fellowship to N. Schroyens, grant number 1114018N) and KU Leuven Research Grant C16/19/002 (to T. Beckers and L. Luyten).

Author contributions

Conceptualization: N.S., T.B. and L.L.; Data curation: N.S.; Formal analysis: N.S.; Funding acquisition: N.S., T.B. and L.L.; Investigation: N.S., L.V., B.Ö. and V.A.O.S.; Methodology: N.S., J.Z., D.D., T.B. and L.L.; Project administration: T.B. and L.L.; Resources: T.B.; Supervision: T.B. and L.L.; Validation: N.S.; Visualization: N.S.; Writing – original draft: N.S.; Writing - review & editing: N.S., L.V., B.Ö., V.A.O.S., J.Z., D.D., T.B. and L.L.

Introduction

The neurotransmitter dopamine has been shown to play a role in the processing of emotional memories (Iemolo et al., 2015). For instance, genetic and pharmacological studies suggest that activation of dopaminergic D1 and D2 receptors immediately after Pavlovian conditioning or inhibitory avoidance training is required for subsequent expression of acquired fear (Castellano et al., 1991; Fadok et al., 2009; Greba et al., 2001; LaLumiere et al., 2004; Manago et al., 2008). In addition, dopamine has been found to modulate long-term potentiation (Bissière et al., 2003; Frey et al., 1991; Lemon & Manahan-Vaughan, 2006; Rosenkranz & Grace, 2002; Rossato et al., 2009), a form of synaptic plasticity that is assumed to be essential for memory formation (Lisman & Grace, 2005). The focus of the present paper is on the effect of dopaminergic signaling after fear conditioning with two cues, differentially predicting safety (CS-) or threat (CS+), on subsequent differential fear responding to those cues.

Although most studies regarding the influence of dopamine on fear memory processing have focused on its effects on memory retention specifically, more recent studies have also investigated dopamine's role in generalization of fear towards safe or novel stimuli after contextual, cued, or differential cued fear conditioning (De Bundel et al., 2016; Jo et al., 2018; Jones et al., 2015; Sarinana et al., 2014; Zweifel et al., 2011). Investigating if and how dopaminergic manipulations during or after learning affect the retention and spreading of fear towards safe situations is highly relevant to understand the neurobiology of anxiety, given that fear generalization is often found to be enhanced in patients with anxiety disorders (Hermans et al., 2013; Lee et al., 2016).

According to a study by Sarinana et al. (2014), knock-out mice lacking hippocampal dopamine D1 receptors (D1Rs) exhibit equal levels of freezing during exposure to a novel context as to a context that was previously paired with shock. In addition, using differential cued fear conditioning procedures in mice, it has been shown that differential responding between CS+ and CS- can be increased by optogenetic stimulation of dopaminergic neurons during conditioning (Jo et al., 2018) or by systemic injection of a D2R agonist immediately after conditioning (De Bundel et al., 2016). De Bundel and colleagues found the dopaminergic effect to be bidirectional, with post-learning injection or intra-amygdala infusion of a D2R antagonist impairing differential responding.

The abovementioned neurobiological studies have proposed a range of different mechanisms that may underlie dopamine's effect on the spreading of fear towards safe situations. For example, Sarinana et al. (2014) suggested that dopamine's role in the encoding of novel *perceptual* information may lie at the basis of its effects on fear generalization. Alternatively, it has been proposed that the generalized fear observed in knockout mice with impaired dopaminergic signaling can be attributed to inadequate learning of CS-US *associations* due to aberrant salience detection (Zweifel et al., 2011)

and the erratic assignment of negative valence to both the CS+ and CS- during learning (Jo et al., 2018). Yet another possible way in which dopamine may affect differential fear responding between stimuli that predict danger versus safety is via enhancement of the *stabilization* or ‘consolidation’ of acquired fear memory (De Bundel et al., 2016). Related to this idea, it has been suggested that ensuring adequate stabilization of the fear memory trace may be required for the establishment of a sufficiently precise memory to prevent generalization to non-threatening situations (Robertson, 2018). Overall, dopamine’s effects on fear memory generalization have thus been attributed to several aspects of learning (i.e., encoding of perceptual information, association formation, differential learning), at different points in time (i.e., during versus shortly after learning), and via various hypothetical mechanisms (i.e., by coding salience or valence, or by enhancing memory stabilization).

Our goal was to further evaluate, in rats rather than mice, to what extent dopamine signaling after differential fear learning plays a role in subsequent differential fear responding and, if so, which aspect(s) of learning (perceptual, associative, and/or differential) would be involved. To this aim, we embarked on a conceptual replication of published findings in mice by De Bundel et al. (2016), in which it was shown that systemic injection of a D2R agonist or antagonist immediately after differential cued fear conditioning can attenuate or enhance subsequent fear generalization (i.e., enhanced fear responding to a safe cue in a new context), respectively. One advantage of administering dopaminergic drugs *after* learning is that it allows to distinguish between the importance of dopaminergic signaling during versus after learning. It may thus provide useful insight into the role of dopamine during the memory stabilization phase specifically.

While all prior evidence in non-human animals on this topic has been obtained in mice (see Frick et al., 2021 for a related study in humans), the translation of those findings to rats remains unclear. Despite the many similarities between both species, rats and mice are characterized by important differences in functional brain anatomy and physiology, including neurochemistry (Ellenbroek & Yoon, 2016). Rats are an important model organism for behavioral neuroscience, in particular in the study of fear learning and interventions to influence fear memory specificity.

We describe a series of five experiments (Exp. 1-5) that investigated the behavioral effects of post-training dopaminergic drug injection on subsequent differential fear responding in rats. These experiments were preceded by a series of protocol optimization studies (reported as Exp. A-C) that provided insight into the effects of shock intensity and testing order on differential fear responding. In a last experiment (Exp. 6), the acute effects on locomotor activity of the drugs used throughout our experiments was evaluated (i.e., 0.3 mg/kg raclopride and 0.05, 1, and 5 mg/kg quinpirole).

Materials and Methods

Preregistration

All study protocols and statistical analyses were preregistered on the Open Science Framework (OSF) at <https://osf.io/bm76c/registrations> (behavioral protocol optimization studies; Experiments A-C), <https://osf.io/kzcyt/registrations> (differential fear conditioning studies with dopaminergic manipulations; Experiments 1-5), and <https://osf.io/9d72h/registrations> (acute drug effects on locomotor activity; Experiment 6).

Data and code availability

All relevant datasets, analysis scripts, and results of all preregistered statistical analyses can be found on the OSF at <https://osf.io/bm76c> (Experiments A-C), <https://osf.io/kzcyt> (Experiments 1-5), and <https://osf.io/9d72h> (Experiment 6).

Behavioral protocol optimization studies (Experiments A-C)

We performed experiments A, B and C to obtain appropriate behavioral protocols to investigate the effects of post-training dopaminergic drugs on fear memory generalization (see **Table 1** for an overview of study characteristics).

Experiment B investigated the effect of presenting tones in blocked versus intermixed order at test on differential fear responding, and used a higher shock intensity during training compared to Exp. A (.8 mA instead of .4 mA). Experiment C investigated the effect of shock intensity (.4 versus 1 mA) on differential fear responding. Smaller pilot studies were conducted to determine the optimal frequency and intensity of the tones; they are not reported here.

All rats received an intraperitoneal injection of saline (1 mg/kg) immediately after differential fear conditioning. The subjects, apparatus and behavioral procedures used in these studies are identical to those described below, except for the details that are listed below.

In Experiment A, a baseline period (i.e., before onset of the first tone) of 1 min. was used. This baseline period was prolonged to 3 min. in all other studies in order to measure baseline responding. Experiment B compared two different test sessions, i.e., with intermixed versus blocked tone presentations. The intermixed test session was as described below in the section '*Test of differential fear responding*'. In the blocked testing group, either the CS+ or the CS- was presented three times (baseline period of 180 s, 80 dB, 30 s, ITI = 80 – 140 s) during a first test session one day after training. Thirty seconds after the last tone, rats were returned to their home cage. The next day, the other tone was tested under the same conditions. The order of tone presentations (i.e., CS- or CS+ as the first tone during test) was counterbalanced.

The behavioral protocol of Exp. A was used in dopamine studies 1-3 (with the addition of extra behavioral testing) and the behavioral protocol of Exp. C was used in dopamine studies 4-5 because we aimed to investigate dopaminergic drug effects when using a protocol that elicits low differential fear responding in control rats.

Subjects

Male Wistar rats (Janvier Labs, Le Genest-Saint-Isle, France) were housed in groups of 3 or 4 rats per cage with food and water available ad libitum on a 12 h/12 h day-night cycle (lights on at 8 am). Cage enrichment, in the form of a tunnel hanging from the top grid, was provided. **Tables 1 & 2** provide overviews of sample sizes, average body weight one day before training, number of days of acclimatization to our animal facility (i.e., time between arrival and start of handling), and other study characteristics. Rats were briefly handled during the 2 days preceding conditioning. Experiments were carried out between 9 am and 5 pm. All experiments were approved by the KU Leuven animal ethics committee (in accordance with the Belgian Royal Decree of 29/05/2013 and European Directive 2010/63/EU).

Drug administration

Depending on the experiment (see **Table 2**), raclopride HCl (RACLO; 0.3 mg/kg; Santa Cruz Biotechnology), (-)-quinpirole HCl (QUIN; 0.05 mg/kg, 1 mg/kg or 5 mg/kg; Tocris), or saline was administered immediately after conditioning. Both drugs were dissolved in saline (0.9%, w/v) and injected intraperitoneally at a volume of 1 ml/kg. Drug doses were based on the study in mice by De Bundel et al. (2016). Similar doses have previously been used in rats (de Lima et al., 2011; Mueller et al., 2010).

Exp. ID	N	Body weight	Acclim. period	Groups	Shock Intensity	Test 1 (Day 2, CTX-B) Order of tone presentations*	Test 2
A	16	315 g	4 days	NA	.4 mA	12112122	NA
B	24	339 g	3 days	Intermixed vs. blocked test	.8 mA	12211212 (intermixed) vs. 111 or 222 (blocked)	CTX-B; Day 3 (only blocked group) 222 or 111
C	24	298 g	11 days	.4 vs. 1 mA	.4 or 1 mA	12211212	NA

Table 1. Behavioral protocol optimization studies. All experiments were preregistered on OSF at <https://osf.io/bm76c/registrations>. Sample size (N) and average body weight one day before training are shown. Acclim. period = interval between the rats' arrival in our animal facility and the start of handling (2 subsequent days of handling preceded the fear conditioning protocol). Rats were trained in context A (CTX-A) and tested in context B (CTX-B). *The order of the tones at test was counterbalanced (1 = CS+, 2 = CS- or 1 = CS-, 2 = CS+). During intermixed tests, 4 CS+ and 4 CS- presentations occurred in intermixed and semi-random order within one session, whereas blocked testing occurred in two separate test sessions with either 3 CS- or 3 CS+ presentations.

Exp.	N	Body weight	Acclim. period	Treatment*	Shock Intensity	Test 1 (Day 2, CTX-B) Order of tone presentations**	Test 2	Test 3
1	36	250 g	4 days	quinpirole 1 mg/kg raclopride .3 mg/kg	.4 mA	12112122	Day 3; CTX-A 21211212	/
2	36	321 g	4 days	quinpirole .05 mg/kg quinpirole 5 mg/kg	.4 mA	12112122	Day 27; CTX-A 21211212	/
3	36	317 g	4 days	quinpirole .05 mg/kg quinpirole 5 mg/kg	.4 mA	12112122	Day 28; CTX-B 21211212	Day 29, CTX-A 12211221
4	48	302 g	11 days	quinpirole .05 mg/kg quinpirole 1 mg/kg	1 mA	12211212	/	/
5	87	314 g	11 days	quinpirole 1 mg/kg	1 mA	12211212	/	/

Table 2. Experiments with saline or dopaminergic drug (quinpirole or raclopride) injection immediately after differential cued fear conditioning. All experiments were preregistered on OSF at <https://osf.io/kzcyt/registrations>. Sample size (N) and average body weight one day before training are shown. Acclim. period = interval between the rats' arrival in our animal facility and the start of handling (two consecutive days of handling preceded the fear conditioning protocol). Rats were trained in context A (CTX-A) and tested in CTX-A or context B (CTX-B). *All experiments contained a saline control group. **The order of the tones at test was counterbalanced (1 = CS+, 2 = CS- or 1 = CS-, 2 = CS+).

Apparatus

Four chambers (Contextual NIR Video Fear Conditioning System for Rats, Med Associates Inc., St. Albans, VT, USA, 30 cm (L) x 25 cm (W) x 21 cm (H)) illuminated by infrared and white light (45 lux), and with built-in ventilation fans (± 67 dB) were used. Fear conditioning took place in context A (CTX-A), consisting of a grid floor (19 rods of 4.8 mm diameter, 16 mm center to center) and a triangular-shaped black insert. Before and/or after each behavioral session, the context was cleaned and scented with diluted cleaning product (5.55 % in water). The first test session took place in context B, consisting of a white plastic floor, a white plastic curved back wall, illuminated with infrared light only, and cleaned and scented with a different cleaning product.

Differential cued fear conditioning

Rats were placed in context A (CTX-A) and, after a baseline period of 120 s, two tones (5 and 10 kHz, counterbalanced) were presented five times each in semi-random order (80 dB, 30 s, ITI = 60 – 160 s, order of tone presentations = CS+, CS-, CS-, CS+, CS-, CS+, CS-, CS-, CS+, CS+) (**Fig. 1**). In Experiments B, C, 4, and 5, the rise time of the tones was changed from .1 s to 1s to prevent interference with freezing behavior due to startle responding at tone onset. One tone (i.e., CS+) always co-terminated with a foot shock (.5 s, .4, .8 or 1 mA, see **Tables 1 & 2** for shock intensities), while the other tone (i.e., CS-) was never paired with shock. Sixty seconds after the last tone, rats were returned to their home cage. Depending on the experiment, rats received an intraperitoneal injection of saline, quinpirole (0.05, 1 or 5 mg/kg) or raclopride (0.3 mg/kg) at a volume of 1 ml/kg (see **Table 2**).

Figure 1. Experimental procedure. Immediately after differential cued fear conditioning in context A (CTX-A), rats were systemically injected with saline, raclopride, or quinpirole (see **Table 2** for details). Twenty-four hours later, behavioral differentiation between CS+ and CS- was assessed in a novel context B (CTX-B, Test 1) using percentage of time spent freezing during tones as an outcome measure. Depending on the experiment, additional behavioral tests took place at least one day after Test 1 (see **Tables 1 & 2**).

Test of differential fear responding

Twenty-four hours after training, rats underwent a drug-free test in a novel context (CTX-B). After a baseline period of 180 s, the CS+ and CS- were repeatedly presented in a semi-random and counterbalanced order (4 presentations of each tone, 80 dB, 30 s, ITI = 80 – 140 s, see **Tables 1 & 2** for the order of tone presentations). Thirty seconds after the last tone, rats were returned to their home cage.

Depending on the experiment, additional drug-free test sessions were performed (see **Tables 1 & 2**). Two (Exp. 1) or 26 days (Exp. 2) after training, rats were re-exposed to the training context (CTX-A, Test 2), and, after a baseline period of 180 s, the CS+ and CS- were repeatedly presented in a semi-random and counterbalanced order (4 presentations of each tone, 80 dB, 30 s, ITI = 80 – 140 s). Thirty seconds after the last tone, rats were returned to their home cage. In Experiment 3, a second test took place in CTX-B at day 28, and a third test in CTX-A on Day 29. The order of the tones and ITIs differed between test sessions.

Statistical analyses

Percentage freezing during tones was scored by an experienced observer, blinded to treatment condition, tone frequency and CS type (CS- or CS+). Average % freezing during CS+ and CS- was calculated for each rat and for each test session. According to a preregistered exclusion criterion (for all studies except Exp. A), rats showing less than 10% freezing during CS+ presentations on average at Test 1 were excluded from the analysis, as these low freezing scores may indicate a lack of acquisition of the CS-US association. Analyses were performed with and without those rats. R was used for frequentist and Bayesian analyses. For Bayesian analyses, we used a default Cauchy prior on the standardized effect size with a scale of 0.707 in the BayesFactor package. Bayes factors indicate how likely the obtained data are under H_A , relative to H_0 (i.e., BF_{10}).

Primary analyses for Experiments 1-5 included mixed ANOVAs with between-subjects factor Treatment (saline or drug) and within-subjects factor CS type (CS- or CS+) to investigate whether treatment affected differential fear responding during tests. Mixed ANOVAs were performed for each drug (versus saline) separately. The Bayes factor (BF) quantifying the evidence for including the Treatment x CS type interaction was calculated by dividing the BF for the model with interaction and main effects by the BF for the model with only main effects, (see doi 10.31234/osf.io/spreb; referred to as 'BF_{inclusion} across matched models'). For Experiments A-C and 1-5, one-sided paired t-tests assessed whether freezing during CS+ was higher compared to CS- for each condition (i.e., classification into conditions based on testing intermixed versus blocked in Exp. B, shock intensity in Exp. C, and treatment in Exp. 1-5). In order to investigate whether shock intensity during training

affected differential fear responding at test in Exp. C, we performed a mixed ANOVA with between-subjects factor Shock intensity (.4 or 1 mA) and within-subjects factor Trial type (CS- or CS+).

Secondary analyses included two-sided t-tests to assess whether treatment affected average freezing (1) during CS+ presentations and (2) during baseline at test. In addition, Chi-squared tests were performed to investigate whether treatment influenced the proportion of generalizers. We preregistered to calculate a generalization index (GENINDEX = average % freezing during CS- divided by average % freezing during CS+ at test) for each rat. Rats were subdivided in ‘discriminators’ (GENINDEX smaller or equal to 0.75) and ‘generalizers’ (GENINDEX larger than 0.75, but smaller than 1.30). Rats showing considerably higher freezing during CS- compared to CS+ (GENINDEX larger or equal to 1.30) were not included in the Chi-Squared tests, as they could not be unambiguously classified as generalizers or discriminators.

Statistical analyses performed for Experiment 5 deviated from those for the other studies. We used a sequential design, in which the total sample size depended on the value of the Bayes factor for the CS type x Treatment interaction observed during interim assessments (see **Table 3**). The first assessment took place after testing a total of 40 rats (batch 1; n = 20 rats/treatment condition) and the second assessment after testing a total of 64 rats (batch 2; 24 additional rats; n = 12 rats/treatment condition). The maximum sample size (N = 88) was determined based on frequentist power calculations using the results obtained in Experiment 4 (CS Type x Treatment interaction; power = 92%, alpha = .05, partial eta squared = .13).

Batch	N	N _{total} (all batches combined)	Obtained power (alpha = .05)
1	40	40	.60
2	24	64	.80
3	24	88	.92

Table 3. Overview of the sequential design used for Experiment 5. The reported power was calculated based on the effect size for the CS Type x Treatment interaction observed in Experiment 4. Interim assessments – performed after collecting data for batch 1 and 2 – included the computation of the Bayes factor for the CS type x Treatment interaction (testing stopped if the obtained BF was larger than 10 or smaller than 1/10 at interim assessment).

Primary analyses for Experiment 5 were the same as for the other studies and as described above. Secondary analyses included calculation of the generalization index (GENINDEX = average % freezing during CS- divided by average % freezing during CS+) for each rat, and rats were subdivided

into ‘discriminators’ (GENINDEX smaller or equal to 0.90) and ‘generalizers’ (GENINDEX larger than 0.90, but smaller than 1.10). Rats showing considerably higher freezing during CS- compared to CS+ (GENINDEX larger or equal to 1.10) were not included in the Chi-Squared tests, as they could not be unambiguously classified as generalizers or discriminators. The rationale for changing the criteria for classification into generalizers and discriminators can be found in the preregistration form for Experiment 5 at <https://osf.io/gnqpw>. As in our previous studies, Chi-Squared tests (quinpirole versus saline) were performed to assess whether quinpirole treatment affected the proportion of generalizers. In addition, we conducted one-sided t-tests to investigate whether the GENINDEX was lower in quinpirole-treated rats than in saline control rats.

Evaluation of the acute effects of raclopride and quinpirole on locomotor activity

Previous research in rats has shown acute effects of systemic administration of raclopride and quinpirole on locomotor activity (Archer et al., 2003; Eilam & Szechtman, 1989; Frantz & Van Hartesveldt, 1995; Hartesveldt et al., 1992; Horvitz et al., 2001; Kropf & Kuschinsky, 1991). As a positive control of drug efficacy, non-naïve rats received intraperitoneal saline or drug injection and acute effects on locomotor activity were assessed (Experiment 6). Based on the previously mentioned literature, we hypothesized that raclopride would generally suppress locomotor activity during a 1-h test session. Regarding quinpirole, it has been suggested that a dose of 1 mg/kg would first activate presynaptic autoreceptors, resulting in a transient decrease in dopamine synthesis and locomotor activity, followed by activation of postsynaptic dopamine D2 receptors, inducing hyperactivity (Kropf & Kuschinsky, 1991). On the other hand, a low dose of quinpirole (0.05 mg/kg) would solely activate presynaptic autoreceptors. Thus, quinpirole was expected to generally suppress locomotor activity at a dose of 0.05 mg/kg, whereas higher doses (1 or 5 mg/kg) would first suppress and later increase locomotor activity.

Subjects

At the age of 15-16 weeks, 35 rats that previously underwent a fear conditioning procedure (identical to the one adopted in Experiment 2) were used to test the acute drug effects during a locomotor activity test.

Drug administration

Rats received an intraperitoneal injection of saline, 0.3 mg/kg raclopride, 0.05 mg/kg quinpirole, 1 mg/kg quinpirole, or 5 mg/kg quinpirole (1 ml/kg; 7 rats/treatment condition).

Apparatus

Five minutes after the injection, rats were exposed to a novel testing box (38 x 38 x 60 (h) cm) for 1 h to assess effects on locomotor activity (distance travelled and % movement). The rats' behavior was recorded by a video camera that was mounted above the experimental set-up. Testing occurred in the dark. Distance travelled (cm) and % movement during the 1h-session were measured in 5-min time bins using Ethovision software XT (version 11.5, Noldus, Netherlands). The software's default criteria, i.e., start velocity of 2 cm/s and stop velocity of 1.75 cm/s, were used to calculate % movement.

Statistical analyses

Mixed Treatment x Time (twelve 5-min time bins) ANOVAs were used to assess treatment effects over time for raclopride (RACLO, SAL) and quinpirole (SAL, QUIN0.05, QUIN1, QUIN5) separately. Significant ANOVAs were followed up by Tukey post hoc tests to assess effects of treatment during each 5-min time bin, except for the first and the last time bin given that we had registered planned comparisons (one-sided t-tests) at those time points based on our *a priori* hypotheses (see <https://osf.io/5bsh7>). Specifically, we performed one-sided t-tests to assess treatment effects during the first and last 5 min of the test session. For the first 5 min (i.e., min 0-5) we expected for distance travelled and % movement a general suppression of locomotor activity by all treatment conditions, compared to saline (i.e., SAL > RACLO, QUIN0.05, QUIN1, QUIN5) and for the last 5 min (i.e., min 55-60) we expected an enhancement of locomotor activity in rats that received 1 and 5 mg/kg quinpirole, compared to saline (i.e., SAL < QUIN1, QUIN5).

Results

Behavioral protocol optimization studies (Experiments A-C)

Experiment A: Inducing intermediate levels of differential fear responding

The aim of Experiment A was to obtain a behavioral protocol that could be used to investigate the effects of post-training dopamine D2 receptor (ant)agonism on differential fear responding. Rats were trained using a differential cued fear conditioning protocol, injected intraperitoneally with saline immediately after training, and tested one day later in a new context B (CTX-B) to assess the amount of fear generalization between CS+ and CS-. About half of the rats showed higher freezing during the CS+ than the CS- at test, whereas the other half showed generalized fear responding (i.e., no or small difference in freezing between CS+ and CS-, see **Fig. 2**). Average freezing was significantly higher during CS+ presentations, compared to CS- presentations (one-sided paired t-test; $t(15) = -2.08$, $p =$

.028, $d = -.33$, $BF = 2.67$). We decided to use this protocol in Experiments 1, 2, and 3 as there were no apparent ceiling or floor effects, thus theoretically allowing the bidirectional modulation of generalization.

Figure 2. Average % freezing during testing (Day 2; CTX-B) of Experiment A.

Experiments B and C: Inducing lower levels of differential fear responding

The aim of Experiments B and C was to obtain a behavioral protocol that would induce lower levels of differential fear responding at test than in Experiment A, i.e., no reliable difference in freezing levels during CS+ versus CS-. This behavioral pattern would be more in line with what was observed in mice in the study by De Bundel et al. (2016) who reported no differential responding in saline control animals (and an enhancement of differential responding by post-training quinpirole injection; see Fig. 1C by De Bundel et al, 2016). To this aim, we increased shock intensity during training (i.e., from .4 mA used in Exp. A (as well as Exp. 1-3) to .8 mA in Exp. B and 1 mA in Exp. C (as well as Exp. 4-5)). In addition, Experiment B aimed to compare freezing levels between two different types of test sessions. Intermixed tone presentations (i.e., CS- and CS+ presented in intermixed and semi-random order, in a single session) were compared to blocked tone presentations (i.e., CS- and CS+ presented in separate test session on different days, similar to De Bundel et al., 2016).

The results of Experiment B (**Fig. 3**) show that, despite the use of a higher shock intensity, rats still showed significantly higher freezing during CS+ than CS- (one-sided t-tests; intermixed test session: $t(11) = -2.62$, $d = -.48$, $p = .012$, $BF = 5.82$; blocked test: $t(11) = -2.67$, $d = -.59$, $p = .011$, $BF = 6.24$). A visual analysis suggested that the overall and differential fear responding during tones observed during the blocked and intermixed test are similar. Exploratory analyses suggested that there was a significant effect of testing order (i.e., whether CS+ or CS- was presented as the first tone during testing) on the amount of fear generalization when using the blocked tests, as suggested by a significant Testing order (CS- first versus CS+ first) x CS type (CS- versus CS+) interaction ($F(1, 68) =$

6.50, $p = .013$, $\eta^2p = .087$, $BF = 3.94$). Visual analysis of supplemental graphs (see <https://osf.io/kg8vb/>, section 5.3) illustrates that, on average, rats that received CS+ presentations during the first test show high rates of differential responding, whereas rats that received CS- presentation during the first test do not show clear differential responding. There was no evidence for such an effect of testing order on generalization when using the intermixed test ($F(1,92) = .42$, $p = .521$, $\eta^2p = .004$, $BF = .36$).

Figure 3. Average % freezing during intermixed testing (Day 2; CTX-B; left panel) and blocked testing (Day 2 and 3; CTX-B; right panel) of Experiment B.

In Experiment C (**Fig. 4**), two different shock intensities were used during training, i.e., .4 mA and 1 mA. There was a statistically significant difference in freezing between CS+ and CS- presentations at test if shocks of .4 mA were used during training, in line with what we observed in Experiment A (one-sided t-test; $t(11) = -7.11$, $d = -1.1$, $p < .001$, $BF = 2380.46$), whereas there was no clear evidence for a difference in freezing between CS+ and CS- when 1-mA shocks were used (one-sided t-test; $t(11) = -1.23$, $d = -.34$, $p = .122$, $BF = 0.92$). In addition, there was a significant Shock intensity x CS type interaction ($F(1,22) = 20.37$, $\eta^2p = .48$, $p < .001$, $BF = 394.35$). To conclude, increasing the shock intensity during training from .4 to 1 mA induced a significant decrease in differential fear responding at test. The behavioral protocol from Experiment C (with 1-mA shocks) was therefore used in Experiments 4 and 5.

Figure 4. Average % freezing scores during testing (Day 2; CTX-B) of Experiment C illustrate that rats receiving 1-mA shocks during training show smaller differential fear responding between CS- and CS+ than rats that received .4-mA shocks.

Effects of systemic dopaminergic drug administration after training on differential cued fear expression (Experiments 1-5)

Experiment 1: No evidence for an effect of post-training injection of raclopride (0.3 mg/kg) or quinpirole (1 mg/kg) on subsequent differential fear responding

Based on the results of De Bundel et al. (2016) in mice, we hypothesized that 0.3 mg/kg raclopride or 1 mg/kg quinpirole would increase or decrease differential fear responding at test, respectively.

Primary analyses provided no evidence for Treatment (dopaminergic drug versus saline) x CS type (CS- versus CS+) interactions at Test 1 (RACLO: $F(1, 22) = .04$, $p = .847$, $\eta^2p < .01$, $BF = .38$; QUIN1: $F(1, 22) = .20$, $p = .662$, $\eta^2p = .01$, $BF = .38$). One-sided paired t-tests suggested higher freezing during CS+ than CS- presentations at test in saline control rats ($t(11) = -2.42$, $p = .017$, $d = -.64$, $BF = 4.35$), and raclopride rats ($t(11) = -2.6$, $p = .012$, $d = -.67$, $BF = 5.66$), but not in quinpirole rats ($t(11) = -1.38$, $p = .098$, $d = -.33$, $BF = 1.09$).

Although freezing during CS+ presentations at Test 1 was on average lower in quinpirole rats than in saline control rats (see **Fig. 5**, left panel), two-sided t-tests (preregistered secondary analysis, see OSF) suggested no evidence for differences in freezing during CS+ presentations between treatment conditions (i.e., QUIN1 versus SAL or RACLO versus SAL). In addition, there was no evidence for differences in baseline freezing (i.e., before onset of the first tone, see OSF) or the proportion of generalizers between treatment conditions.

During Test 2, which took place in the training context on Day 3 (**Fig. 5**, right panel), there was again no evidence for Treatment x CS type interactions (RACLO: $F(1, 22) = .67$, $p = .421$, $\eta^2p = .03$, $BF = .45$; QUIN1: $F(1, 22) = 1.25$, $p = .276$, $\eta^2p = .05$, $BF = .63$) and one-sided paired t-tests suggested higher freezing during CS+ than CS- presentations at test in saline control rats ($t(11) = -2.09$, $p = .03$, $d = -.60$, $BF = 2.75$), raclopride rats ($t(11) = -2.31$, $p = .021$, $d = -.29$, $BF = 3.75$), and in quinpirole rats ($t(11) = -3.75$, $p = .002$, $d = -1.14$, $BF = 30.08$). Secondary analyses suggested no evidence for differences in freezing during CS+ presentations between treatment conditions (i.e., QUIN1 versus SAL or RACLO versus SAL). In addition, there was no evidence for differences in baseline freezing (i.e., before onset of the first tone), average freezing during CS+ presentations, or the proportion of generalizers between treatment conditions.

Figure 5. Average % freezing per treatment condition during Test 1 (Day 2; CTX-B; left panel) and Test 2 (Day 3; CTX-A; right panel) of Experiment 1. QUIN = quinpirole (1 mg/kg), RACLO = raclopride (0.3 mg/kg), SAL = saline.

Experiments 2 & 3: No evidence for an effect of post-training injection of quinpirole (0.05 or 5 mg/kg) on subsequent differential fear responding

As mentioned, locomotor activity studies in rats suggest that quinpirole differentially affects presynaptic versus postsynaptic D2 receptors depending on the applied dose. Experiments 2 and 3 aimed to assess how different doses of quinpirole influence differential fear responding. We hypothesized that a low dose of quinpirole (0.05 mg/kg) would decrease differential responding (due to the assumed activation of presynaptic autoreceptors), whereas a higher dose of 5 mg/kg would increase differential responding (due to the assumed activation of postsynaptic D2Rs). Experiments 2 and 3 adopted the exact same behavioral procedures and drug doses (apart from additional tests 2 and 3), and are therefore presented together.

In Experiment 2 (**Fig. 6**), three rats (1 SAL and 2 QUIN5 rats) were excluded due to the preregistered exclusion criterion of showing less than 10% freezing on average during CS+ presentations at Test 1, so all preregistered analyses were performed with and without those three rats. Statistical results are shown here for the sample in which the exclusion criterion was applied, and results for both samples (i.e., with and without exclusions) are provided only when both analyses reached different overall conclusions. All results can be found at <https://osf.io/kzcyt>.

Primary analyses suggested no evidence for Treatment (dopaminergic drug versus saline) x CS type (CS- versus CS+) interactions (QUIN (0.05 mg/kg): $F(1, 21) = 2.01$, $p = .171$, $\eta^2p = .09$, $BF = .89$; QUIN (5 mg/kg): $F(1, 19) = .53$, $p = .477$, $\eta^2p = .03$, $BF = .48$) at Test 1. Contrary to Experiment 1, in which the same behavioral procedures were used for training and testing, there was no evidence for more freezing during CS+ compared to CS- presentations at test in saline control rats ($t(10) = -.39$, $p = .353$, $d = -.12$, $BF = .40$). A similar pattern of responding was observed in rats treated with the high dose of quinpirole ($t(9) = -1.34$, $p = .106$, $d = -.51$, $BF = 1.1$). However, rats that received the low quinpirole dose did show statistically significant differential responding between tones ($t(11) = -2.67$, $p = .011$, $d = -.59$, $BF = 6.25$). Contrary to our hypotheses, neither of the quinpirole doses thus induced a statistically significant effect on differential fear responding. If anything, the low quinpirole dose (0.05 mg/kg) seemed to *enhance*, rather than impair, differential responding, although this mere trend did not reach statistical significance.

Average freezing during CS+ presentations at Test 1 was numerically higher in the ‘low quinpirole’ condition than in saline control rats (see **Fig. 6**, left panel), but this between-group difference did not receive statistical support when excluding the saline rat showing on average less than 10% freezing during CS+ presentations ($t(21) = 1.79$, $p = .088$, $d = .75$, $BF = 1.16$). When including all rats, the effect was statistically significant, although the obtained evidence was indifferent according to the Bayesian analysis ($t(22) = 2.1$, $p = .047$, $d = .86$, $BF = 1.72$). Furthermore, no between-group differences were found in baseline freezing (i.e., before onset of the first tone) or the proportion of generalizers.

During Test 2, which took place in the training context on Day 27 (**Fig. 6**, right panel), there was again no evidence for Treatment x CS type interactions (QUIN (0.05 mg/kg): $F(1, 21) = .06$, $p = .801$, $\eta^2p < .01$, $BF = .37$; QUIN (5 mg/kg): $F(1, 19) < .01$, $p = .945$, $\eta^2p < .01$, $BF = .38$). Furthermore, both QUIN0.05 and saline rats now showed significantly higher freezing during CS+ than CS- ($t(10) = -2.96$, $p = .007$, $d = -.77$, $BF = 8.98$; $t(11) = -3.85$, $p = .001$, $d = -1.20$, $BF = 34.85$, respectively), whereas QUIN5 rats did not show statistically significant differential responding ($t(9) = -1.61$, $p = .071$, $d = -.63$, $BF = 1.51$). In addition, secondary analyses suggested no evidence for differences in baseline freezing

(i.e., before onset of the first tone), average freezing during CS+ presentations, or the proportion of generalizers between treatment conditions.

Figure 6. Average % freezing per treatment condition during Test 1 (Day 2; CTX-B; left panel) and Test 2 (Day 27; CTX-A; right panel) of Experiment 2. QUIN0.05 = quinpirole (0.05 mg/kg), QUIN5 = quinpirole (5 mg/kg), SAL = saline.

In Experiment 3 (**Fig. 7**), eight rats (2 SAL, 2 QUIN0.05, and 4 QUIN5 rats) were excluded due to the preregistered exclusion criterion of showing less than 10% freezing on average during CS+ presentations at Test 1, so all preregistered analyses were performed with (N = 36) and without (N = 28) those eight rats.

Primary analyses suggested no evidence for Treatment (dopaminergic drug versus saline) x CS type (CS- versus CS+) interactions (QUIN (0.05 mg/kg): $F(1, 18) = .55$, $p = .468$, $\eta^2p = .03$, $BF = .47$; QUIN (5 mg/kg): $F(1, 16) = .88$, $p = .362$, $\eta^2p = .05$, $BF = .54$) at Test 1. One-sided paired t-tests suggested higher freezing during CS+ than CS- presentations at test in all treatment conditions (saline control rats: $t(9) = -3.64$, $p = .003$, $d = -.42$, $BF = 20.35$; QUIN (0.05 mg/kg): $t(9) = -2.82$, $p = .01$, $d = -.76$, $BF = 7.03$; QUIN (5 mg/kg): $t(7) = -3.52$, $p = .005$, $d = -.98$, $BF = 13.27$). However, differential responding between tones was smaller (and non-significant) when including all QUIN5 rats ($t(22) = .08$, $p = .933$, $d = .03$, $BF = .37$), probably due to the fact that two rats showed no freezing during any of the tones, and thus, no differential responding.

During Test 2, which took place in the testing context (CTX-B) on Day 28, there was again no evidence for Treatment x CS type interactions (QUIN0.05: $F(1, 18) = .57$, $p = .458$, $\eta^2p = .03$, $BF = .47$; QUIN5: $F(1, 16) = .03$, $p = .861$, $\eta^2p < .01$, $BF = .39$). Saline control rats ($t(9) = -1.25$, $p = .121$, $d = -.24$, $BF = .99$) and QUIN5 rats ($t(7) = -1.37$, $p = .107$, $d = -.77$, $BF = 1.18$) did no longer show statistically significant differential responding between CS+ and CS-, whereas QUIN0.05 rats did show significant differential responding between tones still ($t(9) = -2.76$, $p = .011$, $d = -.78$, $BF = 6.47$). However, when

including all rats, all groups showed statistically significant differential responding (SAL: $t(11) = -1.86$, $p = .045$, $d = -.33$, $BF = 2.01$; QUIN0.05: $t(11) = -2.48$, $p = .015$, $d = -.61$, $BF = 4.72$; QUIN5: $t(11) = -2.11$, $p = .029$, $d = -.65$, $BF = 2.83$).

During Test 3, which took place in the training context (CTX-A) on Day 29, there was no evidence for Treatment x CS type interactions (QUIN0.05: $F(1, 18) < .01$, $p = .982$, $\eta^2p < .01$, $BF = .41$; QUIN5: $F(1, 16) = .74$, $p = .402$, $\eta^2p = .04$, $BF = .58$) and there was no statistical evidence for differential responding between tones in any of the treatment conditions (SAL: $t(9) = -1.45$, $p = .09$, $d = -.5$, $BF = 1.25$; QUIN0.05: $t(9) = -1.48$, $p = .086$, $d = -.53$, $BF = 1.29$; QUIN5: $t(7) = .08$, $p = .531$, $d = .04$, $BF = .32$).

Secondary analyses suggested no evidence for between-group differences in average freezing during CS+ presentations, baseline freezing, (i.e., before onset of the first tone), or the proportion of generalizers during any of the test sessions.

Figure 7. Average % freezing per treatment condition during Test 1 (Day 2; CTX-B; left panel), Test 2 (Day 28; CTX-B; middle panel), and Test 3 (Day 29; CTX-A; right panel) of Experiment 3. QUIN0.05 = quinpirole (0.05 mg/kg), QUIN5 = quinpirole (5 mg/kg), SAL = saline.

Experiment 4: No evidence for an effect of post-training injection of quinpirole (0.05 or 1 mg/kg) on differential fear responding when using higher shock intensity during training

Experiment 4 aimed to investigate whether quinpirole injection can enhance differential fear responding at a drug-free test when using a higher shock intensity during training, which we expected would result in limited differential responding in saline control rats at test (based on the results of Experiment C). Although we initially hypothesized that the low dose of quinpirole (i.e., 0.05 mg/kg) would decrease differential responding, the results of Exp. 2 suggested that, if anything, this dose may increase differential responding. Therefore, the low dose was also used in this study.

Saline control rats showed differential responding between tones at test ($t(15) = -1.8$, $p = .046$, $d = -.35$, $BF = 1.78$). Numerically even higher levels of differential responding were observed in quinpirole rats, especially in rats that received the dose of 1 mg/kg (QUIN0.05: $t(15) = -2.34$, $p = .017$,

$d = -.44$, $BF = 4.01$; QUIN1: $t(15) = -5.04$, $p < .001$, $d = -.86$, $BF = 410.27$) (**Fig. 8**). There was, however, no reliable evidence for Treatment (dopaminergic drug versus saline) x CS type (CS- versus CS+) interactions (QUIN (0.05 mg/kg): $F(1, 30) = .25$, $p = .622$, $\eta^2p = .01$, $BF = .36$; QUIN (1 mg/kg): $F(1, 30) = 3.28$, $p = .08$, $\eta^2p = .1$, $BF = 1.08$).

Secondary analyses suggested no evidence for between-group differences in average freezing during CS+ presentations, baseline freezing, (i.e., before onset of the first tone), or the proportion of generalizers.

Figure 8. Average % freezing per treatment condition during testing (Day 2; CTX-B) of Experiment 4. QUIN0.05 = quinpirole (0.05 mg/kg), QUIN1 = quinpirole (1 mg/kg), SAL = saline.

Experiment 5: No evidence for an effect of post-training injection of quinpirole (1 mg/kg) on differential fear responding when using higher shock intensity during training and large sample size

In Experiment 4 we observed numerically higher levels of differential responding in rats that received 1 mg/kg quinpirole. Therefore, we aimed to investigate this effect further by taking into account the size of the effect observed in Exp. 4 and using a sample size that is required to detect such an effect with a power of more than 90%. Experiment 5 followed the procedures from Experiment 4, but only included two of the groups from Experiment 4 (i.e., quinpirole 1 mg/kg versus saline). The experiment had a sequential design and was performed in three separate batches. Given that we did not observe the predefined Bayes factor for the CS type x Treatment interaction after testing batch 1 or 2, the maximum sample size of 88 rats was tested.

One saline rat was excluded from analyses because it showed no freezing at all during testing (i.e., 0% freezing; exclusion due to preregistered criterion). In addition, the excluded rat did not show any freezing during training either, and a lack of responding at the time the shocks should have been given, so we decided not to perform analyses with this rat included. Note that we hereby deviate from the preregistration because we planned to perform the analyses with *all* subjects included, apart from

applying the preregistered exclusion criterion. A total of 87 rats was thus included in the analyses described below.

There was no evidence for a CS type x Treatment interaction ($F(1, 85) = .17, p = .68, \eta^2p < .01, BF = .23$), and the obtained Bayes factor suggested substantial evidence for the absence of an interaction (Wetzels et al., 2011) (**Fig. 9**). One-sided paired t-tests suggested statistically significant differential responding between CS+ and CS- in saline control rats ($t(42) = -4.93, p < .001, d = -.62, BF = 3032.43$) and quinpirole rats ($t(43) = -4.31, p < .001, d = -0.56, BF = 502.73$).

Secondary analyses suggested no difference in the proportion of generalizers between saline and QUIN1 rats ($\chi^2(1) < .01, p = 1, BF = .27$). In addition, the generalization index was not significantly lower in QUIN1 rats than in saline rats ($t(85) = .76, p = .777, d = .16, BF = .14$).

Figure 9. Average % freezing per treatment condition during testing (Day 2; CTX-B) of Experiment 5. QUIN1 = quinpirole (1 mg/kg), SAL = saline.

Experiment 6: Acute effects of raclopride and quinpirole on locomotor activity

Raclopride (0.3 mg/kg) and quinpirole (0.05 mg/kg) temporarily suppress locomotor activity

In line with previously published studies and our preregistered hypotheses (see Methods section), raclopride (0.3 mg/kg) and the low dose of quinpirole (0.05 mg/kg) induced suppression of locomotor activity (compared to saline, **Fig. 10**). For both drugs, mixed ANOVAs showed statistically significant main effects of Treatment and Time, and a significant interaction (see **Table 4**). Tukey post-hoc tests showed that the raclopride- and quinpirole-induced differences in distance travelled and % movement compared to saline rats were temporary and became statistically insignificant during the second half of the test session. These findings corroborate previous research and illustrate that the drugs produced the expected acute behavioral effects at the applied doses in Wistar rats.

An intermediate dose of quinpirole (1 mg/kg) first suppresses and later increases locomotor activity

In line with previously published studies and our preregistered hypotheses (see Methods section), the intermediate quinpirole dose (1 mg/kg) first suppressed locomotor activity, followed by a statistically significant increase in locomotor activity compared to saline (**Fig. 10**). One-sided t-tests suggested that during the first 5 min of the test (i.e., 5-10 min after injection), quinpirole rats showed a statistically significant decrease in distance travelled and % movement compared to saline rats, whereas they showed a statistically significant increase compared to saline rats during the last 5 min of the test (see **Table 5**). This is in line with previous research and illustrates that quinpirole (1 mg/kg) produced the expected acute behavioral effects.

Figure 10. Acute effects of raclopride (RACLO; 0.3 mg/kg) and quinpirole (QUIN, different doses in mg/kg) on locomotor activity (means and SEMs) in Experiment 6. Distance travelled (cm) (left panel) and % movement (right panel) were measured to assess locomotor activity.

A high dose of quinpirole (5 mg/kg) temporarily suppresses locomotor activity

We assumed that the biphasic behavioral pattern that was observed after injection of an intermediate 1 mg/kg-dose of quinpirole (i.e., brief suppression followed by an increase in locomotor activity after injection) would be even more pronounced when using a higher dose (5 mg/kg). One-sided t-tests suggested that the highest dose of quinpirole induced a statistically significant suppression of locomotor activity (i.e., distance travelled and % movement) during the first 5 min. of the test session (**Fig. 10, Table 5**). However, contrary to our expectations, its stimulating effects at the end of the test session (i.e., about 1 hour after injection) were only moderate and insignificant when compared to saline.

Treatment	Main effect of Treatment	Main effect of Time	Treatment x Time interaction
RACLO, SAL	$F(1,12) = 52.68$, $\eta^2p = .81$, $p < .001$, BF = 187.45	$F(4.68, 56.19) = 27.67$, $\eta^2p = .70$, $p < .001$, BF = 3.85×10^{18}	$F(4.68, 56.19) = 8.48$, $\eta^2p = .41$, $p < .001$, BF = 2.71×10^8
QUIN0.05, QUIN1, QUIN5, SAL	$F(3, 24) = 6.30$, $\eta^2p = .44$, $p = .003$, BF = 14.83	$F(5.29, 127.04) = 4.71$, $\eta^2p = .16$, $p < .001$, BF = 3.91	$F(15.88, 127.04) = 8.64$, $\eta^2p = .52$, $p < .001$, BF = 3.55×10^{23}

Table 4. Results of preregistered mixed ANOVAs show statistically significant main effects of Treatment and Time, and significant Treatment x Time interactions for raclopride and quinpirole during the locomotor activity test.

Treatment	First 5 min. of test session (min. 0-5)	Last 5 min. of test session (min. 55-60)
RACLO	$t(12) = 5.26$, $d = -2.81$, $p < 0.001$, BF = 103.19	NA
QUIN0.05	$t(12) = 7.66$, $d = -4.10$, $p < 0.001$, BF = 2049.88	NA
QUIN1	$t(12) = 9.45$, $d = -5.05$, $p < 0.001$, BF = 13802.42	$t(12) = 2.55$, $d = 1.36$, $p = 0.013$, BF = 2.76
QUIN5	$t(12) = 10.29$, $d = -5.50$, $p < 0.001$, BF = 31009.8	$t(12) = 0.91$, $d = 0.49$, $p = 0.190$, BF = 0.58

Table 5. Results of preregistered one-sided t-tests during the first and last five minutes of the locomotor activity test.

Discussion

In a series of five experiments, the dopamine D2 receptor (D2R) agonist quinpirole and D2R antagonist raclopride were injected systemically after differential cued fear conditioning in rats. One day after treatment, differential expression of acquired fear was assessed by comparing average % freezing during CS- versus CS+ presentations in a new context. Our studies aimed to evaluate in rats the role of dopaminergic signaling in fear memory processing as established in mice, emulating the protocol of a previously published study by De Bundel et al. (2016). They showed that post-training systemic injection in mice of quinpirole or raclopride affects differential responding to a learned danger cue (CS+) versus safety cue (CS-). Based on those published results, we expected quinpirole (1

or 5 mg/kg) to increase differential fear responding, and raclopride (0.3 mg/kg) to impair differential fear responding. In addition, we initially hypothesized that a low dose of quinpirole (0.05 mg/kg) would decrease differential responding due to the assumed activation of presynaptic autoreceptors. We found no evidence in rats for an effect of systemically administered quinpirole or raclopride on differential fear responding at test. In addition, there were no effects of treatment on freezing levels during CS+ presentations or during the baseline period at test (i.e., before tone onset) in any of the 5 experiments. We did, however, replicate previously described acute behavioral effects of quinpirole (0.05, 1 and 5 mg/kg) and raclopride (0.3 mg/kg) during a locomotor activity test.

In the light of the inconsistent results between our studies (in rats) and the published studies by De Bundel et al. (in mice), a number of differences between these two studies should be acknowledged. First and foremost is of course the difference in species, given the reported species-dependent variations in ventral tegmental area (VTA) dopamine signaling (Courtney et al., 2012). The fact that DA neurons in mice and rats show different sensitivity to D2-mediated inhibition could have important implications for presynaptic or postsynaptic effects of D2R modulation. Similarly, differences in DA agonist-induced locomotor effects, prepulse inhibition, or yawning between rats and mice have been described (Halberda et al., 1997; Li et al., 2010; Ralph & Caine, 2005). Another relevant question is whether mice and rats express fear in the same manner (e.g., comparable levels of freezing versus escape behavior) during presentations of tones with different frequencies. If existent, between-species differences in prototypical fear behavior may obviously complicate the comparison of studies in rats versus mice when differential freezing responses are used as a measure of generalization.

Furthermore, and as expected, pilot studies in our lab indicated the need for different behavioral parameters than those used by De Bundel et al. in order to allow for the study of differential fear responding in rats in our lab (e.g., tones of 5 and 10 kHz rather than 2.5 and 7.5 kHz, shocks of .5 s rather than 2 s, and intermixed rather than blocked testing). In addition, we opted to leave out the habituation session prior to conditioning, and used a semi-random rather than a fixed order of CS+ and CS- presentations during learning. Finally, our saline control animals showed, on average, a different behavioral pattern than those in the De Bundel study. The control rats in most of our dopamine studies (except for Experiment 2) showed statistically significant higher freezing during CS+ presentations than during CS-, whereas the control mice in the quinpirole study by De Bundel et al. (2016) showed a lack of differential responding (see Fig. 1C of De Bundel et al., 2016).

As far as the effects of raclopride concern, our conclusions rely on a single study with a sample size of 12 rats per treatment condition, yielding only anecdotal evidence for the absence of a CS type x Treatment interaction effect. In contrast, we performed five studies with quinpirole, in which we

used three different doses of the drug (0.05, 1, or 5 mg/kg), two different shock intensities (.4 or 1 mA), and including a final study with a large sample size yielding a power of over 90% to detect an effect if any. Moreover, Experiment 3 (N = 36) was a replication of Experiment 2 (N = 36) (except for additional tests), and Experiment 5 (N = 88) was a replication of Experiment 4 (N = 48) (except for the omission of the 0.05-mg/kg quinpirole group in Exp. 5). The final experiment provided substantial evidence (Bayesian analyses) for the absence of an effect of quinpirole (1 mg/kg) on differential fear responding. In other words, we have gathered evidence *against* an effect of quinpirole on differential fear responding under the applied conditions in rats.

It should be noted that our studies are limited to the use of systemic drug injection and that we did not directly target a particular brain region via, for example, drug infusion or optogenetic manipulation. Given the apparent lack of generalizability to rats of the results of De Bundel et al. (2016) of *systemic* application of dopaminergic drugs, a possible follow-up study to investigate the involvement of post-learning D2R signaling in differential fear responding could include the use of *intracerebral* drug administration. In this case, a replication in rats of previous experiments in which the D2R antagonist raclopride was infused into the central or basolateral amygdala (see Fig. 2I and 3I by De Bundel et al., 2016) would be optimal to inform about the replicability of the effect specifically and the role of post-learning D2R signaling in differential fear responding in rats more generally.

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