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## Brief Communication

# D1/5 dopamine receptors are necessary for learning a novel context

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Dopamine participates in encoding memories and could either encode rewarding/aversive value of unconditioned stimuli or act as a novelty signal triggering contextual learning. Here we show that intraperitoneal injection of the dopamine D1/5R antagonist SCH23390 impairs contextual fear conditioning and tone-shock association, while intrahippocampal injection only impairs contextual fear conditioning. By using the context pre-exposure facilitation effect test, we show that SCH23390 is able to block the encoding of the context during the pre-exposure phase. Thus, we provide additional evidence that dopamine is involved in encoding conjunctive representations of new contexts.

Although dopamine has been thought of for decades as encoding the rewarding value of stimuli (Schultz 1997), it is currently considered that both aversive events and exposure to novel environments are also able to stimulate dopaminergic neurons (Brischoux et al. 2009; Matsumoto and Hikosaka 2009; Gore et al. 2014; McNamara et al. 2014). Exposure to a novel environment leads to dopamine release in the hippocampus (Ihalainen et al. 1999), and the intrahippocampal administration of dopamine receptor 1 and 5 (D1/5R) antagonists before learning impairs long-term memory in a wide variety of behavioral tests, including classical eyelid conditioning (Ortiz et al. 2010), inhibitory avoidance (Broussard et al. 2016), fear conditioning (Heath et al. 2015; Tsetsenis et al. 2021), Morris Water maze (da Silva et al. 2012), spatial learning of new goal locations (McNamara et al. 2014), or learning a new pair of odor/location in the Event Arena (Bethus et al. 2010). Thus, dopamine is required for encoding of long-term memories, whether they are aversive or appetitive.

However, it is still unclear whether dopamine provides the hippocampus with a rewarding/aversive value of the unconditioned stimuli or acts as a teaching signal triggered by novelty that enables the formation of new cognitive maps (Harley 2004; Lisman et al. 2011; Duzskiewicz et al. 2019). In order to tackle this issue, we used a combination of experiments involving either classical Pavlovian conditioning coupled to contextual fear conditioning or a variation of this test, named contextual pre-exposure facilitation effect (CPFE), in which learning the novel context and associating it with the electric shock occur on two consecutive days (Barrientos et al. 2002).

In experiments 1 and 2, we first confirmed the role of D1/5R in contextual fear conditioning and in classical Pavlovian conditioning by performing intraperitoneal (ip) injections or intrahippocampal (ih) infusions of D1/5R antagonist SCH23390. In experiment 3, we validated the CPFE test and determined the pre-exposure duration necessary for mice to acquire and store a conjunctive representation of a context. In experiment 4, we injected SCH23390 just before the pre-exposure in order to test the role of D1/5R in learning a new context independently of any rewarding or aversive stimulus.

All experiments were performed on C57BL/6J female mice aged 12–16 wk obtained from Charles River Laboratories. The experiments were performed in strict accordance with the recommendations of the European Union (86/609/EEC) and the French National Committee (87/848). Fear experiments were performed using the Panlab s.l. Startfear 1.06 system. Freezing was scored every 5 sec by two independent experimenters blind to the experimental condition and is expressed as a percentage of the sampled time spent freezing. In order to respect parametric requirements for ANOVA tests, percentages (P) were transformed using the equation  $Q = \text{Asin}(\sqrt{P/100})$  (Zar 2010).

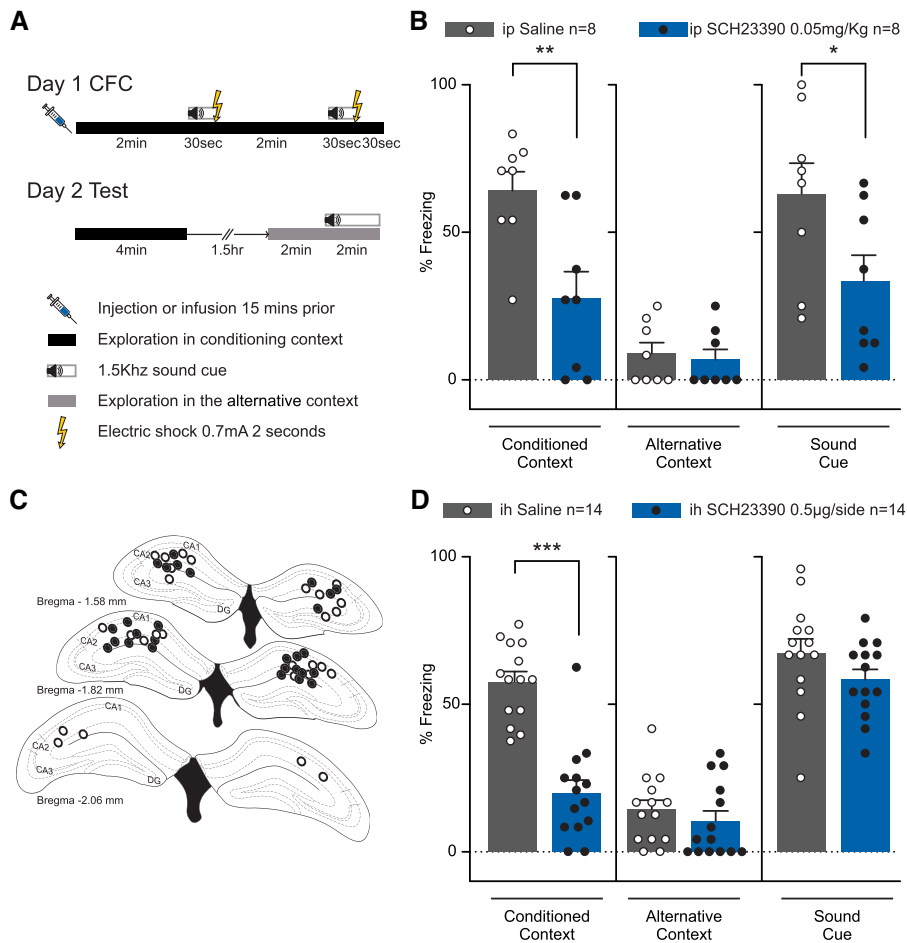
Contextual fear conditioning was performed as described in Daumas et al. (2005). Briefly, on the first day, mice were placed in the conditioned context for 2 min (27 × 27 × 27 cm, with horizontal black and white stripes on one wall and vertical ones on the opposite, metal rod floor, white light). Then, a sound cue (1.5 kHz, 85 dB, 30 sec) was emitted, ending with an electric shock (0.7 mA, 2 sec) delivered by a grid floor. This sequence was repeated and followed by 30 sec of exploration before taking the mouse back to its home cage. The next day, freezing behavior was measured in the same context for 4 min to assess contextual memory. An hour and a half later, freezing was measured in an alternative context (triangular shape, transparent Plexiglas walls and floor, red light) for 2 min to assess generalized fear, followed by two more minutes with the sound cue to assess tone fear memory (Fig. 1A). Although other laboratories reported a competition between the context and the sound cue leading to reduced contextual freezing when the sound cue was paired with the shock (Trifilieff et al. 2007), the experimental settings used in our laboratory were chosen in order to allow us to obtain similar and high freezing rates for both the context and the sound cue. This allowed us to assess the effects of treatments on both types of memory in the same animals (Daumas et al. 2005; Ceccom et al. 2013; Remaud et al. 2014).

In experiment 1, mice received an intraperitoneal injection of either SCH23390 (0.05 mg/kg) or saline (0.9% NaCl) 15 min before the conditioning session. Freezing in the conditioned context

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**Figure 1.** Hippocampal D1/5R dopamine receptors are necessary for contextual fear memory but not for tone fear memory. (A) Representative schema describing the contextual fear conditioning protocol used to assess contextual and tone fear memory. (B) Twenty-four hours after training, freezing to the context is lower for mice treated with intraperitoneal (ip) injection of SCH23390 15 min before training (blue bar, black circles) compared with those injected with saline (gray bar, white circles). Both groups show comparable low levels of freezing behavior in the alternative context. Tone fear memory is also reduced by SCH23390 intraperitoneal injection. (C) Intrahippocampal (ih) infusion sites of saline (white circles) or SCH23390 (0.5 µg/side, black circles). (D) Twenty-four hours after training, freezing to the context is lower for mice treated with intrahippocampal infusion of SCH23390 (blue bar, black circles) 15 min before training, compared with those injected with saline (gray bar, white circles). Both groups show comparable low levels of freezing behavior in the alternative context. Tone fear memory is preserved even after SCH23390 infusion. (\*)  $P < 0.05$  *t*-test, (\*\*)  $P < 0.01$  *t*-test, (\*\*\*)  $P < 0.001$  *t*-test.

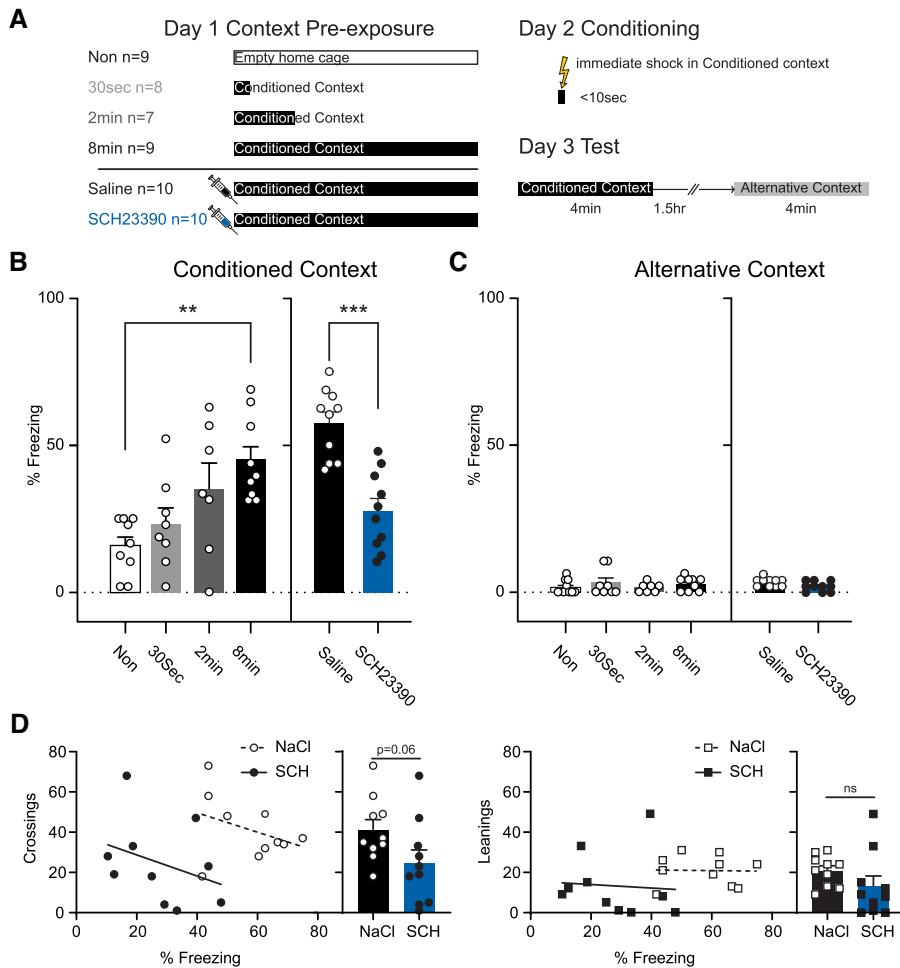
dropped from  $64\% \pm 6\%$  for the saline group to  $27\% \pm 9\%$  for the SCH23390 mice (*t*-test:  $P < 0.01$ ) (Fig. 1B). Similarly, freezing dropped in response to the tone, from  $63\% \pm 10\%$  to  $33\% \pm 9\%$  (*t*-test:  $P < 0.05$ ;  $n = 8$  per group) (Fig. 1B). Freezing behavior in the alternative context was  $<15\%$  and comparable between both experimental groups (*t*-test:  $P = 0.67$ ), indicating that freezing behavior was specific to acquired memories and that the dopamine D1/5R antagonist only affected memory-driven freezing. Here we replicated in mice previous studies showing that, in rats, injection of SCH23390 prior to training impairs long-term memory of contextual fear conditioning (Inoue et al. 2000; Heath et al. 2015). Interestingly, these two studies also show that injecting SCH23390 right after the training session had no effect. Our first experiment shows that D1/5R are involved in the acquisition of both contextual and tone-driven fear memories.

In experiment 2, we used the same fear conditioning protocol, but this time SCH23390 was delivered through intrahippocampal

infusion (0.5 µg/side, 0.35 µL/side); the control group received infusions of the same volume of saline. For this purpose, 2 wk prior to conditioning, we implanted guide cannulas aimed at CA1 (as described in Artinian et al. 2008). Infusion site locations were verified by infusing Chicago blue (2% in 0.5 M acetate) 3–5 d after the last behavioral session, which showed that all implanted mice had their infusions correctly centered in the hippocampus ( $n = 14$  per group) (Fig. 1C). Freezing in the conditioned context dropped from  $57\% \pm 4\%$  for the saline group to  $20\% \pm 4\%$  for the SCH23390 group (*t*-test:  $P < 0.001$ ) (Fig. 1D). Conversely, freezing in response to the auditory cue was preserved at  $67\% \pm 5\%$  and  $58\% \pm 3\%$  for saline and SCH23390 infusion, respectively. This difference was not significant (*t*-test:  $P = 0.123$ ) (Fig. 1D). Freezing behavior in the alternative context was  $<15\%$  and comparable between both experimental groups (*t*-test:  $P = 0.4$ ), showing that freezing behavior was specific to acquired memories and that SCH23390 only affected memory-driven freezing. Here we confirm that hippocampal D1/5R receptors are required for encoding contextual fear conditioning in mice, replicating previous data obtained in rats (Heath et al. 2015) and very recent data in mice (Tsetsenis et al. 2021). Interestingly, although hippocampal infusions of SCH23390 impaired contextual fear memory, it completely spared the concomitant Pavlovian tone-shock conditioning. Since D1/5R in the amygdala have been shown to be necessary for encoding tone-induced fear (Guarraci et al. 1999), it seems likely that D1/5R in the hippocampus are involved in the contextual fear memory but not in tone-driven memory, which probably involves D1/5R in the amygdala.

In experiment 3, we validated the CPFE. In this modified version of contextual fear conditioning, on day 1, mice were allowed to explore the context during

30 sec, 2 min, or 8 min. We used as control a non-pre-exposed group that explored an empty home cage for 8 min. On day 2, all animals received a very brief conditioning session ( $<10$  sec), during which they received one electric shock (0.7 mA, 2 sec) in the conditioning context without any sound cue. Tests took place on day 3. Mice were placed for 4 min in the context associated with the shock in order to test contextual memory. Ninety minutes later, they were placed for 4 min in the alternative context in order to test for generalized fear (Fig. 2A). To make sure that odor association did not happen, we used nonalcoholic makeup remover wipes (Ysiance, aloe vera) to clean the contexts on the first 2 d and alcohol solution on day 3. The experimenter who conducted the conditioning session on day 2 was different from the experimenter on days 1 and 3 in order to avoid any association between the context, the experimenter, and the shock. As expected, the non-pre-exposed animals exhibited very limited freezing behavior in response to the associated context ( $15\% \pm 3\%$ ); this low freezing



**Figure 2.** D1/5R dopamine receptors are involved in contextual memory formation. (A) Representative schema describing the CPFE protocol used to separate contextual from associative memory components of contextual fear conditioning. (B) Progressive increase in fear memory with increased pre-exposure time, as shown by the increase in freezing behavior in the conditioning context. Mice pre-exposed for 8 min (black bar, white circles) freeze significantly more than non-pre-exposed mice. Injection of SCH23390 (blue bar, black circles) significantly prevents the effect of CPFE, as compared with saline-injected animals (black bar, white circles). (C) All six groups of mice show comparable low levels of freezing behavior in the alternative context, indicating that fear memory is specific of the conditioned context. (D) A slight decrease in locomotion and exploration is observed during pre-exposure in SCH23390-injected mice that does not correlate with freezing measured on the day of test. (\*\*) $P < 0.01$  Dunnett's test following one-way ANOVA ( $P < 0.01$ ), (\*\*\*) $t$ -test  $P < 0.001$ .

response is supposed to be due to the short duration of exposure to the context on day 2, which is not sufficient to initiate a conjunctive representation of the context (Fanselow 2000; Barrientos et al. 2002). However, freezing behavior gradually increased with longer pre-exposure time; indeed, mice froze for  $23\% \pm 5\%$ ,  $35\% \pm 9\%$ , and  $45\% \pm 5\%$  of the time in the conditioned context when pre-exposed for 30 sec, 2 min, or 8 min, respectively (one-way ANOVA,  $P < 0.01$ ). The group pre-exposed for 8 min was the only one to freeze significantly more than the control group (Dunnett's multiple comparison for non-pre-exposed vs. 30 sec:  $P = 0.68$ ; vs. 2 min:  $P = 0.15$ ; vs. 8 min:  $P < 0.01$ ) (Fig. 2B). Freezing behavior in the alternative context remained  $<10\%$  for all groups, showing that freezing was related to a specific memory of the context even for the non-pre-exposed group. Altogether, we validated the CPFE experiment and identified that mice need 2–8 min to encode a new context without any aversive or appetitive stimuli.

In experiment 4, mice underwent the CPFE experiment with a pre-exposure lasting 8 min and received an intraperitoneal injection

of either SCH23390 (0.05 mg/kg) or saline 15 min prior to pre-exposure. During the pre-exposure, SCH23390 reduced the locomotion, calculated by separating the context floor into four sections and counting how many times the animal crosses from one section to another ( $41.2 \pm 5.1$  vs.  $24.6 \pm 6.6$  crossings) (Fig. 2D) and the exploration ( $20.9 \pm 2.4$  vs.  $13.2 \pm 5.0$  leanings against the walls) (Fig. 2D). However, this effect was highly variable and did not reach statistical significance ( $t$ -tests:  $P = 0.06$  and  $P = 0.19$ , respectively). Mice injected with SCH23390 froze significantly less than mice receiving saline ( $27\% \pm 4\%$  and  $57\% \pm 4\%$ , respectively;  $t$ -test:  $P < 0.001$ ) (Fig. 2B). In this case also, freezing in the alternative context remained  $<10\%$ , showing that freezing was elicited by contextual memory (Fig. 2C). There was no correlation between locomotion or exploration during the pre-exposure and the freezing level observed during the test session in any group (Pearson's  $P$ :  $[-0.36; -0.07]$  and  $P$ :  $[0.31; 0.94]$ ) (Fig. 2D). Thus, the impact of SCH23390 on locomotor activity during context pre-exposure cannot account for its effect on learning. While this experiment does not rule out a possible role for dopamine in encoding the value of rewarding or aversive stimuli, it clearly shows that dopamine is involved in encoding a new context by itself, in the absence of an aversive or rewarding outcome.

We show, on the one hand, that local injection of SCH23390 in the hippocampus impairs contextual fear conditioning while sparing Pavlovian tone-shock conditioning. On the other hand, intraperitoneal injection before the pre-exposure to the context impairs the pre-exposure facilitation effect. It would thus be tempting to conclude that hippocampal D1/5 receptors are mandatory for conjunctive contextual learning. However, since we did not assess

the effect of a local injection of SCH23390 in the hippocampus during the pre-exposure, we cannot rule out that the effects of SCH23390 observed in experiment 4 could rely on the inhibition of dopamine receptors outside the hippocampus.

Encoding a new context, as our mice did during the pre-exposure session, corresponds to what Duzsikiewicz et al. (2019) called "distinct novelty." According to these investigators, it relies on dopamine released in the hippocampus from axons of locus coeruleus noradrenergic neurons. However, Tsetsenis et al. (2021) elegantly showed that midbrain dopamine neurons innervating the hippocampus are actually able to promote contextual fear conditioning, while genetic depletion of monoamine synthesis in the locus coeruleus has no influence on either a weak or a strong contextual fear conditioning protocol. According to this latest report, the effects of SCH23390 observed in our study would most likely rely on the inhibition of the dopamine inputs arising from the midbrain. Since dopamine originating from the locus coeruleus and from the midbrain is supposed to exert its effects on memory

through activating D1/5R, pharmacological experiments cannot address this issue. To further characterize the pathways involved in encoding new contexts, optogenetic experiments will be required.

In conclusion, consistent with previous data, we show that D1/5R in the dorsal hippocampus are essential for contextual fear conditioning, whereas D1/5R located outside the hippocampus, most likely in the amygdala, are required for Pavlovian tone-shock association. Using the CPFE procedure, we provide additional evidence that D1/5R dopamine receptors are required to encode a conjunctive representation of a new context, without any reward or punishment.

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