# Dopamine D2 receptor gate generalization of conditioned threat responses through mTORC1 signaling in the extended amygdala

## Supplementary Data

Dimitri De Bundel[1,2,3]*, Charleine Zussy[1,2,3], Julie Espallergues[1,2,3], Charles R Gerfen[4], Jean-Antoine Girault[5,6,7], Emmanuel Valjent[1,2,3].

[1]CNRS, UMR5203, Institut de Génomique Fonctionnelle, Montpellier, F-34094, France.

[2]Inserm, U1191, Montpellier, F-34094, France.

[3]Universités de Montpellier, UMR-5203, Montpellier, F-34094, France.

[4]Laboratory of Systems Neuroscience, National Institute of Mental Health, Bethesda, Maryland 20892, USA.

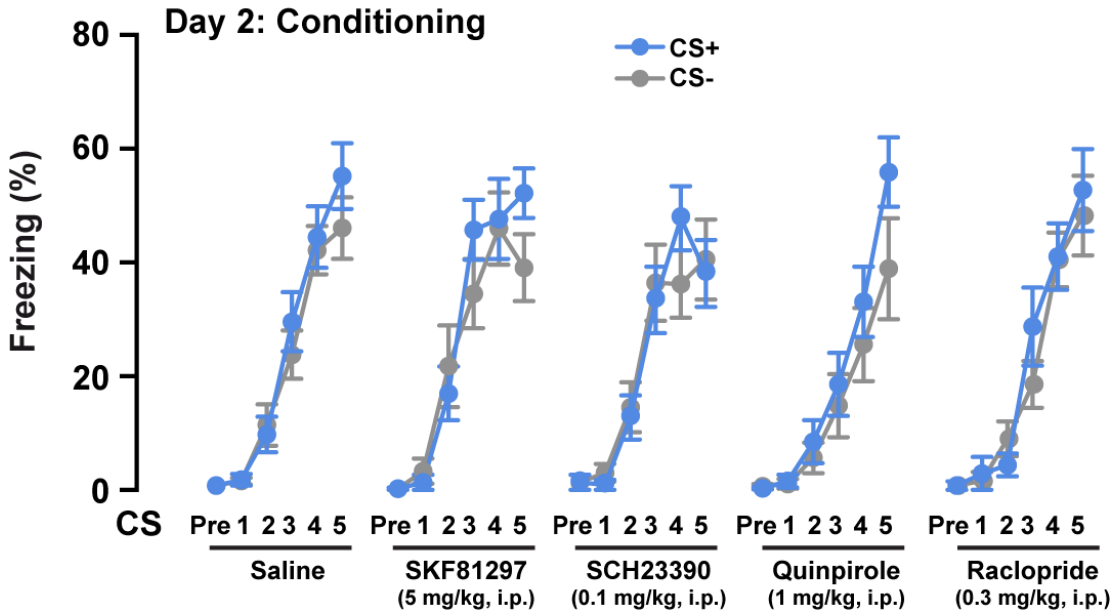
[5]Inserm, UMR-S 839, F-75005, Paris, France.

[6]Institut du Fer à Moulin, F-75005, Paris, France.

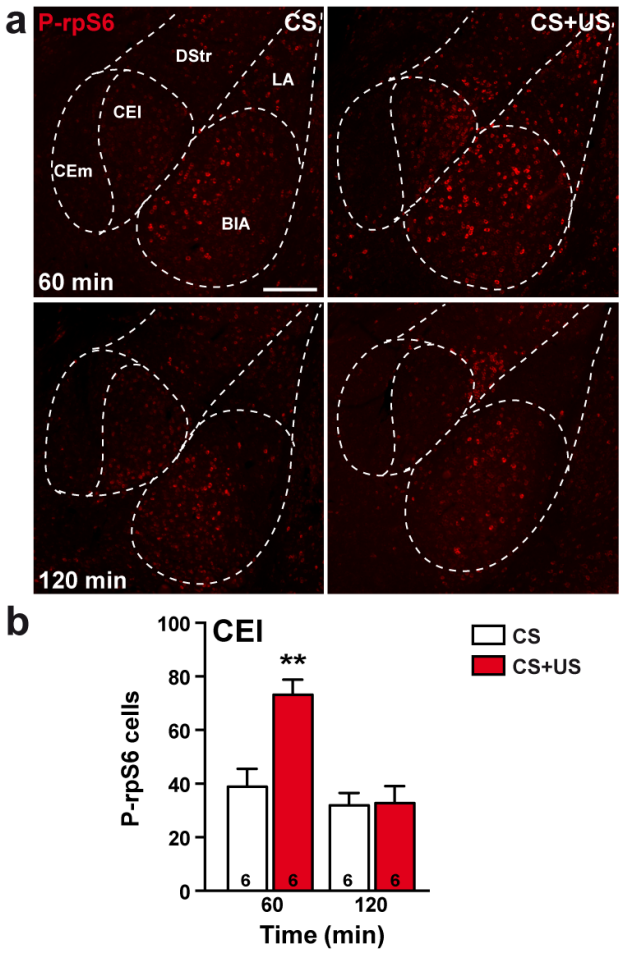
[7]Sorbonne Universités, UPMC, Université Paris 06, F-75005, Paris, France.

*present address: Department of Pharmaceutical Chemistry and Drug Analysis, Center for Neurosciences, Vrije Universiteit Brussel, 103 Laarbeeklaan, 1090 Brussels, Belgium.

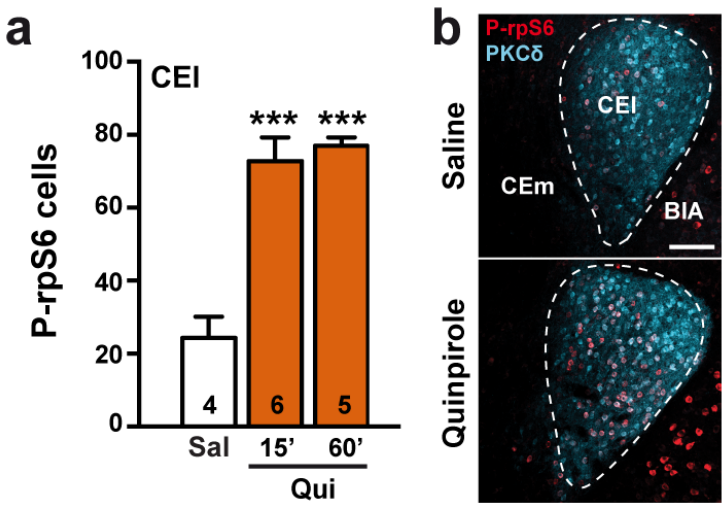
Correspondence should be addressed to: Emmanuel Valjent, Inserm U1191, CNRS UMR 5203, Institut de Génomique Fonctionnelle, 141 rue de la Cardonille, 34094 Montpellier Cedex 05, France. E-mail: emmanuel.valjent@igf.cnrs.fr; emmanuel.valjent@gmail.com or Dimitri De Bundel, Dept of Pharmaceutical Chemistry & Drug Analysis, Center for Neurosciences, Vrije Universiteit Brussel, 103 Laarbeeklaan, 1090 Brussels, Belgium. E-mail: ddebunde@vub.ac.be




**Supplementary Figure 1: Auditory Pavlovian conditioning.** Conditioning phase during which mice were exposed to CS+ or CS- cues during 15 min. Drugs were injected after conditioning (see legend to Fig. 1). The number of animals in each condition is indicated in the bars of Fig. 1a. Values are means ± s.e.m. Statistical analysis, repeated measure ANOVA (values in Supplemental Table 1: S1).

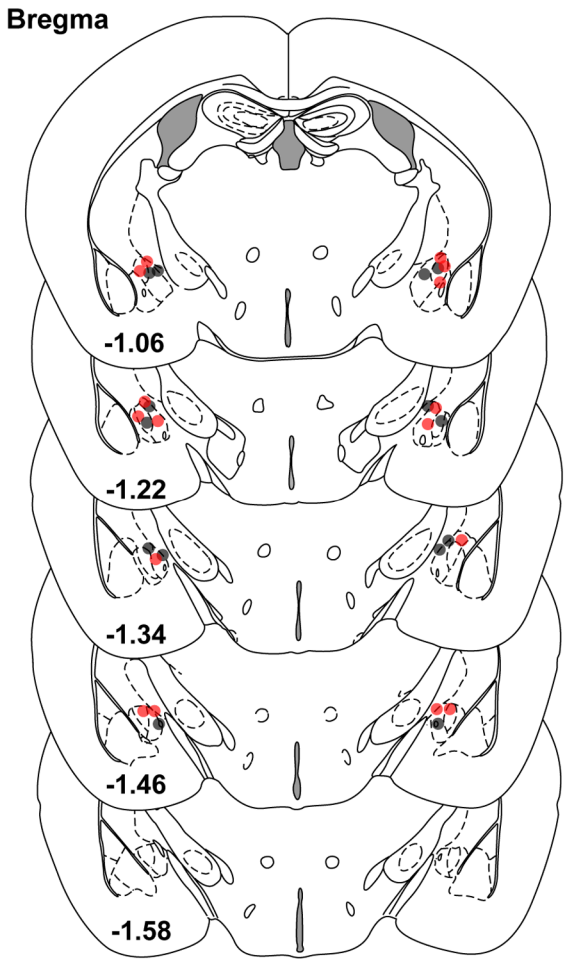



**Supplementary Figure 2: Pavlovian conditioning increases P-rpS6 immunoreactivity in CEA.** (**a**) P-rpS6 immunofluorescence in the CEA from mice perfused at different time point after conditioning (60 and 120 min). Scale bar, 200 μm. (**b**) Number of P-rpS6 positive cells in the CEl of mice exposed to CS alone (white bars) or paired with US (red bars). The number of animals in each condition is indicated in the bars. Values are means + s.e.m. Statistical analysis, two-way ANOVA (values in Supplemental Table 1: S2b) and Tukey's test, ** $p < 0.01$ CS vs CS+US.

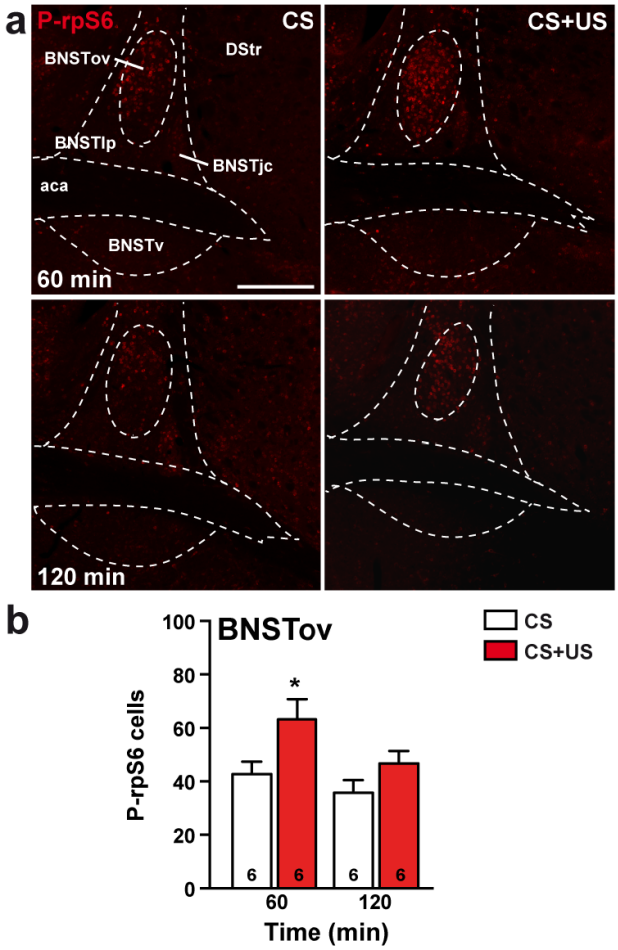



**Supplementary Figure 3: Quinpirole increases the P-rpS6 in CEl PKCδ$^{+}$ cells.**

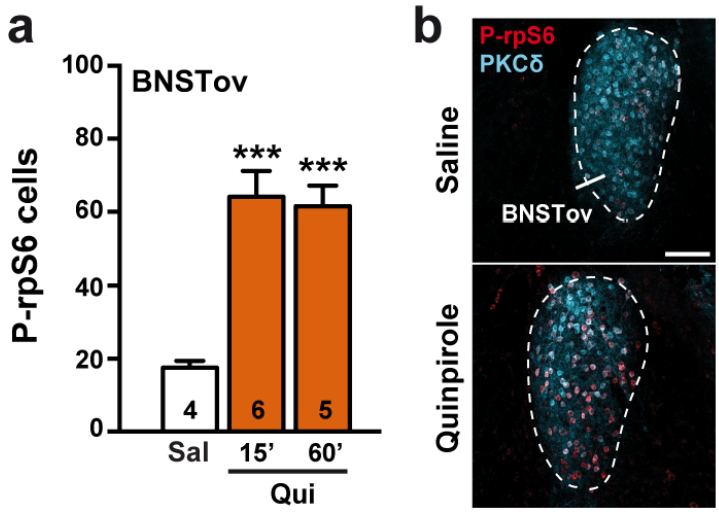
(**a**) Number P-rpS6 positive cells in the CEl of mice treated with saline or quinpirole (1 mg/kg, i.p.). Mice were treated with saline or quinpirole and perfused 15 min (15') or 60 min (60') later. Values are means + s.e.m. Statistical analysis, one-way ANOVA (values in Supplemental Table 1: S3a) and Tukey's test, *** $p < 0.001$ saline vs quinpirole. (**b**) Double immunostaining for P-rpS6 (red) and PKCδ (cyan) in the CEl following quinpirole administration (1 mg/kg, i.p.). Scale bar, 100 μm.



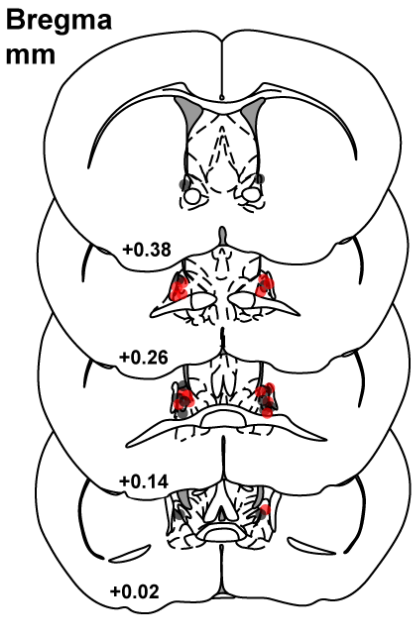

**Supplementary Figure 4: Intra-CEA raclopride infusion sites.** Localization of cannula hits for bilateral infusion of raclopride into the CEA. Mice microinjected with saline (black dots, n = 7 mice) and raclopride (red dots, n = 8 mice).



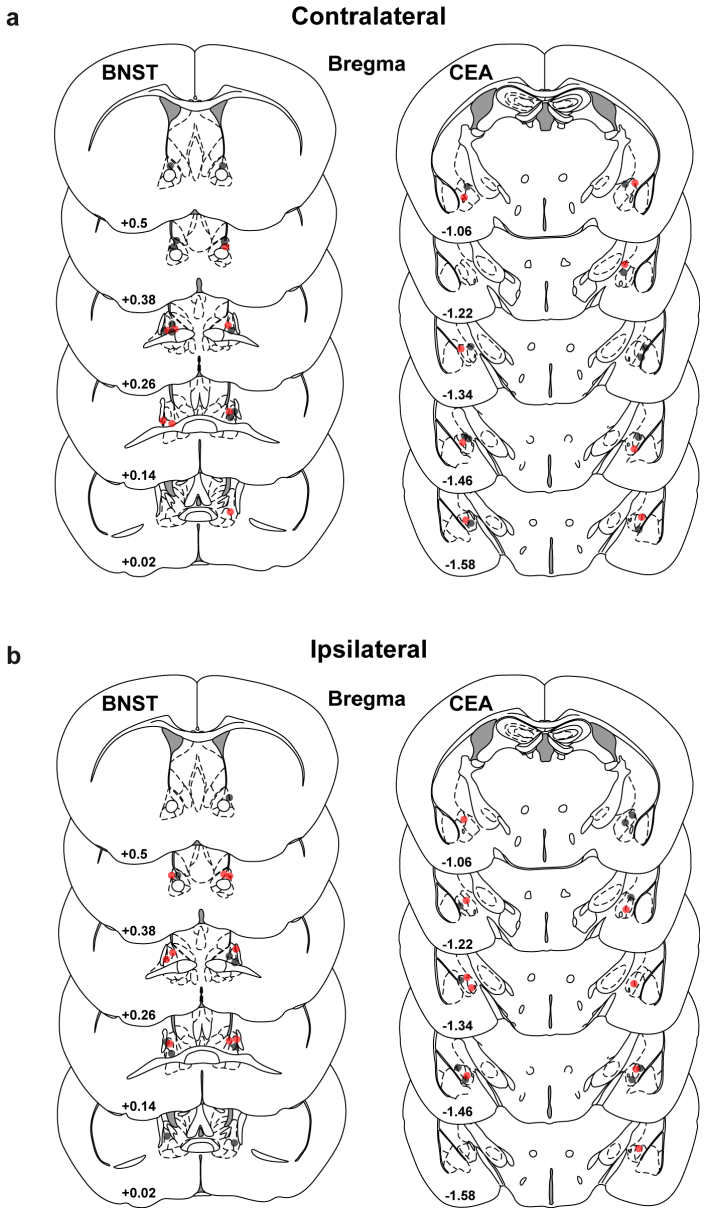

**Supplementary Figure 5: Pavlovian conditioning increases P-rpS6 in the BNST.** (**a**) P-rpS6 immunofluorescence in the BNST from mice perfused at different time point after conditioning (60 and 120 min). Scale bar, 200 μm. (**b**) Number of P-rpS6 positive cells in the BNSTov of mice exposed to CS alone (white bars) or paired with US (red bars). The number of animals in each condition is indicated in the bars. Values are means + s.e.m. Statistical analysis, two-way ANOVA (values in Supplemental Table 1: S5b) and Tukey's test, * $p < 0.05$ CS vs CS+US.



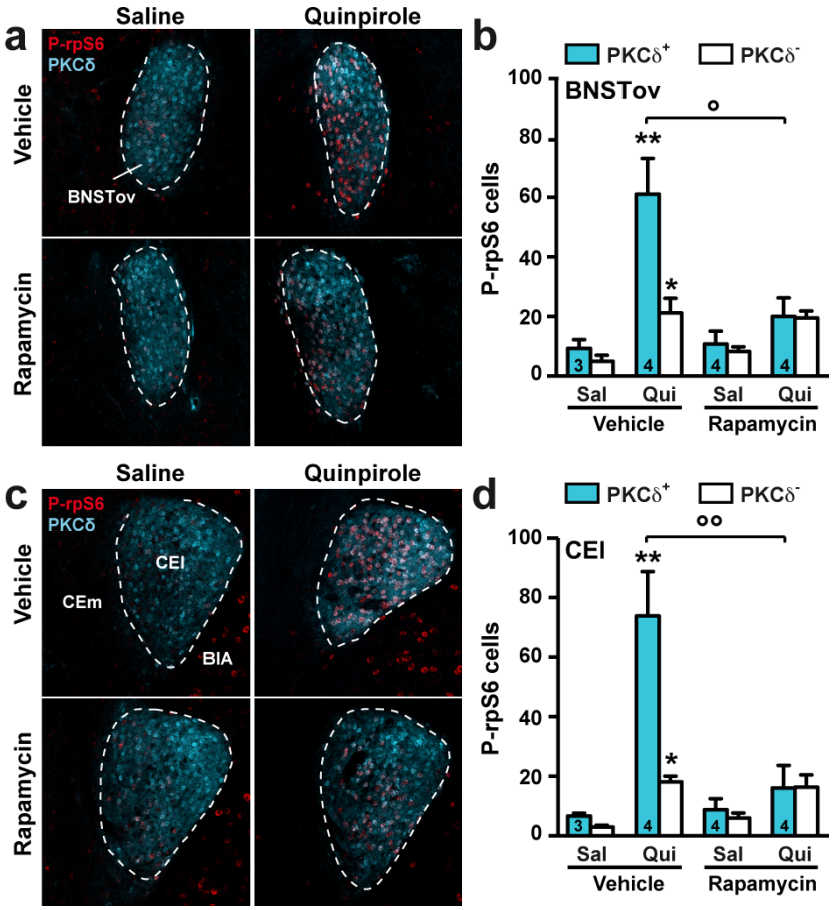

**Supplementary Figure 6: Quinpirole increases P-rpS6 immunofluorescence in BNSTov PKCδ$^{+}$ cells.** (**a**) Number P-rpS6 positive cells in the BNSTov of mice treated with saline or quinpirole (1 mg/kg, i.p.). Mice were treated with saline or quinpirole and perfused 15 min (15') or 60 min (60') later. Values are means + s.e.m. Statistical analysis, one-way ANOVA (values in Supplemental Table 1: S6a) and Tukey's test, *** $p < 0.001$ saline vs quinpirole. (**b**) Double immunostaining for P-rpS6 (red) and PKCδ (cyan) in the BNSTov following quinpirole administration (1 mg/kg, i.p.). Scale bar, 100 μm.




**Supplementary Figure 7: Intra-BNST raclopride infusion sites.** Localization of cannula hits for bilateral infusion of raclopride into the BNST. Mice microinjected with saline (black dots, n = 7 mice) and raclopride (red dots, n = 8 mice).




**Supplementary Figure 8: Pharmacological disconnection between the CEA and the BNST.** (**a**) Localization of cannula hits for contralateral infusion (i.e. disconnection) of raclopride into the BNST and CEA. Saline-infused mice (black dots, n = 11 mice), raclopride-infused mice (red dots, n = 8 mice). (**b**) Localization of cannula hits for ipsilateral infusion of raclopride in the BNST and the CEA. Saline infused mice (black dots, n = 9 mice), raclopride infused mice (red dots, n = 9 mice).




**Supplementary Figure 9: mTORC1 inhibition prevents quinpirole-induced rpS6 phosphorylation.** (**a**) Immunofluorescence of P-rpS6 (red) and PKCδ (cyan) in the BNSTov following saline or quinpirole administration (1 mg/kg, i.p.) in mice pre-treated with vehicle or rapamycin (5 mg/kg, i.p.). Scale bars, 100 μm. (**b**) Number of P-rpS6-positive cells in $PKC\delta^+$ or $PKC\delta^-$ neurons in the CEl of mice treated as in (**a**). * $p < 0.05$, ** $p < 0.01$ for comparison between saline and quinpirole; ° $p < 0.05$, °° $p < 0.01$, for comparison between vehicle and rapamycin. In (**b**) and (**d**), values are means + s.e.m., the number of animals in each condition is indicated in the bars, and statistical analysis done with two-way ANOVA (Values in Supplemental Table 1: 9b and 9d) followed by Tukey's test.

**Supplementary Table 1: Statistical Analysis**

| **Figure** | **Groups** (n: number of mice) | **Statistical Analysis** |
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| | | |
| 1b | Saline (n = 10)<br>SCH23390 (n = 9)<br>SKF81297 (n = 9)<br>Raclopride (n = 12)<br>Quinpirole (n = 12) | Repeated measures ANOVA<br>Cue $F_{(2, 115)} = 115.12$, $p < 0.0001$<br>Treatment $F_{(4, 56)} = 0.56$, $p = 0.69$<br>Interaction $F_{(8, 115)} = 2.71$, $p = 0.0091$ |
| 1c | Saline (n = 10)<br>Quinpirole (n = 10) | Repeated measures ANOVA<br>Cue $F_{(2, 40)} = 36.17$, $p < 0.0001$<br>Treatment $F_{(1, 20)} = 0.30$, $p = 0.59$<br>Interaction $F_{(2, 40)} = 5.89$, $p = 0.0057$ |
| | | |
| 2f | Saline CS (n = 6)<br>Saline CS+US (n = 6)<br>Raclopride CS (n = 6)<br>Raclopride CS+US (n = 6) | Two-way ANOVA ($PKC\delta^+$)<br>Cue $F_{(1, 20)} = 14.42$, $p = 0.0011$<br>Treatment $F_{(1, 20)} = 11.34$, $p = 0.0031$<br>Interaction $F_{(1, 20)} = 8.284$, $p = 0.0093$<br><br>Two-way ANOVA ($PKC\delta^-$)<br>Cue $F_{(1, 20)} = 2.381$, $p = 0.1385$<br>Treatment $F_{(1, 20)} = 1.121$, $p = 0.3023$<br>Interaction $F_{(1, 20)} = 0.2645$, $p = 0.6127$ |
| 2g | Quinpirole 0 mg/kg (n = 6)<br>Quinpirole 0.02 mg/kg (n = 4)<br>Quinpirole 0.1 mg/kg (n = 5)<br>Quinpirole 1 mg/kg (n = 4) | Repeated measures ANOVA<br>Cell-type $F_{(1, 15)} = 35.07$, $p < 0.0001$<br>Treatment $F_{(3, 15)} = 19.66$, $p < 0.0001$<br>Interaction $F_{(3, 15)} = 8.56$, $p = 0.0015$ |
| 2i | Saline (n = 7)<br>Raclopride (n = 8) | Repeated measures ANOVA<br>Cue $F_{(2, 24)} = 18.50$, $p < 0.0001$<br>Treatment $F_{(1. 12)} = 1.49$, $p = 0.25$<br>Interaction $F_{(2, 24)} = 2.16$, $p = 0.13$ |
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| 3f | Saline CS (n = 6)<br>Saline CS+US (n = 6)<br>Raclopride CS (n = 6)<br>Raclopride CS+US (n = 6) | Two-way ANOVA ($PKC\delta^+$)<br>Cue $F_{(1, 20)} = 6.679$, $p = 0.0177$<br>Treatment $F_{(1, 20)} = 16.92$, $p = 0.0005$<br>Interaction $F_{(1, 20)} = 9.349$, $p = 0.0062$<br><br>Two-way ANOVA ($PKC\delta^-$)<br>Cue $F_{(1, 20)} = 0.4362$, $p = 0.5165$<br>Treatment $F_{(1, 20)} = 1.212$, $p = 0.2841$<br>Interaction $F_{(1, 20)} = 2.185$, $p = 0.1549$ |
| 3g | Quinpirole 0 mg/kg (n = 6)<br>Quinpirole 0.02 mg/kg (n = 4)<br>Quinpirole 0.1 mg/kg (n = 5)<br>Quinpirole 1 mg/kg (n = 4) | Repeated measures ANOVA<br>Cell-type $F_{(1, 15)} = 218.5$, $p < 0.0001$<br>Treatment $F_{(3, 15)} = 49.37$, $p < 0.0001$<br>Interaction $F_{(3, 15)} = 25.27$, $p < 0.0001$ |
| 3i | Saline (n = 7)<br>Raclopride (n = 8) | Repeated measures ANOVA<br>Cue $F_{(2, 26)} = 18.34$, $p < 0.0001$<br>Treatment $F_{(1, 13)} = 0.53$, $p = 0.46$<br>Interaction $F_{(2, 26)} = 3.77$, $p = 0.036$ |
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| 4a | Saline (n = 11)<br>Raclopride (n = 8) | Repeated measures ANOVA<br>Cue $F_{(2, 36)} = 23.02$, $p < 0.0001$ |

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| | | Treatment $F_{(1, 18)} = 0.23$, $p = 0.64$<br>Interaction $F_{(2, 36)} = 7.62$, $p = 0.087$ |
| 4b | Saline (n = 9)<br>Raclopride (n = 9) | Repeated measures ANOVA<br>Cue $F_{(2, 32)} = 13.08$, $p < 0.0001$<br>Treatment $F_{(1, 16)} = 0.00023$, $p = 0.99$<br>Interaction $F_{(2, 32)} = 0.063$, $p = 0.94$ |
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| 5b | Vehicle CS (n = 6)<br>Vehicle CS+US (n = 6)<br>Rapamycin CS (n = 7)<br>Rapamycin CS+US (n = 8) | Two-way ANOVA ($PKC\delta^+$)<br>Cue $F_{(1, 20)} = 14.39$, $p = 0.0009$<br>Treatment $F_{(1, 20)} = 3.720$, $p = 0.0662$<br>Interaction $F_{(1, 20)} = 13.05$, $p = 0.0015$<br><br>Two-way ANOVA ($PKC\delta^-$)<br>Cue $F_{(1, 23)} = 0.005$, $p = 0.9451$<br>Treatment $F_{(1, 23)} = 0.8199$, $p = 0.3746$<br>Interaction $F_{(1, 23)} = 1.066$, $p = 0.3125$ |
| 5d | Vehicle CS (n = 6)<br>Vehicle CS+US (n = 6)<br>Rapamycin CS (n = 7)<br>Rapamycin CS+US (n = 8) | Two-way ANOVA ($PKC\delta^+$)<br>Cue $F_{(1, 23)} = 9.366$, $p = 0.0055$<br>Treatment $F_{(1, 23)} = 3.202$, $p = 0.0867$<br>Interaction $F_{(1, 23)} = 4.577$, $p = 0.0432$<br><br>Two-way ANOVA ($PKC\delta^-$)<br>Cue $F_{(1, 23)} = 3;369$, $p = 0.0794$<br>Treatment $F_{(1, 23)} = 0.8351$, $p = 0.3703$<br>Interaction $F_{(1, 23)} = 1.411$, $p = 0.2471$ |
| 5e | Vehicle (n = 7)<br>Rapamycin (n = 8) | Repeated measures ANOVA<br>Cue $F_{(2, 28)} = 15.02$, $p < 0.0001$<br>Treatment $F_{(1, 14)} = 1.171$, $p = 0.2975$<br>Interaction $F_{(2, 28)} = 5.218$, $p = 0.0119$ |
| 5f | Vehicle (n = 7)<br>Rapamycin (n = 8) | Repeated measures ANOVA<br>Cue $F_{(2, 28)} = 45.45$, $p < 0.0001$<br>Treatment $F_{(1, 14)} = 2.669$, $p = 0.1246$<br>Interaction $F_{(2, 28)} = 3.036$, $p = 0.0641$ |
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| S1 | Saline (n = 10)<br>SCH23390 (n = 9)<br>SKF81297 (n = 9)<br>Raclopride (n = 12)<br>Quinpirole (n = 12) | Repeated measures ANOVA<br>Time $F_{(5, 280)} = 114.8$, $p < 0.0001$<br>Treatment $F_{(4, 56)} = 1.20$, $p = 0.32$<br>Interaction $F_{(20, 280)} = 1.44$, $p = 0.10$ |
| S2b | CS 60 min (n = 6)<br>CS+US 60 min (n = 6)<br>CS 120 min (n = 6)<br>CS+US 120 min (n = 6) | Two-way ANOVA<br>Cue $F_{(1, 20)} = 8.779$, $p = 0.0077$<br>Time $F_{(1, 20)} = 15.94$, $p = 0.0007$<br>Interaction $F_{(1, 20)} = 7.97$, $p = 0.011$ |
| S3a | Saline (n = 4)<br>Quinpirole 15 min (n = 6)<br>Quinpirole 60 min (n = 5) | One-way ANOVA<br>$F_{(2, 11)} = 23.25$, $p = 0.0003$ |
| S5b | CS 60 min (n = 6)<br>CS+US 60 min (n = 6)<br>CS 120 min (n = 6)<br>CS+US 120 min (n = 6) | Two-way ANOVA<br>Cue $F_{(1, 20)} = 5.95$, $p = 0.024$<br>Time $F_{(1, 20)} = 10.70$, $p = 0.0038$<br>Interaction $F_{(1, 20)} = 0.97$, $p = 0.33$ |
| S6a | Saline (n = 4)<br>Quinpirole 15 min (n = 6)<br>Quinpirole 60 min (n = 5) | One-way ANOVA<br>$F_{(2, 11)} = 17.03$, $p < 0.0004$ |

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| S9b | Vehicle Saline (n = 3)<br>Vehicle Quinpirole (n = 4)<br>Rapamycin Saline (n = 4)<br>Rapamycin Quinpirole (n = 4) | Two-way ANOVA ($PKC\delta^{+}$)<br>Quinpirole $F_{(1, 11)} = 15.35$, $p = 0.0024$<br>Rapamycin $F_{(1, 11)} = 6.512$, $p = 0.0269$<br>Interaction $F_{(1, 11)} = 7.471$, $p = 0.0195$<br><br>Two-way ANOVA ($PKC\delta^{-}$)<br>Quinpirole $F_{(1, 11)} = 18.89$, $p = 0.0012$<br>Rapamycin $F_{(1, 11)} = 0.0562$, $p = 0.817$<br>Interaction $F_{(1, 11)} = 0.6245$, $p = 0.4461$ |
| S9d | Vehicle Saline (n = 3)<br>Vehicle Quinpirole (n = 4)<br>Rapamycin Saline (n = 4)<br>Rapamycin Quinpirole (n = 4) | Two-way ANOVA ($PKC\delta^{+}$)<br>Quinpirole $F_{(1, 11)} = 16.01$, $p = 0.0021$<br>Rapamycin $F_{(1, 11)} = 8.959$, $p = 0.0122$<br>Interaction $F_{(1, 11)} = 10.36$, $p = 0.0082$<br><br>Two-way ANOVA ($PKC\delta^{-}$)<br>Quinpirole $F_{(1, 11)} = 22.58$, $p = 0.0006$<br>Rapamycin $F_{(1, 11)} = 0.05533$, $p = 0.8184$<br>Interaction $F_{(1, 11)} = 0.7989$, $p = 0.3905$ |