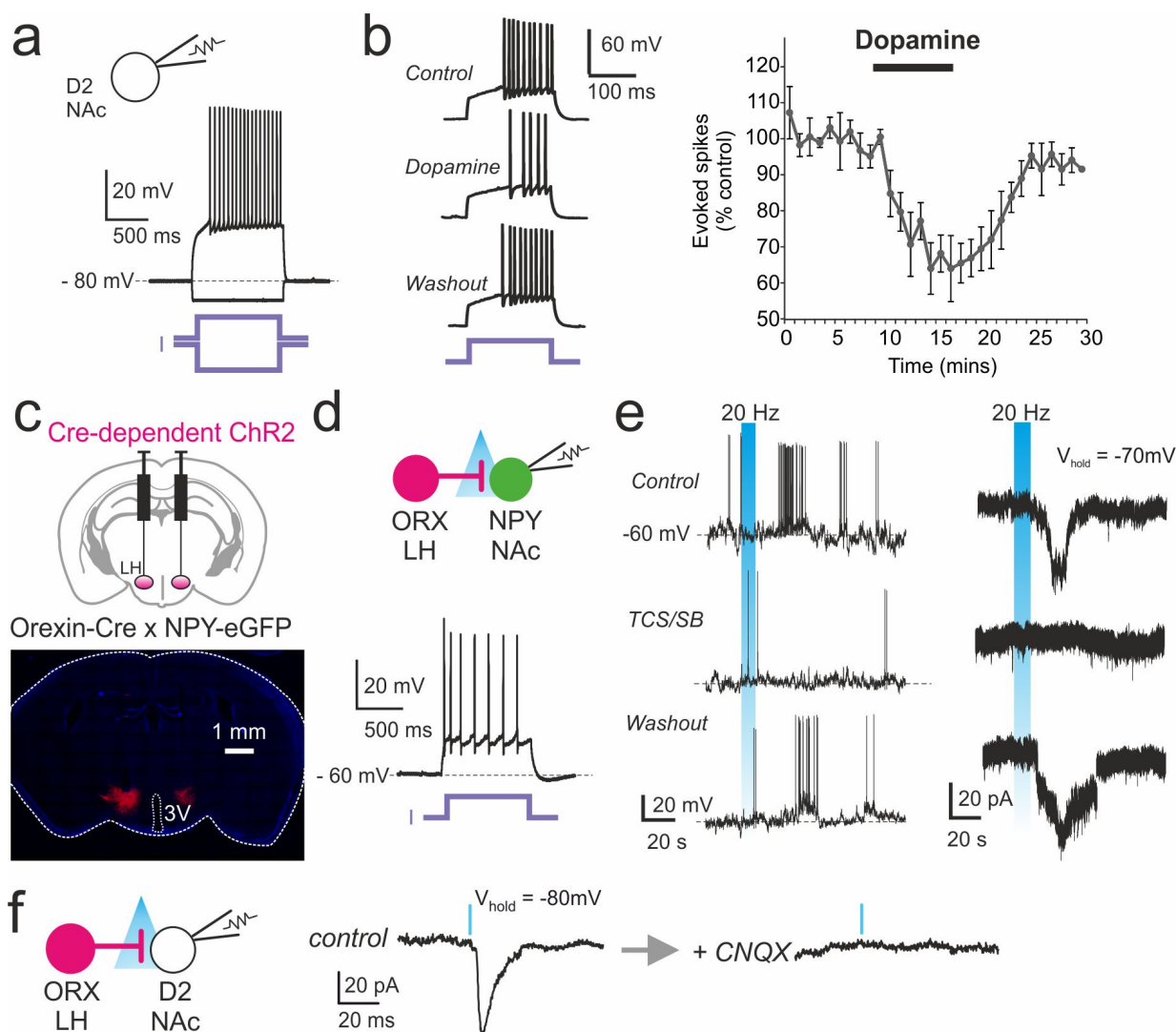


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Accumbal D2 cells orchestrate innate risk-avoidance according to orexin signals

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Supplementary Figure 1

Supplementary optogenetic and electrophysiological data

A. Studying electrical properties of NAc D2 neurons. Top: recording schematic. Bottom: classical electrophysiological dynamics (responses to current injections, purple) used to identify NAc D2R neurons (performed in every cell studied).

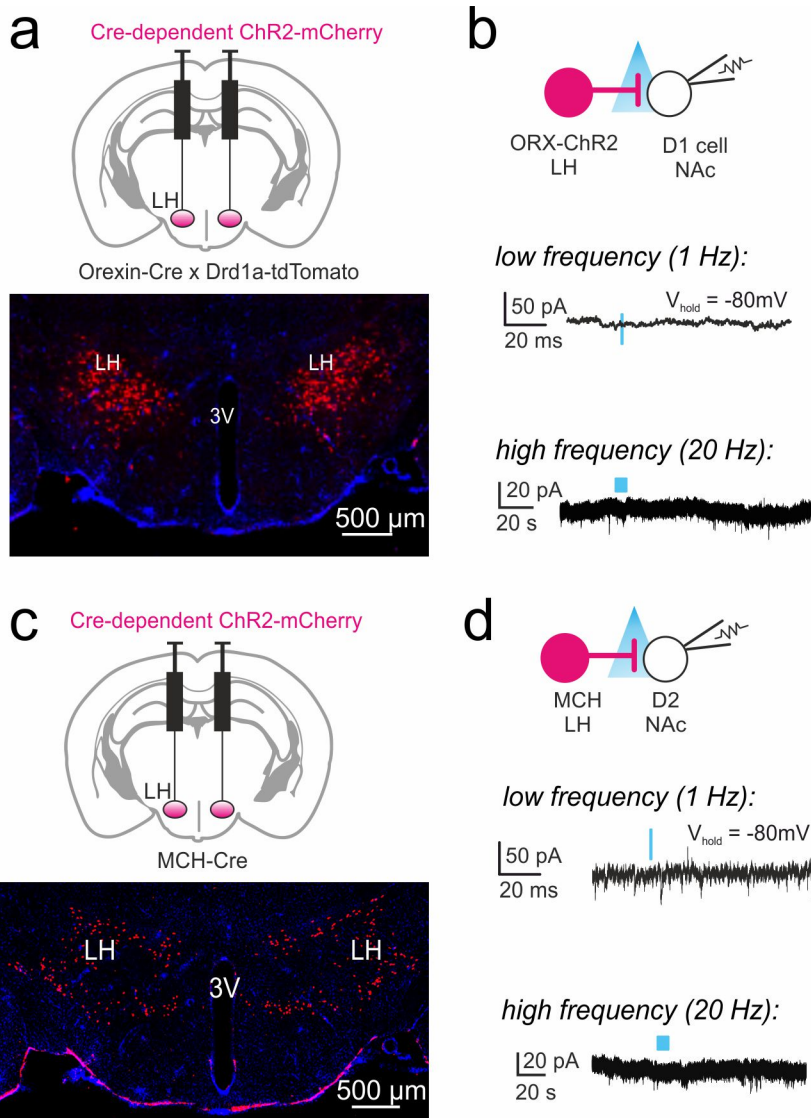
B. Left: effect of bath-application of dopamine (40 mM) on D2 cell membrane potential responses to excitatory current injection (purple). Representative example of n = 8/8 cells (all cells responded). Right: group data (means±sem) illustrating inhibition of the evoked spiking for dopamine, n = 8 cells.

C. Top: targeting scheme for studying responses of NAc NPY interneurons to photo-stimulation of LH orexin cells. Bottom: Expression of ChR2 in the LH (typical example of n = 5 brains).

D. Top: recording schematic for studying LH orexin→NAc NPY circuit. Bottom: confirmation that NAc NPY-eGFP neurons display defining electrical signatures of NAc NPY interneurons (response to current injection, purple, performed in every cell studied).

E. Effect of orexin axon photostimulation on NAc NPY interneuron membrane potential (left) and current (right), in the presence of orexin receptor antagonists TCS/SB (see Supplementary Methods). Representative responses of n = 11 cells.

F. Effect of low-frequency (1 Hz) orexin cell stimulation of NAc D2 cells (individual flash is shown here; this effect is representative of all flashes in the 1 Hz 10 s train), revealing a low-probability (n = 13/64 cells), CNQX-sensitive input.



Supplementary Figure 2

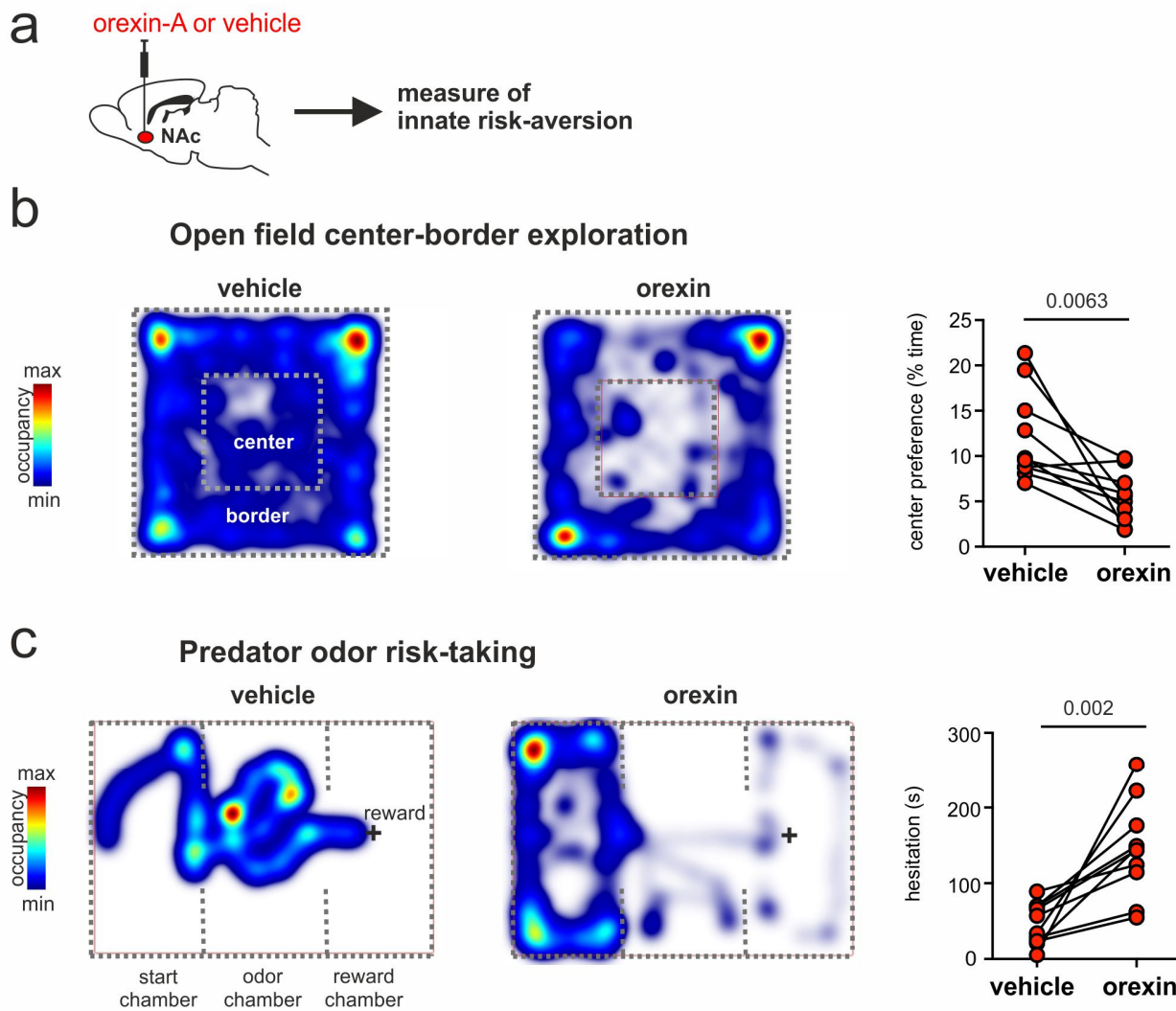
Supplementary data relating to cell-type-specific LH→NAc functional connectivity mapping

A, B. Probing orexin cell→D1 cell interaction. A, Targeting strategy (top) and resulting expression of mCherry (red) in LH (bottom), representative example of $n = 5$ brains showing expression localization typical of orexin cells.

B, Recording scheme (top), and effects of low and high frequency orexin axon photo-stimulation on D1 cells (representative example of $n = 48$ neurons, 48/48 cells showed no detectable responses).

C, D. Probing MCH→D2 cell interaction. C, Targeting strategy (top) and resulting expression of mCherry (red) in LH (bottom), representative example of $n = 5$ brains showing expression localization typical of MCH cells.

D, Recording scheme (top), and effects of low and high frequency MCH axon photo-stimulation on D2 cells (representative example of $n = 24$ neurons, 24/24 cells showed no detectable responses).



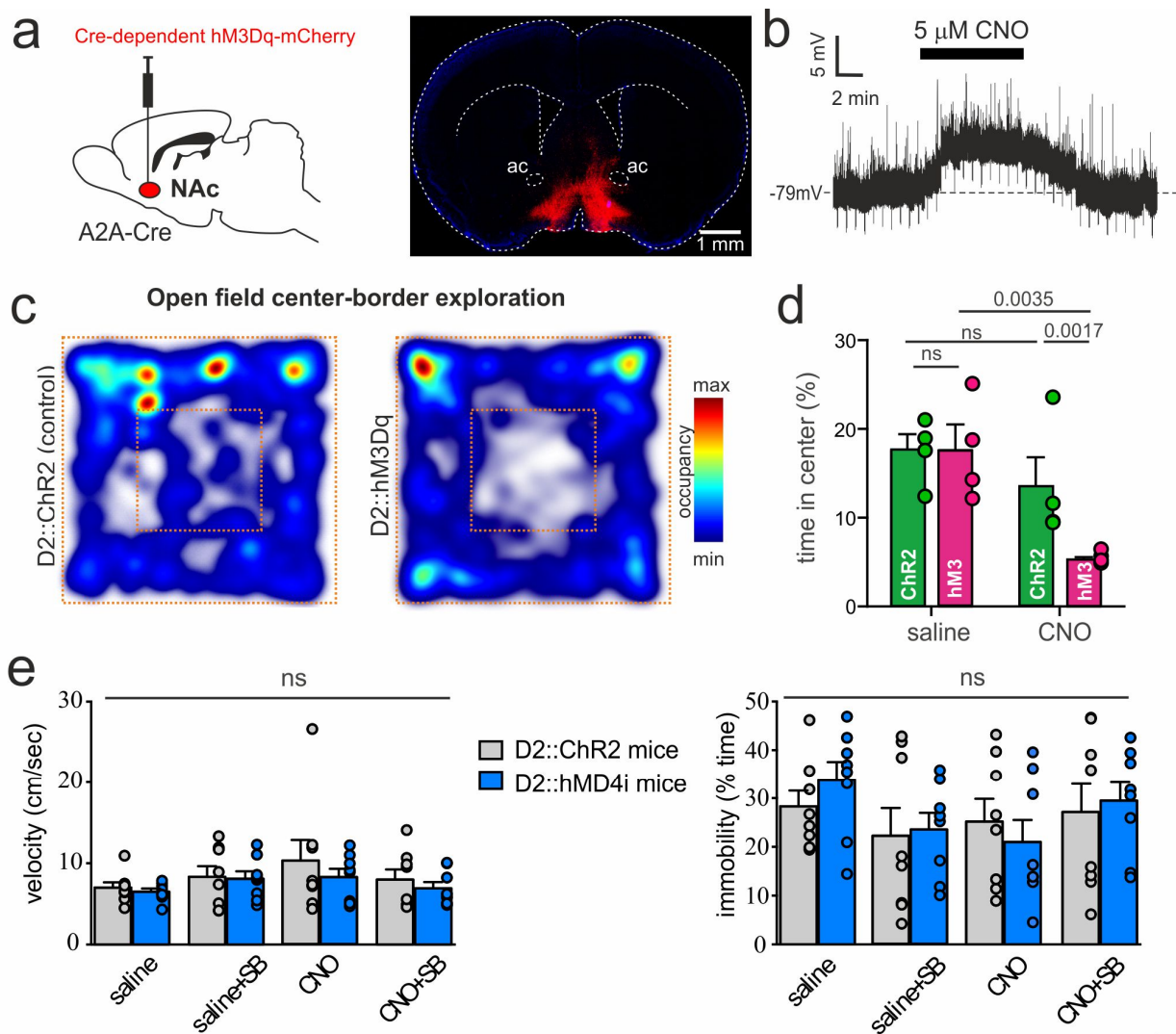
Supplementary Figure 3

Probing sufficiency of NAc orexin for innate risk-avoidance behaviors

A. Experimental paradigm: orexin or control vehicle solutions were infused bilaterally into accumbens via implanted cannulae, followed by behavioral assays, see Supplementary Methods.

B. Effect of orexin on center preference in the open-field test. Left and center, examples of spatial exploration heatmaps from the same mouse infused with orexin or vehicle solutions. Right, effect of orexin or vehicle infusion on centre occupancy (% time during the first 15 min of arena exploration) in $n = 10$ mice. P value is from a two-tailed paired t test, $t = 3.545$ $df = 9$. In the same tests, orexin did not significantly affect locomotion velocity (vehicle = 7.24 ± 3.5 cm/sec; orexin = 6.04 ± 0.96 cm/s; $p = 0.176$ by two-tailed paired t-test, $t = 1.468$ $df = 9$).

C. Effect of orexin on hesitation in the PORT test. Left and center, examples of spatial exploration heatmaps from the same mouse infused with orexin or vehicle solutions. Right, effect of orexin or vehicle infusion on hesitation to entry of middle (odor) chamber in $n = 10$ mice. P value is from a two-tailed paired t test, $t = 4.281$ $df = 9$.



Supplementary Figure 4

Effect of NAc D2 cell chemogenetic excitation on innate risk-avoidance behavior, and control experiments ruling out locomotor sedation as a confounding factor in open field experiments.

A. Left, targeting scheme for hM3Dq-mCherry; Right, localization of hM3Dq-mCherry (red); ac = anterior commissure; representative example of $n = 4$ brains.

B. Effect of CNO on membrane potential of a D2-hM3Dq cell (representative example of $n = 10$ cells; mean $\pm$ sem depolarization = 7.92 ± 0.91 mV, $p < 0.0001$ by two-tailed one-sample t-test; $t, df = 8.666, 9$).

C. Example of spatial exploration heatmaps from CNO-injected mice expressing ChR2 or hM3Dq in NAc D2 cells.

D. Effect of CNO on centre occupancy (% time during the first 15 min of arena exploration) in mice expressing hM3Dq or ChR2 in NAc D2 cells. 4 mice per group, values are individual mice and means $\pm$ sem. Two-way repeated measures ANOVA $F(1,6) = 9.715$, $p = 0.0207$; numbers above bars are p-values from Sidak's post tests (ns = > 0.2).

E. Effect on drug treatments on measures of sedation (locomotion velocity and immobility) in the open field test used to assess center-border preference. $n = 8$ mice in each group, values are individual mice and means $\pm$ sem. No significant effects were found: Right plot, two-way repeated-measures ANOVA $F(3,42) = 0.2342$, $p = 0.872$ (ns); Left plot, two-way repeated-measures ANOVA $F(3,42) = 0.6046$, $p = 0.616$ (ns).