Figures and figure supplements

A genetic link between discriminative fear coding by the lateral amygdala, dopamine, and fear generalization

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Figure 1. Impaired fear discrimination and c-Fos activation in the LA of DAT-NR1 KO mice. (A) Fear conditioning paradigm. Mice were probed for freezing in context B (top right) to the CS+ and CS− with three presentations of each stimulus (middle) prior to fear conditioning (Pre) and 24 hr after each conditioning session (Test 1 and Test 2). Mice were conditioned in context A (top left) with 10 presentations of a CS− or CS+ co-terminating with US delivery (bottom). (B) Freezing behavior (% Time Immobile) during presentation of the CS+ and CS− during pre-conditioning and Test 1 and Test 2. (C) Brain atlas image (left) (Paxinos and Franklin, 2013) illustrating LA subdivisions (gray shading) analyzed for c-Fos induction following fear conditioning. Representative c-Fos immunoreactive cells in the LA of control (control, left) and DAT-NR1KO (KO, right) mice following a single fear conditioning session. Scale bar = 250 μm (D) Average c-Fos positive cells in the LA of Ctrl and KO mice following fear conditioning (n = 3 mice each group, 8 sections/mouse). p < 0.01, unpaired Student’s T-test.
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Figure 2. Population activity in the LA is not enhanced in DAT-NR1 KO mice following footshock. (A) Brain atlas image (Paxinos and Franklin, 2013) illustrating bilateral tetrode implantation (top) and location of recording electrodes in Ctrl and KO mice. (B) Average waveform of recorded units in Ctrl and KO mice across days of conditioning. (C) Baseline firing rate of individual units in Ctrl and KO mice across days of conditioning (Control: n = 55, 52, and 54 Days 1–3, respectively; DAT-NR1 KO: n = 48, 57, and 58 Days 1–3, respectively). (D) Heat plot of normalized activity in concatenated CS+ trials from Ctrl (top) and KO (bottom) mice. (E) Percent change from baseline activity during CS+ trials following presentation of the US across days of conditioning. (F) Heat plot of normalized activity in concatenated CS− trials from Ctrl (top) and KO (bottom) mice. (G) Percent change from baseline activity during CS− trials following presentation of the US across days of conditioning. (E, G) Data are presented as the mean ± S.E.M. Repeated measures ANOVA, p < 0.001 and p < 0.05, Bonferroni post-test. DOI: 10.7554/eLife.08969.004
Figure 2—figure supplement 1. Population activity during fear conditioning. (A) Histogram of firing rate of an example neuron on day one of fear conditioning illustrating increased activity following presentation of the US. Arrow indicates onset of first footshock. (B) Proportion of activated neurons is highest in control mice on day 1 and decreases with subsequent conditioning. \( p < 0.001 \), Chi-square. (C, D) Heat plots of normalized firing rate for individual cells in control and DAT-NR1 KO mice during CS+/US (C) and CS− (D) presentation and subsequent ITI. DOI: 10.7554/eLife.08969.005
Figure 3. Transient plasticity in US-activated LA neurons is absent in DAT-NR1 KO mice. (A) Average waveform of recorded units in Ctrl and KO mice that were activated by the US. (B) Baseline firing rate of individual units in Ctrl and KO mice that were activated by the US. (C) Proportion of neurons from Ctrl and KO mice that were activated or inhibited by the US. (D) Average normalized firing rate of US activated neurons in Ctrl mice across days of conditioning. (E) Average normalized firing rate of US-activated neurons in KO mice across days of conditioning. (F) Average area under the curve (AUC) of activated response for Ctrl and KO mice across days. (G–I) Comparison of activated responses of Ctrl and KO mice during day 1 (G), day 2 (H), and day 3 of conditioning (I). Data are presented as the mean ± S.E.M. Repeated measures ANOVA, p < 0.001, Bonferroni post-test. DOI: 10.7554/eLife.08969.006
Figure 3—figure supplement 1. US-inhibited LA neurons do not change across days of conditioning. (A) Average normalized firing rate of US-inhibited neurons in control mice across days of conditioning (Control: n = 8, 16, and 14 Days 1–3, respectively; DAT-NR1 KO: n = 8, 8, and 9 Days 1–3, respectively). (B) Average normalized firing rate of US-inhibited neurons in DAT-NR1 KO mice across days of conditioning. (C) Average area under the curve (AUC) of inhibited response for control and DAT-NR1 KO mice across days. (D–F) Comparison of inhibited responses of control and DAT-NR1 KO mice during day 1 (D), day 2 (E), and day 3 of conditioning (F). Data are presented as the mean ± S.E.M.
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Figure 4. Plasticity in CS activated LA neurons is absent in DAT-NR1 KO mice. (A) Average waveform of recorded units in Ctrl and KO mice that were activated by the CS+ and CS−. (B) Baseline firing rate of individual units in Ctrl and KO mice that were activated by the CS+ and CS− (Control: n = 21, 22, and 19 Days 1–3, respectively; DAT-NR1 KO: n = 14, 23, and 23 Days 1–3, respectively). (C) Proportion of neurons from Ctrl and KO mice that were activated by the CS+ and CS−. (D) Average normalized firing rate of CS+ and CS− activated neurons in Ctrl and KO mice on day 1 of conditioning. (E) Average normalized firing rate of CS+ and CS− activated neurons in Ctrl and KO mice on day 3 of conditioning. Data are presented as the mean ± S.E.M. Repeated measures ANOVA, p < 0.001 and p < 0.01, Bonferroni post-test.
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Figure 4—figure supplement 1. Plasticity in CS activated LA neurons is absent in DAT-NR1 KO mice. (A) Average normalized firing rate of CS+ activated neurons in control mice across days of conditioning. (B) Average normalized firing rate of CS+ activated neurons in DAT-NR1 KO mice across days of conditioning. (C) Average normalized firing rate of CS− activated neurons in control mice across days of conditioning. (D) Average normalized firing rate of CS− activated neurons in DAT-NR1 KO mice across days of conditioning. (E) Average normalized firing rate of activated neurons during presentation of unpaired CS+ in control mice across days of conditioning. (F) Average normalized firing rate of activated neurons during presentation of unpaired CS− in control mice across days of conditioning. (G) Average normalized firing rate of CS+ and CS− activated neurons in unpaired Ctrl mice on day 1 of conditioning. (H) Average normalized firing rate of CS+ and CS− activated neurons in unpaired Ctrl mice on day 3 of conditioning. Data are presented as the mean ± S.E.M. Repeated measures ANOVA, ****p < 0.0001, Bonferroni post-test.
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Figure 4—figure supplement 2. CS activated LA neurons are not different at the start of conditioning. (A) Average normalized firing rate of CS+ and CS− activated neurons in Ctrl and KO mice during trial 1 of the first day of conditioning. (B) Average normalized firing rate of CS+ and CS− activated neurons in Ctrl and KO mice during trial 10 of the first day of conditioning.

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Figure 4—figure supplement 3. Differential response latencies in CS activated neurons between control and DAT-NR1 KO mice. (A) Average cumulative distribution of latency to increase activity in response to CS+ presentation in control mice does not change across days. (B) Average cumulative distribution of latency to increase activity in response to CS+ presentation in DAT-NR1 KO mice decrease across days. (C) Average cumulative distribution of latency to increase activity in response to CS− presentation in control mice decrease across days. (D) Average cumulative distribution of latency to increase activity in response to CS− presentation in DAT-NR1 KO mice decrease across days. (E) Average cumulative distribution of latency to increase activity in response to CS+ presentation in control vs DAT-NR1 KO mice on day 1. (F) Average cumulative distribution of latency to increase activity in response to CS+ presentation in control vs DAT-NR1 KO mice on day 3. (G) Average cumulative distribution of latency to increase activity in response to CS− presentation in control vs DAT-NR1 KO mice on day 1. (H) Average cumulative distribution of latency to increase activity in response to CS− presentation in control vs DAT-NR1 KO mice on day 3. Average normalized firing rate of CS+ and CS− activated neurons in unpaired Ctrl mice on day 3 of conditioning. Data are presented as the mean ± S.E.M. Repeated measures ANOVA, *p < 0.05, ****p < 0.0001, Bonferroni post-test. DOI: 10.7554/eLife.08969.011
Figure 5. Plasticity in CS inhibited LA neurons is absent in DAT-NR1 KO mice. (A) Average waveform of recorded units in Ctrl and KO mice that were inhibited by the CS+ and CS−. (B) Baseline firing rate of individual units in Ctrl and KO mice that were inhibited by the CS+ and CS− (Control: n = 21, 15, and 22 Days 1–3, respectively; DAT-NR1 KO: n = 13, 20, and 18 Days 1–3, respectively). (C) Proportion of neurons from Ctrl and KO mice that were inhibited by the CS+ and CS−. (D) Average normalized firing rate of CS+ and CS− inhibited neurons in Ctrl and KO mice on day 1 of conditioning. (E) Average normalized firing rate of CS+ and CS− inhibited neurons in Ctrl and KO mice on day 3 of conditioning. Data are presented as the mean ± S.E.M. Repeated measures ANOVA, p < 0.05, Bonferroni post-test.
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Figure 5—figure supplement 1. Plasticity in CS inhibited LA neurons is absent in DAT-NR1 KO mice. (A) Average normalized firing rate of CS + inhibited neurons in control mice across days of conditioning. (B) Average normalized firing rate of CS + inhibited neurons in DAT-NR1 KO mice across days of conditioning. (C) Average normalized firing rate of CS− inhibited neurons in control mice across days of conditioning. (D) Average normalized firing rate of CS− inhibited neurons in DAT-NR1 KO mice across days of conditioning. (E) Average normalized firing rate of CS+ and CS− inhibited neurons in Ctrl and KO mice during trial 1 of the first day of conditioning. (F) Average normalized firing rate of CS+ and CS− inhibited neurons in Ctrl and KO mice during trial 10 of the first day of conditioning. Data are presented as the mean ± S.E.M. Repeated measures ANOVA, **p < 0.01 and , ***p < 0.001, Bonferroni post-test.

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Figure 6  Synaptic plasticity in LA neurons is impaired in DAT-NR1 KO mice. (A) Brain atlas image illustrating placement of stimulating electrodes in the IC and EC and recording electrode in the LA. (B) Representative compound PSP following thalamic stimulation (left) and isolated EPSP and IPSP from Ctrl (middle) and KO (right) mice. (C, D) Excitation/inhibition ratios of EPSP/IPSPs of individual neurons from Ctrl and KO mice following cortical (C, Control: n = 7 naive, n = 8 shock; DAT-NR1: KO n = 9 naive, n = 11 shock) and thalamic (D, Control: n = 6 naive, n = 10 shock; DAT-NR1: KO n = 11 naive, n = 7 shock) stimulations. (E, F) Representative mEPSCs (E) and mIPSCs (F) from naive (black) and fear conditioned (gray) Ctrl and KO mice. (G, H) Cumulative distribution of mEPSC frequency from naive and fear conditioned control (G, n = 18 naive, n = 14 shock) and KO mice (H, n = 14 naive, n = 13 shock). (I, J) Cumulative distribution of mIPSC frequency from naive and fear conditioned control (I) and KO mice (J). Data are presented as the mean ± S.E.M. Repeated measures ANOVA, p < 0.0001, Bonferroni post-test.

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Figure 6—figure supplement 1. mEPSC and mIPSC amplitude does not change in LA neurons following fear conditioning. (A) Average amplitude of mEPSCs in naive and fear conditioned (shock) control and DAT-NR1 KO mice. (B) Average amplitude of mIPSCs in naive and fear conditioned (shock) control and DAT-NR1 KO mice. DOI: 10.7554/eLife.08969.015