

Figure S1. Supplemental information related to Figure 1. (**A**) Chromogenic *in situ* hybridization (ISH) analysis of the expression of Egr2 throughout the anterior-posterior axis of the claustrum. The boundaries of the claustrum are indicated on coronal sections corresponding to the atlas images located above the images. Numbers represent distance from Bregma (mm). (**B-C**) Coronal sections representing the overlap between frontal cortical afferents to the claustrum and CL_{Egr2+} neurons. Green labeling corresponds to AAV-CMV-GFP labeled projections from the ACA/ORB, while magenta represents the AAV-DIO-mCherry expression in CL_{Egr2+} neurons. (**B**) Afferents to the claustrum from the anterior cingulate cortex (ACA). (**C**) Afferents to the claustrum from the Orbitofrontal cortex (ORB). (**D**) Single-molecule fluorescent ISH (smFISH), demonstrating co-expression of vGlut1 and GAD65 in CL_{Egr2+} neurons. Individual channels are shown, as well as the merge of the three channels with DAPI (white background). Putative outlines of cell bodies are demarcated. (**E**) Representative images of CL_{Egr2+} anterograde tracing, demonstrating the scoring scale and color scheme used the qualitative numerical representation of anterograde tracing (**Figure 1J, N**).

C Delay time

(ms)

(fraction)

05

0.0

0.5

Side errors

Late responses **m**

G

Visit counr per day

Median RT relative to

80

1.00

0.7

0.75 0.50 0.25 0.00

fraction) 0.0

Delay time (ms): 160





Figure S2. Supplemental information related to Figure 2. (A) Summary of virus expression locations in experimental mice (light pink; n=6 per group). Infection spread was marked on histological sections (left insets), and digitally overlaid on an atlas image (right). (B) Representative traces of whole-cell current-clamp recordings from Kir2.1 expressing CL_{Egr2+} neurons, in comparison to adjacent control cells (top panels), demonstrating Kir2.1 suppression of firing in response to 150-300 pA current injections (bottom panels). (C-J) No effect of Kir2.1 inhibition of CL_{Enr2+} neurons on multiple behavioral parameters during variation of cue duration or delay time: (C) fraction of side errors; (D) fraction of premature responses; (E) fraction of late responses; (F) fraction of omitted trials; (G) visit count; (H) lick count; (I) median reaction time (RT) relative to cue onset; (J) median inter-trial interval (ITI). (K) Breakdown of error types during distractor session. CL_{Egr2+} deficient mice (Kir2.1) performed more premature responses in the presence of the distractor than in its absence. Marginal effect of the distractor p=0.015 (linear mixed effect model), contrast: Kir2.1.On-Kir2.1.Off p=0.025 (Tukey post hoc comparison with Bonferroni correction). (L-N) No effect of Kir2.1 inhibition of CL_{Enr2+} neurons was observed on multiple behavioral parameters in the session in which the distractor was included: (L) mean latency to premature responses; (M) mean visit count; (N) mean lick count. Bars represent group averages ± SEM.



Figure S3. Efficacy and efficiency of transduction of hMDi infection of CL_{Egra}, neurons (Related to Figure 2). (A) Representative traces of whole-cell current-clamp recordings from CL_{Egra}, neurons expressing the hM4Di DREADD, in the presence or absence of CNO (clozapine-N-oxide; 1 µM). 50-250 pA current injections (right) demonstrate the reduction of excitability in cells expressing hM4Di before (middle) and after (right) application of CNO. (B) Summary graph of the impact of CNO on the excitability of neurons expressing hM4Di. (C) Verification of viral expression in mice used for transient inhibition experiments (n=4). The site of expression in individual mice (light pink) was digitally overlayed on an atlas image. Right inset depicts a representative infection from a single mouse.



Figure S4. Analysis of the activity of dams during pup retrieval (Related to Figure 3). (A) Mean total distance travelled per single trial of pup retrieval. (B) Distance travelled to collect the pup (meter), marginal effect for injection type p=0.0588 (generalized mixed effect linear model). (C) Distance travelled returning to the nest (meter). (D) Average velocity to collect the pup (m/s). (E) Average velocity returning to the nest (m/s). (F) Fraction of time spent in the nest zone. Marginal effect for injection type p=0.0228 (generalized mixed effect linear model), contrast Saline.On-CNO. ON p=0.0402, (Tukey post hoc comparisons with Bonferroni corrections). (G) Example trajectories of a representative dam under different experiment conditions. Top: trajectory towards the pup. Bottom: trajectory returning to the nest. (H) Verification of viral expression in mice. Summary of virus expression locations in mice involved in the maternal pup retrieval assay during chemogenetic inhibition of CL_{Egr2+} neurons (light pink; n=5). Infection spread was marked on histological sections, and digitally overlaid on an atlas image.