Incubation of palatable food craving is associated with brain-wide neuronal activation in mice

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Abstract (245 words)

Studies using rodent models have shown that relapse to drug or food seeking increases progressively during abstinence, a phenomenon termed 'incubation of craving'. Mechanistic studies of incubation of craving have focused on specific neurobiological targets within pre-selected brain areas. Recent methodological advances in whole-brain immunohistochemistry, clearing, and imaging now enable unbiased brain-wide cellular resolution mapping of regions and circuits engaged during learned behaviors. However, these whole brain imaging approaches were developed for mouse brains while incubation of drug craving has primarily been studied in rats and incubation of food craving has not been demonstrated in mice. Here, we established a mouse model of incubation of palatable food craving and examined food reward seeking after 1, 15, and 60 abstinence days. We then used the neuronal activity marker Fos with intact brain mapping procedures to identify corresponding patterns of brain-wide activation. Relapse to food seeking was significantly higher after 60 abstinence days than after 1 or 15 days. Using unbiased ClearMap analysis, we identified increased activation of multiple brain regions, particularly corticostriatal structures, following 60, but not 15 abstinence days. We used orthogonal SMART2 analysis to confirm these findings within corticostriatal and thalamocortical subvolumes and applied expert-guided registration to investigate subdivision and layer-specific activation patterns. Overall, we (1) identified novel brain-wide activity patterns during incubation of food seeking using complementary analytical approaches, and (2) provide a single-cell resolution whole-brain atlas that can be used to identify functional networks and global architecture underlying incubation of food craving.

Significance Statement

Relapse to reward seeking progressively increases during abstinence, a phenomenon termed incubation of craving. Mechanistic studies of incubation can lead to novel relapse treatments. However, previous studies have primarily used rat models and targeted region-by-

region analyses and a brain-wide functional atlas of incubation of reward seeking is lacking. We established a behavioral procedure for incubation of palatable food seeking in mice and applied whole-brain activity mapping with Fos as a neuronal activity marker to identify the functional connectome of this incubation. Like rats, mice showed incubation of food seeking during abstinence. Using two complementary activity mapping approaches, we identified a brain-wide pattern of increased neural activation that mirrored incubation of food seeking after 60, but not 15, days of abstinence.

Main Text

Introduction

Studies using rodent models have shown that non-reinforced drug or food seeking progressively increases during abstinence in the home cage (1-3). This phenomenon, termed *'incubation of craving'*, was first identified in rats after cocaine self-administration (4-7), and has since been demonstrated for other drugs such as heroin (8), methamphetamine (9), alcohol (10), and nicotine (11), as well as for non-drug rewards such as sucrose (12, 13), standard chow pellets (14), and high-carbohydrate pellets (15). These preclinical findings mirror reports of incubation of cue-induced drug craving and physiological responses in human drug-users (16-18) and could provide avenues to identify cellular and molecular mechanisms underlying persistent relapse vulnerability to both unhealthy palatable food (3, 19) and addictive drugs (1, 2, 20-22).

Immediate early gene (IEG, eg. Fos, Arc, Zif) expression serves as a proxy for strongly activated neurons, and quantification of Fos-positive (Fos+) cells is routinely used to identify changes in neural activation patterns after exposure to different unconditioned and conditioned stimuli (23-27). Previous activity-mapping studies of incubation of drug and food craving have identified several brain regions (Supplementary Table S1) relevant to (1) relapse (increased Fos expression during relapse tests vs. home cage controls), and/or (2) incubation (higher Fos expression during late abstinence test vs. early abstinence test) (1, 3, 5, 28, 29). These studies used targeted one-by-one regional quantification of Fos+ cell counts in thin sectioned tissue

samples and focused on changes in specific pre-determined brain areas. Thus, it is currently unknown whether brain-wide activity patterns, including multi-regional patterns, are altered during incubation of reward seeking during abstinence.

To address this knowledge gap, we leveraged recent developments in brain-wide activity mapping approaches, including whole mouse brain immunofluorescent staining and clearing (30-35), light sheet fluorescence microscopy (LSFM) (36, 37), and open-source analysis tools (38-41) to investigate changes in brain-wide activation patterns during incubation of palatable food seeking in mice.

We trained food-sated CD-1 male mice to self-administer palatable high-carbohydrate food pellets (42, 43) for 7 days and then tested them for relapse to food seeking after 1, 15, or 60 abstinence days. We perfused and extracted their brains 90 min after the relapse tests (or directly from homecage as a baseline activity control), labeled 'active' Fos+ nuclei across intact mouse brains using an optimized iDISCO+ Fos immunofluorescent staining protocol (41), and imaged Fos immunofluorescence at single cell resolution using LSFM. We used the ClearMap pipeline (39, 40) for unbiased mapping of "incubation-associated" neural activation patterns across the entire anterior-posterior axis of the mouse brain. We also updated the SMART analysis package (41) to conduct targeted analysis of neural activation patterns within LSFM coronal subvolumes and used SMART2 to cross-validate and extend our ClearMap findings within a subset of corticostriatal and thalamocortical brain regions and subdivisions.

Materials and Methods

Subjects

We used male (n = 60) 4-6-month-old sexually experienced CD-1 mice (Charles River Lab, CRL), weighing ~40 g prior to food self-administration training. We confirmed with CRL animal-facility staff that all sexually experienced CD-1 males had equal access to receptive females. Per CRL's procedure male mice are pair-housed with several females from PD28 until purchase. Pregnant females are switched with new non-pregnant females, with no break between cycles

and male mice that do not successfully breed are removed from the breeding pool and not made available for purchase. We excluded 14 mice due to failure to acquire food self-administration. The mice had free access to food and water in the homecage and were maintained on a reverse 12:12 h light-dark cycle (light off at 8 am). We only used male mice because the behavioral study was performed before the implementation of the NIH Sex as a Biological Variable Guideline.

Apparatus

We trained and tested all mice in standard Med Associates operant chambers, enclosed in ventilated sound-attenuating cubicles. Each chamber was equipped with a stainless-steel grid floor and two side-walls, each with three modular operant panels. We used a houselight located on one side of the chamber to illuminate the chamber during the training and test sessions. Two levers served as operant manipulanda – (1) a non-retractable lever on the same side as the houselight served as the inactive lever and (2) a retractable lever on the side opposite the houselight served as the active lever; both levers were positioned 2.4 cm above the grid floor. We placed a yellow LED light for the food-paired conditioned stimulus (CS) above the active lever and equipped the central panel on the same side with a pellet receptacle connected to a pellet dispenser. Presses on the active lever (only extended during food self-administration sessions or food-seeking tests) resulted in delivery of 20-mg food pellets and a 2-s light CS (bright yellow LED), while presses on the inactive lever had no programmed consequences.

Behavioral procedures

The experimental timeline is shown in Figure 1A. Details of the food self-administration procedure, abstinence phase, and relapse test are provided below.

Food self-administration

The food self-administration procedure is based on our previous study (43). We gave mice free access to regular chow and water in their homecages during this phase and other phases of the experiment. Prior to the operant training sessions, we gave all mice one 30-min session of

food-magazine training. During this session mice received 15 evenly spaced (every 2 min) deliveries of a 20-mg palatable food pellet (TestDiet, Catalogue #1811142, 12.7% fat, 66.7% carbohydrate, and 20.6% protein) paired with a 2-s discrete cue light (Food-paired CS). Next, we trained the mice to lever press for palatable food reward during one 1-h session per day. The start of a session was signaled by the illumination of the house light followed 10-s later by the presentation of the central retractable active lever for 60 min. The houselight remained on for the duration of the session and served as a discriminative stimulus that signaled availability of the palatable food upon lever press.

Throughout the session, responses on the active lever were rewarded under a fixed-ratio-1 (FR1), 20-s timeout (TO) reinforcement schedule – active lever presses resulted in illumination of the food-paired CS for 2-s followed by the delivery of a palatable food pellet. Additional active responses during the 20-s timeout had no programmed consequence. Responses on the inactive lever had no programmed consequences throughout the session. We recorded (1) the total number of active lever presses, (2) the total number of inactive lever presses and (3) the total number of food pellet rewards earned during the entire session. Lever-press data for initial training sessions was not recorded for 2 mice due to technical malfunction so we recorded their values as zero to include them in statistical analysis. We gave mice at least 7 training sessions to acquire stable food self-administration behavior before moving to the homecage forced abstinence phase.

We used the above mentioned TestDiet pellets, because both mice and rats prefer this pellet over other nutritional or flavor compositions and show reliable acquisition of food self-administration without any food deprivation (42, 44). Additionally, food-stated CD-1 male mice strongly preferred these food pellets over operant aggression self-administration (43), and food-sated male and female rats strongly prefer these pellets over methamphetamine, heroin, and fentanyl self-administration in rats (45, 46).

Forced abstinence

During the abstinence phase, we housed mice in individual cages in the animal facility for 1, 15 or 60 days with no access to palatable food pellets. We gave mice ad libitum access to regular chow and water during this phase and handled them once per week.

Relapse tests

Following abstinence, we tested mice for non-reinforced food seeking during a 30-min relapse test. During the test, responses on the active lever resulted in presentation of the Food-paired CS on the same FR1 – 20-s TO reinforcement schedule but were not reinforced with food pellets (extinction conditions). After the test, we returned mice to their homecage for 60-min prior to perfusions and brain tissue collection (n=11 for Day 1, n=12 for Day 15 and n=10 for Day 60). At each incubation timepoint, we also collected brains from food-trained mice directly from the homecage (n=4 for Homecage Day 1, n=6 for Homecage Day 15, n=3 for Homecage Day 60) and collapsed them into a single group (Homecage, n=13) to serve as baseline controls for whole-brain analysis. We matched mice from the four groups for food-reinforced responding during the training phase.

Whole brain Fos immunohistochemistry (IHC)

We used a modified version of the iDISCO+ protocol for intact mouse brain Fos IHC (41). We processed 37 brains across the 4 groups following perfusions (n=11 for Homecage, n=9 for Day 1, n=9 for Day 15 and n=8 for Day 60) based on perfusion quality, level of intactness, and behavioral data. Details of the sample collection, pretreatment, immunolabeling and clearing steps are provided in the sections below.

Sample collection

We anesthetized the mice with isoflurane and perfused them transcardially with 200 ml of 0.1 M phosphate buffered saline (PBS, pH 7.4) followed by 400 ml of 4% paraformaldehyde in PBS (4% PFA, pH 7.4). We extracted brains, post-fixed them for an additional 24-h in 4% PFA at 4°C, and then stored them in PBS with 0.1% sodium azide at 4°C prior to processing.

Sample pretreatment with methanol

We used 15 ml conical tubes for sample pretreatment and gently mixed tubes on a rotating mixer (Daigger Scientific, EF24935). We first washed the brains in PBS (3 x 30 min) at room temperature (RT) to remove 4% PFA. We then dehydrated samples in ascending concentrations of methanol (MeOH) in deionized H_2O (dH_2O) – 0%, 20%, 40%, 60%, 80%, 100%, 100% MeOH (RT, 1.5 h each). Next, we incubated samples in 66% Dichloromethane (DCM)/33% MeOH (RT, 1 x 8 h followed by overnight) for delipidation. We then washed samples in 100% MeOH (RT, 2 x 3 h each), and bleached them by incubating in a chilled H_2O_2 /H_2O/MeOH solution (1 volume 30% H_2O_2 to 5 volumes 100% MeOH) overnight at 4°C. Next, we rehydrated the samples in descending concentrations of MeOH in dH_2O - 80%, 60%, 40%, 20%, 0% MeOH (RT, 1.5 h each). We then washed the samples first in PBS (RT, 1 x 1 h), then three times in a buffer containing PBS with 0.5% TritonX-100 (PTx0.5) at 37°C (2 x 1 h, followed by 1 x overnight).

Immunolabeling

We used 1.5 ml Nalgene cryotubes for immunolabeling and 15 ml conical centrifuge tubes for permeabilization, blocking and wash steps. We performed staining over 7 days – we started with a lower initial concentration on day 1 (1° - 1:1000; 2° - 1:500), stepped up the concentration of antibody using booster doses (1° - 1:1000; 2° - 1:500) over the next 4 days, and let the samples incubate at the final concentration (1° - 1:200; 2° - 1:100) for an additional 2 days. We gently mixed the sample containers by rotation and always filled to the top to prevent oxidation. We first incubated the samples in permeabilization buffer containing 78.6% PTx0.5, 1.4% Glycine, and 20% Dimethyl Sulfoxide (DMSO), and then in blocking buffer containing 84% PTx0.5, 6% Normal Donkey Serum (NDS), and 10% DMSO (37°C, 2 d each). Next, we incubated the samples in primary (1°) antibodies (anti-cFos: Phospho-c-Fos (Ser32) (D82C12) XP® Rabbit mAb, Cell Signaling Technology, #5348S Lot 1; RRID#: AB_10557109) diluted in 1° antibody buffer containing 92% PTwH0.5, 3% NDS, and 5% DMSO (37°C, 7 d). We then performed washes in a buffer containing PBS with 0.5% Tween-20, and 10ug/ml Heparin (PTwH0.5) over 4

183 days (37°C, 4 x 12 h followed by 2 x 1 d). We then incubated the samples in secondary (2°)
184 antibodies (Alexa Fluor® 647 AffiniPure F(ab')$_2$ Fragment Donkey Anti-Rabbit IgG (H+L), Jackson
185 ImmunoResearch Labs, 711-606-152, Lot 128806, RRID:AB_2340625; Alexa Fluor® 488
186 AffiniPure F(ab')$_2$ Fragment Donkey Anti-Chicken IgY (IgG) (H+L), Jackson ImmunoResearch
187 Labs, 703-546-155, Lot 127495, RRID:AB_2340375) diluted in 2° antibody buffer containing 97%
188 PTwH0.5, and 3% NDS (37°C, 7 d). Finally, we performed washes in PTwH0.5 over 4 days
189 (37°C, 4 x 8-12 h followed by 2 x 1 d).

190 Clearing

191 We used 15 ml conical centrifuge tubes for dehydration and delipidation, followed by glass
192 vials with Teflon (PTFE) caps for clearing and refractive index matching. We dehydrated samples
193 using ascending concentrations of MeOH in dH$_2$O – 0%, 20%, 40%, 60%, 80%, 100%, 100%
194 MeOH (RT, 1.5 h each) and performed delipidation using 66% dichloromethane (DCM)+33%
195 MeOH (RT, 2 x 3 h, followed by 1 x overnight). Next, we washed the samples in 100% DCM (RT,
196 2 x 3 h) to remove MeOH and incubated them in 100% dibenzyl ether (DBE) for clearing and
197 refractive index matching (RT, 2 x 3 h, followed by 1 x overnight).

198 **Whole-brain imaging and analysis**

199 We imaged stained and cleared intact mouse brains using LSFM. We used the 3D rendering
200 software Arivis Vision 4D (3.0.0) to stitch image tiles, manually corrected coronal alignment where
201 necessary, and exported the images as TIFF files for whole-brain analysis. We analyzed the data
202 using ClearMap (40) and SMART (41) analysis pipelines as described below.

203 Light-sheet fluorescent microscopy imaging (LSFM)

204 We used a light-sheet microscope (UltraMicroscope II with Infinity Corrected Objective
205 Lenses, Miltenyi Biotec) with an attached camera (Andor Zyla sCMOS), a 1.1x/0.1NA objective
206 (MI PLAN; LaVision BioTec), and a non-corrected dipping cap. Imaging parameters and
207 acquisition order were controlled through ImspectorPro software (v 7.1.4). We mounted cleared

Fos-stained brains in coronal orientation (olfactory bulb side up) using a custom sample platform and imaged at 2.2x effective magnification (1.1x objective x 2x magnification slider) in DBE. We acquired images for autofluorescence (Excitation: 488 nm laser, Emission: 535/43 bandpass filter) and Fos-IHC (Excitation: 647 nm laser, Emission: 690/50 bandpass filter) in separate 2 x 1 tiled scans (scan order: z-x-y). We used the following fixed parameters for acquisition: exposure = ~100 ms; sheet NA = 0.16; sheet thickness = 3.89 um; sheet width = 70%; zoom = 2x; dynamic horizontal focus = 5 (Fos channel only); dynamic horizontal focus processing = blend; merge light-sheet = blend; 488 nm laser power = 20%; 647 nm laser power = 50%. Final image pixel resolution was 2.956 um X x 2.956 um Y x 3 um Z. Resulting tiles were stitched into full size coronal planes using Arivis Vision 4D (3.0.0) and exported as TIFFs. During the analyses, we observed that illumination of the entire coronal plane by the light-sheet during the first imaged tile scan led to significant photo-bleaching of the second imaged tile in all samples. Therefore, we only used the first imaged hemisphere from each sample for analysis and mirrored outputs for all visualizations. We excluded 3 brains due to insufficient clearing and staining, and 2 brains due to technical issues during image acquisition. We analyzed LSFM data for 32 brains across the 4 groups (n=11 for Homecage, n=8 for Day 1, n=8 for Day 15, and n=5 for Day 60).

ClearMap analysis

We used the open-source program ClearMap 1.0 (40) for whole-brain volumetric analysis on a dedicated machine (Intel Xeon® CPU E5-2650 v4 @ 2.20GHz x 48; 4 x GeForce GTX 1080 Ti/PCIe/SSE2; 256GB RAM). We downsampled autofluorescence image stacks for each sample and registered them to a common 25 µm isotropic serial two-photon (STP) tomography reference template (47). We manually validated registration for each sample by post-hoc inspection of overlaid reference template and post-transformation image stacks in ImageJ. Three brains failed registration and were excluded from further analysis in ClearMap.

Next, we used the spot-detection method in ClearMap to automate Fos+ cell detection across all images. We used the same spot detection filter parameters for Fos+ cell detection across all samples (illumination correction: mean scaling; cell shape detection: threshold (150);

Find intensity: Mean and size (3,3,3); Background removal: pixel size (5,5); DoG filter: pixel size (6,6,11); Detect cell shape: threshold (150) and then applied a voxel size threshold (50,200000) to constrain the size of detected Fos+ cells.

We validated this Fos+ cell detection procedure against ground truth manual cell detection performed by 2 expert raters across five separate 300 um x 300 um regions of interest (ROIs) from 3 sample image volumes. For each sample, we first isolated an 81 mm coronal image stack (26 image z-stack) at ~1.46 from bregma along the anterior-posterior axis, and within it selected 5 ROIs encompassing a wide range of Fos+ cell density and background fluorescence signal. Two expert raters performed ground truth manual annotation of Fos+ cells in each FOV using ITK-SNAP software (48) version 3.8.0 (http://www.itksnap.org), resulting in ~3500 manually annotated Fos+ cells. We used the FIJI image analysis package (49) to overlay the automated ClearMap annotation over the expert annotation and used Analyze Objects and Image Calculator plugins to determine expert-rated Fos+ cell counts, ClearMap-rated Fos+ cell counts and overlap. We calculated precision (ratio of correctly predicted Fos+ cells to all predicted cells), recall (ratio of correctly predicted Fos+ cells to expert annotated Fos+ cells), and F-score (harmonic mean of precision and recall) in Microsoft Excel (Supplementary Figure S1A).

We warped all ClearMap detected Fos+ cells into the reference space by applying transformation coordinates from the registration step and obtained counts for individual brain regions based on the Allen Brain Institute atlas ontology provided with the ClearMap installation package. We extracted Fos+ cell counts for all 1205 annotations and used custom python scripts to generate summed counts within regions of interest (ROIs) for analysis of activity changes between groups.

SMART2 analysis

We used an updated version of the open-source R package SMART (41) (SMART2) for expert-guided registration and volumetric activity mapping within two coronal subvolumes selected from ClearMap analysis (subvolume 1: AP +1.55 to AP +1.75 relative to Bregma; subvolume 2: AP -1.08 to AP -1.28 relative to Bregma). SMART extends the WholeBrain

analytical framework (38) to volumetric analysis using mouse LSFM datasets and allows user-guided refinement of registration prior to automated Fos+ cell detection. We followed the steps outlined in the updated online tutorial (https://github.com/sgoldenlab/SMART2) for analysis. First, we set up sample information (animal ID, initials, paths, z spacing, registration step (space between z images), most anterior AP coordinate and z image number, most posterior AP coordinate and z image number), and file paths for each sample volume using the functions setup_pl(), im_sort(), and get_savepaths(). Next, we used the function choice() to align the entire sample volume to internal reference atlas plates along the anterior-posterior axis and used the function interpolate() to identify z-stack numbers corresponding to substacks of interest.

Next, we used the interpolated values to select 4 consecutive reference plates (100 mm spacing) for analysis of each coronal subvolume. We selected 2 'internal plates' to register the subvolume of interest (subvolume 1: ABA atlas plate numbers 36 and 37 corresponding to AP +1.6 and AP +1.7; subvolume 2: ABA atlas plate numbers 65 and 66 corresponding to AP -1.13 and AP -1.23) and specified 2 additional 'outer plates' to enable identification of Fos+ cells corresponding to 50 mm on either side of the registered internal plates.

This resulted in a final analyzed subvolume of 200 mm thickness in atlas space. We used the functions regi_loop() and filter_loop() for user-guided registration correction between autofluorescence channel images and atlas reference plates. We used the same initial parameters for all samples [alim = c(50, 50), threshold.range = c(50000, 60000L), eccentricity = 999L, Max = 3025, Min = 0, brain.threshold = 400, resize = 0.25, blur = 7, downsample = 2] and applied additional correspondence points to improve the automated registration output.

We then used the function seg_loop() for Fos+ cell detection throughout the subvolume of interest and the function clean_duplicates() to correct for duplicate detection of Fos+ cells across adjacent z-slices. We applied the same segmentation filter parameters for all samples [alim = c(4, 100), threshold.range = c(500, 2200), eccentricity = 300, Max = 2200, Min = 100, brain.threshold = 400, resize = 0.25, blur = 7, downsample = 2]. We validated the automated Fos+ cell detection

against ground truth expert annotation as described in the previous section and calculated precision, recall, and F-score in Microsoft Excel (Supplementary Figure S1B).

Finally, we used the forward_warp() function to warp detected Fos+ cells into the reference atlas space and obtained counts for individual brain regions based on the Allen Mouse CCF ontology provided in the WholeBrain package. We extracted Fos+ cell counts and used the get_rois() function to generate summed counts by ROI. We also implemented additional isolate_dataset(), cell_count_compilation(), get_groups(), and voxelize() functions to generate Fos+ cell counts and Fos+ cell density heatmaps for individual samples, and to output summary counts by experimental group. We provide these additional scripts as part of the updated SMART2 package repository (https://github.com/sgoldenlab/SMART2). We also provide a Docker installation image for rapid and user-friendly installation (https://hub.docker.com/repository/docker/goldenneurolab/wholebrain_smart2).

Statistical analyses

We analyzed behavioral and whole-brain Fos data using IBM SPSS Statistics (version 28) and GraphPad Prism (version 9.3). For the behavioral and whole brain analysis, alpha (significance) level was set at 0.05, two-tailed. We tested the data for sphericity and homogeneity of variance when appropriate. When the sphericity assumption was not met, we adjusted the degrees of freedom using the Greenhouse-Geisser correction. Because our analyses yielded multiple main effects and interactions, we report only those that are critical for data interpretation. See Supplementary Table S2 for a listing of number of subjects/samples included in each phase of the study, and Supplementary Tables S3-S11 for statistical analyses and summary statistics.

Behavior

We analyzed two behavioral measures during food self-administration training - (1) the total number of lever presses on active and inactive lever (denoted as *lever presses*), and (2) the total number of food pellet rewards earned (denoted as *rewards*) during a 1-h self-administration session. Following training, we tested separate groups of mice for palatable food reward-seeking

after 1, 15 or 60 days of abstinence and analyzed non-reinforced responding (*lever presses*) during the 30-min food seeking test. We describe the within- and between-subjects factors in the mixed ANOVAs we used to analyze the behavioral data in the Results section and in Supplementary Table S3.

ClearMap analysis

We processed the 32 brains using the ClearMap pipeline, of which 3 failed the registration to the reference atlas. We analyzed the remaining 29 brains (n=9 for Homecage, n=7 for Day 1, n=8 for Day 15 and n=5 for Day 60) as described below. We computed Fos+ cell counts within regions at two levels of the atlas hierarchy - (1) 10 major anatomical divisions, and (2) 56 subregions across the brain based on hierarchical relationships defined in the Allen Mouse Common Coordinate Framework (50). We selected regions within each level such that there were no parent-child relationships and/or overlapping spatial footprints between them. We transformed the raw Fos+ cell counts to Z-scores prior to the statistical analyses to normalize the data and account for differences in volume across regions of interest. We computed Z-scores for each region of interest relative to the homecage group's values using the formula $z = (x - \mu) / \sigma$, where x is the sample Fos+ cell count, μ is the mean Fos+ cell count of the homecage group, and σ is the standard deviation of the homecage group. For each level, we first used 2-way mixed ANOVA (GLM procedure in SPSS) with the within-subject factor of Region and the between-subjects factor of Group (Homecage, Day 1, Day 15, Day 60). We followed up on significant main effects and interactions with 1-way ANOVAs within each region and used Tukey HSD test for post-hoc comparisons between Groups. We provide the statistical outputs for all analysis pertaining to ClearMap in Supplementary Tables S4 and S5.

SMART2 analysis

In addition to the 29 brains used for ClearMap analysis, we used manual registration correction within each subvolume to recover and analyze brains that failed ClearMap registration. We analyzed 32 brains across the 4 groups (n=11 for Homecage, n=8 for Day 1, n=8 for Day 15

and n=5 for Day 60) for subvolume 1 and 31 brains across the 4 groups (n=10 for Homecage, n=8 for Day 1, n=8 for Day 15 and n=5 for Day 60) for subvolume 2. We extracted Fos+ cell counts within regions of interest at three levels of the atlas hierarchy - (1) major anatomical regions (subvolume 1: 5 regions; subvolume 2: 8 regions), (2) main subregions (subvolume 1: 18 subregions; subvolume 2: 28 regions), and (3) internal subdivisions within the subregions (subvolume 1: 43 subdivisions; subvolume 2: 64 subdivisions). Similar to ClearMap analysis, we selected regions within each level such that there were no parent-child relationships and/or overlapping spatial footprints between them. We transformed the raw Fos+ cell counts to Z-scores prior to statistical testing. For each level, we first used 2-way mixed ANOVA (GLM procedure in SPSS) with the within-subject factor of Region and the between-subjects factor of Group (Homecage, Day 1, Day 15, Day 60). We followed up on significant main effects and interactions with 1-way ANOVAs within each region and used Tukey HSD test for posthoc comparisons between Groups. We also generated spatial maps of Fos+ cell densities (mean across group) for each subvolume to aid visualization of differences in activation patterns between groups. We provide statistical outputs for all analysis pertaining to SMART2 subvolumes 1 and 2 in Supplementary Tables S6-S8 and S9-S11, respectively.

Results

The experimental design and timeline of behavioral training, intact mouse brain activity labeling, and brain-wide activity mapping is shown in Figure 1A. See Supplementary Table S2 for a listing of number of subjects/samples included in each phase of the study.

Incubation of palatable food seeking in male CD1 mice

We determined whether the time-dependent increases in food seeking during abstinence (incubation of food craving), previously observed in rats (3, 19), generalize to mice. The experimental timeline is shown in Figure 1A-B. We trained food sated CD-1 male mice to lever press for high carbohydrate food pellets for 7 days. Next, we tested different groups for relapse to

366 food seeking in the presence of contextual (food self-administration chamber) and discrete (light
367 cue paired with food delivery) cues after 1, 15, or 60 days of homecage forced abstinence.
368 Statistical outputs for all analyses pertaining to training and relapse tests are provided in tabular
369 format as Supplementary Table S3.

370 Training phase: The mice showed reliable food self-administration as indicated by increased
371 responding on the food-paired lever during the daily sessions (Figure 1C). The repeated-
372 measures ANOVA of *rewards*, which included the within-subjects factor of Training session
373 (sessions 1-7), showed a significant effect of this factor ($F_{3.2,144.7}$= 67.2, p<0.001). The repeated
374 measures ANOVA of *lever presses*, which included the within-subjects factors of Training session
375 and Lever (inactive, active), showed a significant interaction between the two factors ($F_{3.6,160.3}$=
376 28.9, p<0.001).

377 Relapse (incubation) tests: Non-reinforced presses on the previously active lever were
378 significantly higher after 60 abstinence days than after 1 or 15 days (Figure 1D), indicating
379 'incubation of food craving.' The mixed ANOVA of total lever presses, which included the
380 between-subjects factor of Abstinence day (1, 15, 60) and Lever, showed a significant interaction
381 between the two factors ($F_{2,30}$=3.4, p=0.045). This incubation effect was primarily due to higher
382 lever presses in the day 60 group during the first 10 min of the test session (Figure 1D right
383 panel). Post-hoc group differences (Tukey test) are depicted in Figure 1D.

384 **Unbiased intact brain-wide activity mapping of incubation of palatable food-seeking**

385 We processed brains using the ClearMap pipeline and extracted Fos+ cell counts within
386 regions of interest (ROIs) at two levels of the atlas hierarchy - (1) 10 major anatomical divisions
387 (Fig. 2A, Fig. 2B right panel), and (2) 56 subregions of interest (Fig. 2C) based on hierarchical
388 relationships defined in the Allen Mouse Common Coordinate Framework (50). We selected
389 regions within each level such that there were no parent-child relationships and/or overlapping
390 spatial footprints between them and performed z-score normalization (relative to homecage group
391 mean) for each ROI prior to statistical analyses (Fig. 2C, center and left panels).

Main regions: For Level 1, the mixed ANOVA, using the between-subjects factor of Group (homecage, day 1, day 15, day 60) and the within-subjects factor of Region, showed significant main effects of Group ($F_{3,25}$=4.4, p=0.013) and Region ($F_{1.9,47.7}$=18.2, p<0.001), and an interaction between the two factors ($F_{5.7,47.7}$=6.1, p<0.001). Follow up one-way ANOVAs (between-subjects factor Group) were significant for 6 out of 10 tested regions (denoted by * in Figure 2B, left panel). These data indicate increased brain-wide activation after the relapse test after 60 abstinence days, but not 1 or 15 days. Detailed statistical reporting of the ClearMap analysis at Level 1 are provided in Supplementary Table S4.

Sub-regions: For Level 2, the mixed ANOVA showed significant main effects of Group ($F_{3,25}$=6.0, p=0.003), and Sub-region ($F_{1.8,45.3}$=18.7, p<0.001) and an interaction between the two factors ($F_{5.4,45.3}$=8.0, p<0.001). Follow up one-way ANOVAs (between-subjects factor Group) were significant for 33 out of 56 tested subregions, reflecting selective increased activation following relapse test after 60 but not 1 or 15 days of abstinence. The most activated areas were olfactory, cortical, cortical subplate, and striatal subregions - collectively designated as 'corticostriatal', with sparse subregional activation in the retrohippocampal region of the hippocampus and medulla of the hindbrain. Increased Fos expression in the day 60 group was not observed in subregions of thalamus, hypothalamus, and midbrain structures. Detailed statistical reporting of ClearMap analysis at Level 2 are provided in Supplementary Table S5.

Targeted analysis of the corticostriatal coronal subvolume using SMART2

We processed a 200 μm thick coronal subvolume spanning AP +1.55 to AP +1.75 relative to Bregma using the SMART2 pipeline. Spatial maps of group-wise Fos+ cell densities within the subvolume are shown in Figure 3A to aid visualization of differences in activation patterns between groups. We extracted Fos+ cell counts within regions of interest (ROIs) at three levels of the atlas hierarchy - (1) 5 major anatomical regions (Fig. 3B), (2) 18 main subregions (Fig. 3C) and (3) 43 internal subdivisions within the subregions (Fig. 3D). We performed z-score normalization (relative to homecage mean) for each ROI prior to statistical analysis. One-way

ANOVA and Tukey HSD p-values for all Level 2 ROIs and a subset of Level 3 ROIs are shown as heatmaps in Figure 3C and Figure 3D.

Main regions: For Level 1, the mixed ANOVA showed significant main effects of Region ($F_{1.6,4.7}$=6.5, p=0.006) and Group ($F_{3,28}$=6.9, p=0.001), and an interaction between the two factors ($F_{4.7,44.0}$=4.7, p=0.002). Follow-up one-way ANOVAs were significant for all 5 tested regions, reflecting increased activation following the relapse test after 60 but not 1 or 15 days of abstinence. Detailed statistical reporting for SMART2 analysis at Level 1 are provided in Supplementary Table S6.

Sub-regions: For Level 2, the mixed ANOVA showed significant main effects of Sub-region ($F_{1.2,32.7}$=9.5, p=0.003) and Group ($F_{3,28}$=7.2, p=0.001), and an interaction between the two factors ($F_{3.5,32.7}$=6.3, p=0.001). Follow-up one-way ANOVAs were significant for all 18 tested subregions, reflecting increased activation following the relapse test after 60 but not 1 or 15 days of abstinence. Detailed statistical reporting for SMART2 analysis at Level 2 are provided in Supplementary Table S7.

Sub-divisions: For Level 3, the mixed ANOVA showed significant main effects of Sub-division ($F_{1.2,32.2}$=10.3, p=0.002) and Group ($F_{3,28}$=7.1, p=0.001), and an interaction between the two factors ($F_{3.5,32.2}$=6.5, p=0.001). Follow-up one-way ANOVAs were significant for all 43 tested subdivisions, reflecting increased activation following the relapse test after 60 but not 1 or 15 days of abstinence. Detailed statistical reporting for SMART2 analysis at Level 3 are provided in Supplementary Table S8.

Targeted analysis of the thalamocortical coronal subvolume using SMART2

We processed a 200 μm thick coronal subvolume spanning AP -1.08 to AP -1.28 relative to Bregma using the SMART2 pipeline. Spatial maps of group-wise Fos+ cell densities within the subvolume are shown in Figure 4A to aid visualization of differences in activation patterns between groups. We extracted Fos+ cell counts within ROIs at three levels of the atlas hierarchy - (1) 8 major anatomical regions (Fig. 4B), (2) 28 main subregions (Fig. 4C), and (3) 64 internal

444 subdivisions within the subregions (Fig. 4D). One-way ANOVAs and Tukey HSD p-values for all
445 Level 2 ROIs and a subset of Level 3 ROIs are shown as heatmaps in Figure 4C and Figure 4D.
446 Main regions: For Level 1, the mixed ANOVA showed significant main effects of Region
447 ($F_{1.2,32.3}$=14.0, p<0.001) and Group ($F_{3,27}$=3.2, p=0.040), and an interaction between the two
448 factors ($F_{3.6,32.3}$=5.5, p=0.002). Follow-up one-way ANOVAs were significant for 3 of 8 tested
449 regions, reflecting increased activation following the relapse test after 60 but not 1 or 15 days of
450 abstinence. Detailed statistical reporting for SMART2 analysis at Level 1 are provided in
451 Supplementary Table S9.
452 Sub-regions: For Level 2, two-way ANOVA showed significant main effects of Sub-region
453 ($F_{1.1,30.8}$=9.5, p=0.003) and Group ($F_{3,27}$=3.4, p=0.033), and an interaction between the two factors
454 ($F_{3.4,30.8}$=3.5, p=0.024). Follow-up one-way ANOVAs were significant for 12 of 18 tested
455 subregions, reflecting increased activation following relapse test after 60 but not 1 or 15 days of
456 abstinence. Detailed statistical reporting for SMART2 analysis at Level 2 are provided in
457 Supplementary Table S10.
458 Sub-divisions: For Level 3, the mixed ANOVA showed significant main effects of Region
459 ($F_{1.2,31.2}$=7.8, p=0.007) and Group ($F_{3,27}$=3.1, p=0.044), and an interaction between the two factors
460 ($F_{3.5,31.2}$=3.0, p=0.038). Follow-up one-way ANOVAs were significant for 20 of 64 tested
461 subdivisions, reflecting increased activation following the relapse test after 60 but not 1 or 15
462 days of abstinence. Detailed statistical reporting for SMART2 analysis at Level 3 are provided in
463 Supplementary Table S11.
464

465 **Discussion**

466 We used unbiased intact-brain mapping of Fos expression to investigate brain-wide
467 activation patterns during incubation of palatable food seeking in mice. Relapse to food seeking
468 was higher after 60 abstinence days than after 1 or 15 days, indicating incubation of food
469 seeking. More importantly, unbiased whole-brain analysis of Fos expression using ClearMap

showed a strong induction of neural activity across multiple brain regions that mirrored incubation of food seeking after 60, but not 15, days of abstinence. Targeted coronal slice analysis of Fos expression using SMART2 replicated and validated the time-dependent increases in activation patterns within corticostriatal and thalamocortical subvolumes and enabled detailed analysis of subdivision and layer-specific changes during abstinence. Overall, our data indicate that incubation of palatable food craving in male mice correlates with widespread activation of many brain regions beyond those previously implicated in incubation of food or drug craving (see Supplementary Table S1).

Outbred mice show incubation of palatable food seeking similar to outbred rat models

Food-seeking in the male mice increased following 60, but not 15, days of abstinence from food self-administration training. Previous studies in rats reported incubation of food seeking but with a different time course; robust incubation of sucrose craving was observed in rats after 7, 15, and 30 abstinence days, but not 60 days (5). Incubation of food craving has also been reported in rats trained to self-administer standard chow pellets after 30 abstinence days (14), and high-carbohydrate pellets after 21 abstinence days (15). By extending the model to mice we were able to leverage mouse-optimized procedures to investigate brain-wide activation patterns of incubation of food craving. A question for future research is whether a similar timecourse of incubation of food craving (and pattern of brain activation) will be observed in CD-1 female mice.

Notably, we used outbred CD-1 mice in these experiments rather than more commonly used inbred C57BL/6J. In our experience, outbred and hybrid mice exhibit a more complex spectrum of behavior and acquire learned behavior more robustly than their inbred counterparts(51-54). Additionally, an inbred genetic background limits the generalizability of genotype-phenotype relationships(55) and display highly heritable stain-specific phenotypes in brain volume, scalar diffusion tensor imaging metrics, and quantitative connectomes(56). Similarly, contrary to the assumed relationship, meta-analysis of coefficients of variation do not find evidence of greater trait stability in inbred mice than outbred mice, and hybrid mice show enhanced properties

desired for neurobiological research such as reduced anxiety-like behavior, improved learning, and enhanced long-term spatial memory(57). Taken within the context of developing a resource whole brain atlas for incubation of food craving in mice, outbred populations likely provide a more generalizable and robust platform.

Brain-wide patterns of increased neural activation following incubation of food seeking during abstinence

Previous targeted region-by-region analyses in rats have identified several brain regions that are activated during incubation of food and drug craving during abstinence (1, 3, 21, 28, 29). However, an unbiased brain-wide interrogation of regions engaged during 'incubated' reward seeking has not previously been performed. We used a modified version of the iDISCO+ procedure to label active (Fos+) neurons in intact mouse brains and employed light-sheet fluorescence microscopy to image these potentially behaviorally relevant neurons across the entire brain volume at single cell resolution. Our unbiased ClearMap analysis revealed a time-dependent increase in activation of multiple brain regions (e.g., prelimbic, infralimbic, orbitofrontal, and insular cortices, central amygdala and basolateral amygdala, ventral and dorsal striatum) similar to that shown in rats following incubation of food and drug seeking (58-64) (see Supplementary Table S1). However, this time-dependent increase of neural activity was not restricted to these previously identified incubation-related regions. Indeed, over half of the tested main regions (6 out of 10 main anatomical divisions, and 33 of 56 subregions), including most regions within the isocortex, olfactory areas, cortical subplate, and striatum, showed statistically significant increased activation after 60, but not 15 abstinence days. Additionally, compared to 'resting state' activity in the homecage, most regions showed an initial dip in activation following food seeking tests on days 1 and 15, which preceded strong induction on day 60.

Of note, it is possible that a small number of the statistically significant activated regions reflect false positive results, because we did not correct the statistical analyses for multiple one-way ANOVA comparisons. On the other hand, the fact that statistically significant increases in

Fos expression in multiple brain regions were detected using a relatively small n per group (n=5 to 8 for abstinence days 1, 15, and 60) speaks to the robustness of our results.

Two recent studies used a similar unbiased approach to investigate brain-wide activation (assessed by Fos) patterns of short-term alcohol abstinence after two-bottle choice and chronic intermittent ethanol vapor exposure (65) and acute withdrawal following experimenter-administered psychostimulants (66). They used hierarchical clustering techniques to demonstrate a strong decrease in modularity after abstinence/withdrawal compared to drug-naïve controls and employed graph theory approaches to identify hub regions that might drive this functional restructuring. It is possible that this observed decrease in modularity might be a result of the recruitment of networks of regions like that seen in our study following food seeking in mice. However, it is important to note that in these studies, brains were collected directly from homecage and not after behavioral testing. Thus, the observed changes likely reflect shifts in 'resting-state' functional brain architecture and not behaviorally evoked differential network engagement during reward seeking.

Targeted subvolume analysis reveals variations in time-dependent brain activation patterns

We followed-up on our global volumetric ClearMap findings by analyzing activation patterns within two coronal volumes using an updated version of the SMART pipeline (41). This approach allowed us to use expert-guided registration and Fos-segmentation to (1) isolate ClearMap effects to specific coronal plates along the anterior-posterior axis, (2) validate our data against previous incubation studies that used selected coronal slices for Fos-mapping, (3) include brains that failed ClearMap due to physical damage during processing, and (4) extend our analysis to subdivisions and layers within these subvolumes for future mechanistic investigation. In agreement with the ClearMap analyses, SMART2 analysis identified several subregions with increased activation (following the relapse tests on abstinence day 60) within the isocortex, olfactory areas, cortical subplate, and striatum across the two selected subvolumes, several of which have been previously identified after incubation of food and drug seeking (e.g., prelimbic cortex, infralimbic

548 cortex, orbitofrontal cortex, basolateral amygdala, central amygdala nucleus, nucleus accumbens,
549 somatosensory cortex) and drug seeking (e.g., prelimbic cortex, infralimbic cortex, agranular
550 insular area, basolateral amygdala, central amygdalar nucleus, nucleus accumbens) in rat
551 models (58-64, 67-69) (see Supplementary Table S1). However, while some identified subregions
552 showed similar increases in activation across both subvolumes (e.g., somatomotor and
553 somatosensory areas, claustrum, piriform area), others were only present in one subvolume (e.g.,
554 prelimbic and infralimbic cortex, basolateral and central amygdala) or showed differential
555 engagement along anterior-posterior axis (e.g., anterior cingulate and agranular insular cortices).
556 Even within a subvolume and subregion, activation patterns were not uniform but sometimes
557 graded across layers (e.g., layers of piriform area, dorsal peduncular area, infralimbic and
558 prelimbic areas) or isolated to specific subdivisions (e.g., central but not medial or lateral
559 subdivisions of central amygdala), suggesting different degrees of engagement across multiple
560 brain regions and likely circuits after 60 abstinence days.

561 Conclusions

562 We demonstrated that incubation of palatable food reward seeking is accompanied by an
563 induction of neural engagement in multiple brain regions, many of them extend beyond the
564 traditional brain areas and circuits involved in incubation of food and drug craving. We extend the
565 rat incubation of food seeking model to male CD1 mice and leverage mouse-specific unbiased
566 whole-brain staining, clearing, and analysis pipelines to generate a single-cell resolution whole-
567 brain atlas of incubation food seeking during prolonged abstinence. The results of our study
568 suggest that the overarching neural mechanism underlying incubation of reward seeking is more
569 anatomically widespread than suggested by the published literature and likely not localized to a
570 particular brain area or circuit. Whatever neural mechanisms mediate incubation of reward
571 seeking, our findings suggest that these mechanisms affect acute neural responses throughout
572 the brain, either through widespread alterations in all these brain areas or in key brain areas that
573 regulate brain-wide circuitry. And finally, the ‘incubation atlas’ here provides a mineable dataset

574 for better understanding system-level alterations related to incubation of food craving and
575 relapse.

576

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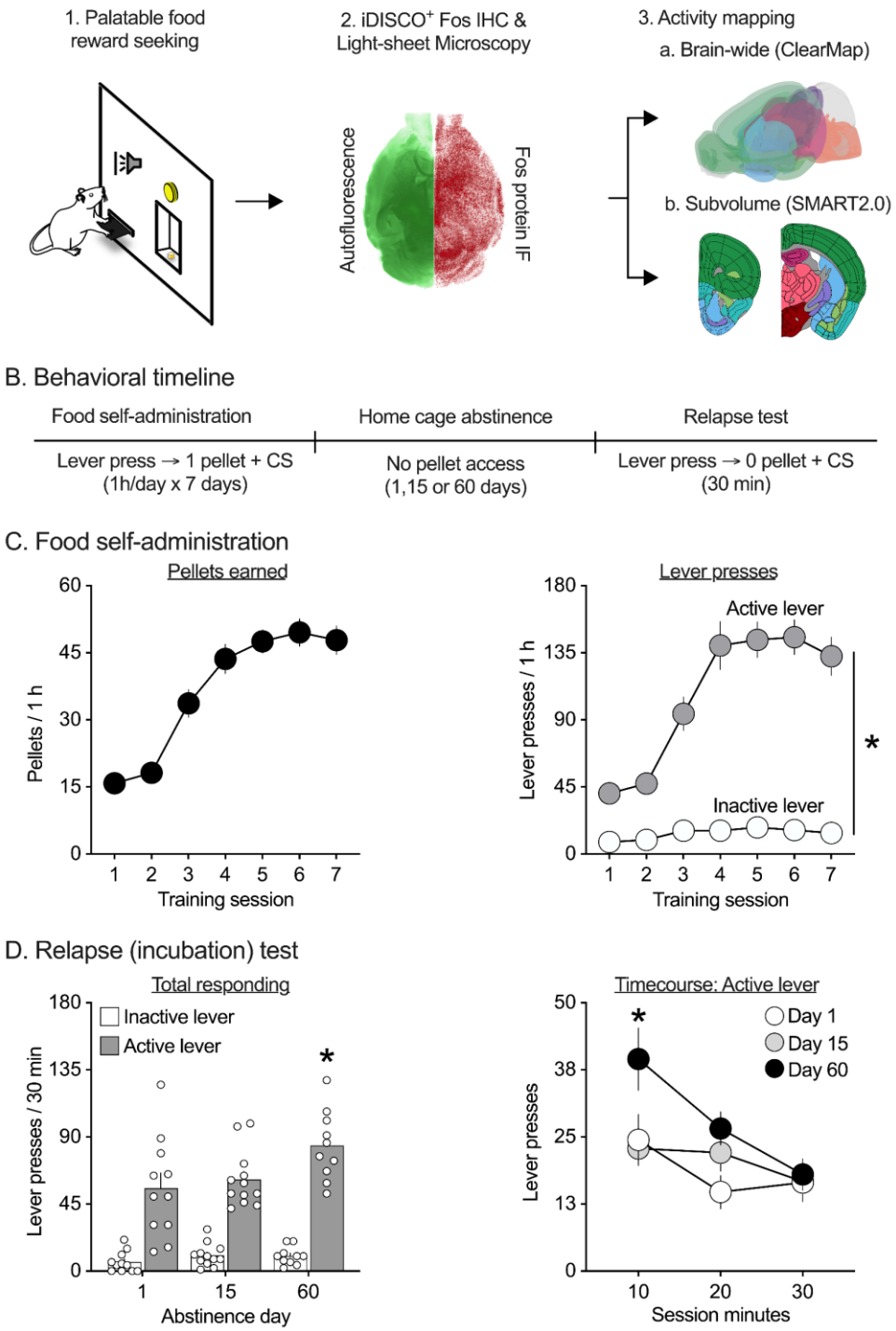
753

754 **Figures and Tables**

Figure 1



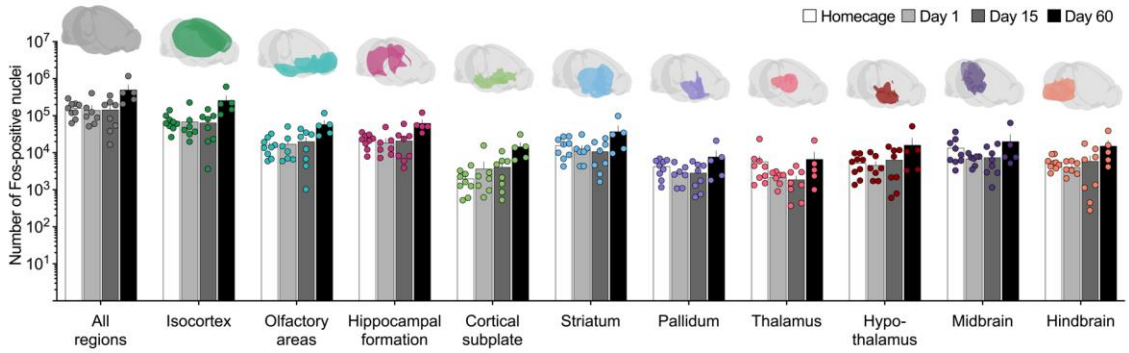
755

Figure 1. Incubation of palatable food seeking in male CD1 mice.

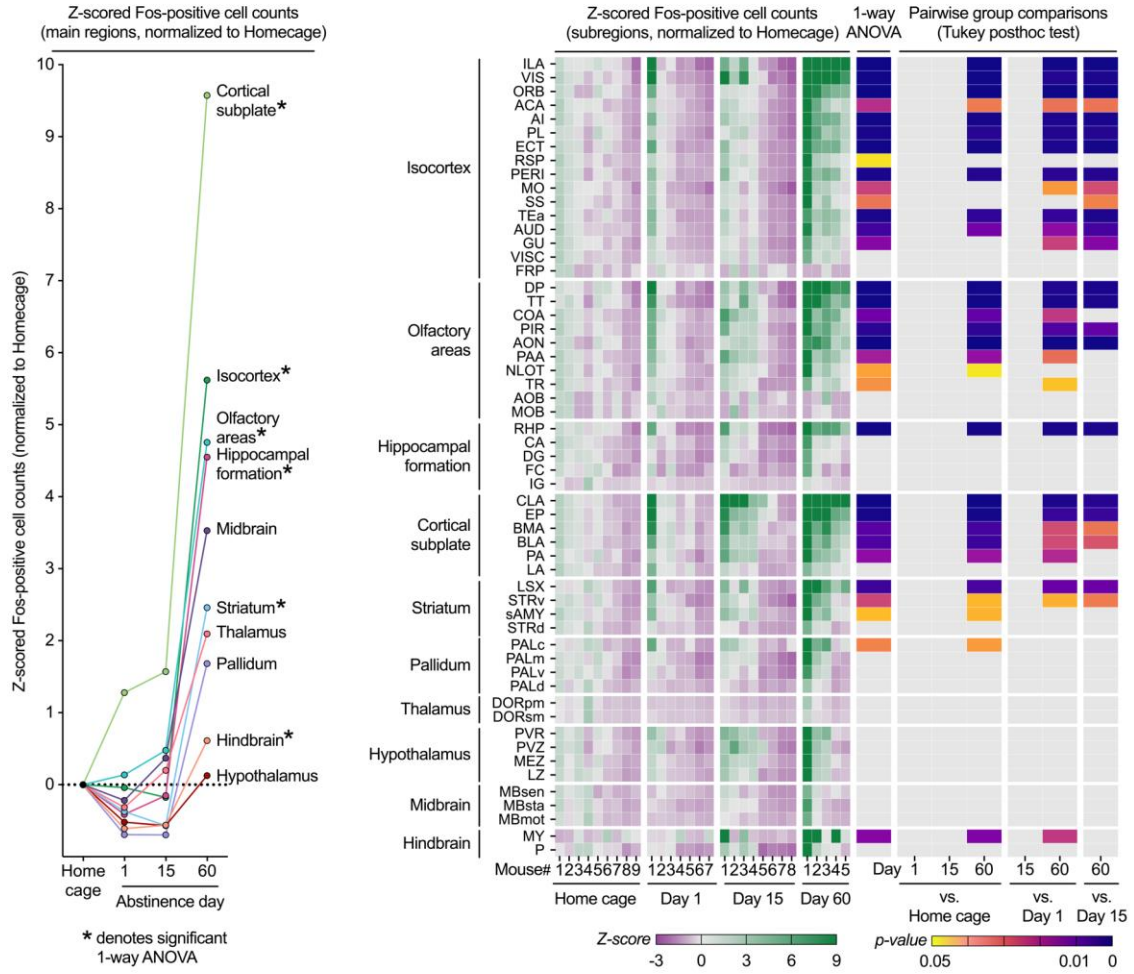
(**A**) Experimental overview. (**B**) Timeline of food self-administration training, abstinence, and relapse tests. (**C**) Food self-administration training. Mice learned to self-administer palatable food pellets over 7 sessions. Mean (±SEM) number of food pellets earned (left panel) and lever presses (right panel) during each 1-h session. * Significant difference ($p<0.05$) between active and inactive lever presses (n=46). (**D**) Relapse (incubation) test. Responding on active but not inactive lever progressively increased during abstinence. Mean (±SEM) number of lever presses during the entire 30-min relapse test session (left panel) and binned 10-min timecourse of active lever presses (right panel). * Significant difference ($p<0.05$) from day 1. See Supplementary Table S3 for a detailed listing of all statistical outputs relating to this figure.

Figure 2

A. Fos-positive cell counts across major anotomical sub-divisions



B. Brain-wide changes in activation during abstinence (counts z-scored normalized to Homecage distribution)



766

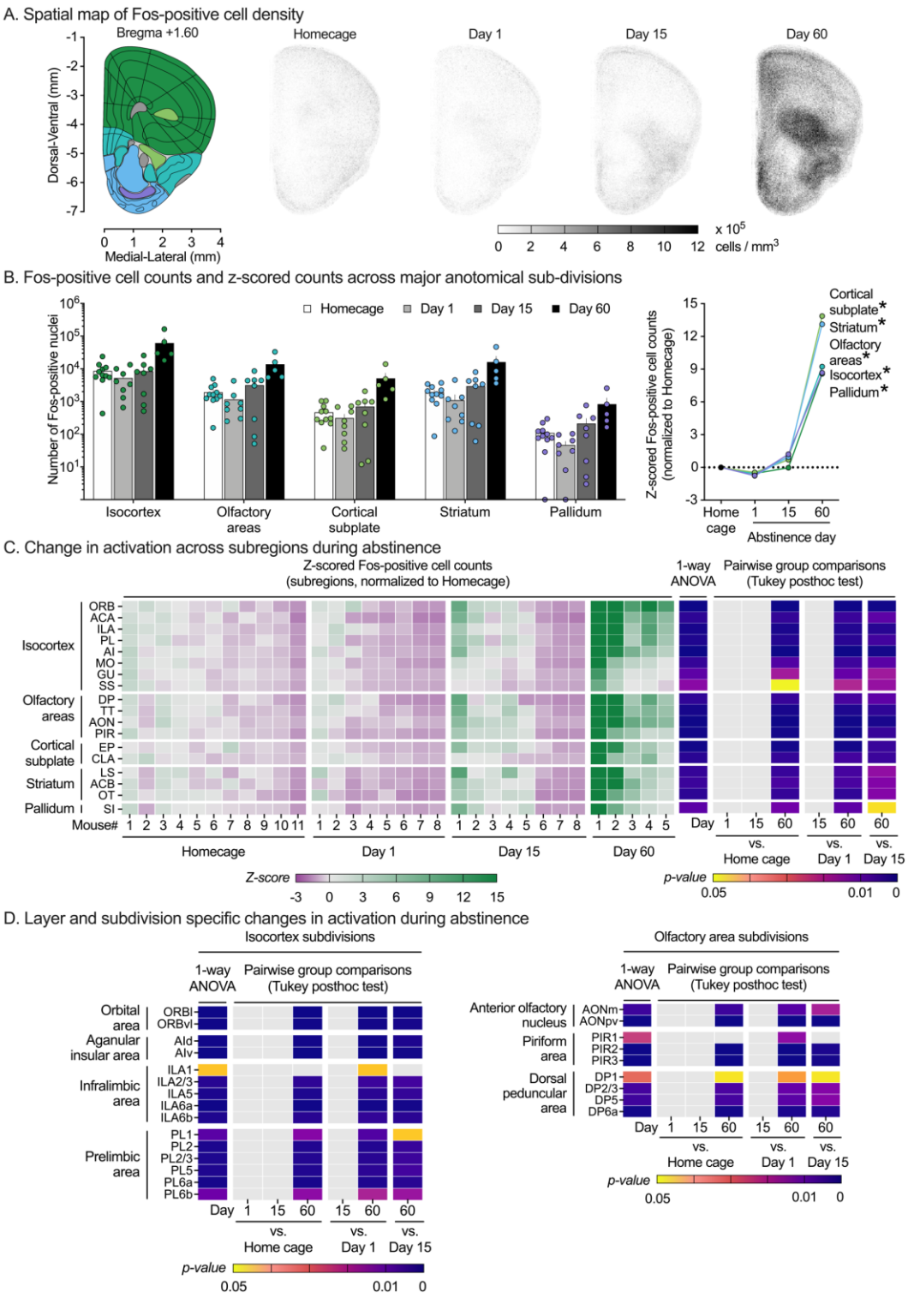
767

768 **Figure 2.** Unbiased brain-wide activity mapping of incubated relapse to palatable food-seeking
769 using ClearMap.

770 (**A**) Mean (±SEM) Fos+ cell counts across the whole brain and for 10 major anatomical sub-
771 divisions. (**B**) Brain-wide changes in activation during abstinence. Raw Fos+ cell counts for each
772 region are z-score normalized to the homecage group distribution for statistical analysis. Mean z-
773 scored cell counts for 10 major anatomical sub-divisions showing time-dependent changes in
774 activation pattern in multiple brain regions induced by the relapse tests (left panel). * Significant
775 differences ($p<0.05$) 1-way ANOVA. Heatmap of individual z-scored Fos+ cell counts, 1-way
776 ANOVA p-value and Tukey HSD pairwise group comparison p-values for 56 subregions across
777 the analyzed brain volume (right panel). Individual data is sorted by group and ranked in
778 descending order of activation level within each group. Subregions are organized by 10 parent
779 anatomical sub-divisions and ranked in descending order of mean day 60 group activation level.
780 See Supplementary Tables S4-S5 for a detailed listing of all statistical results associated with this
781 figure.

782

Figure 3

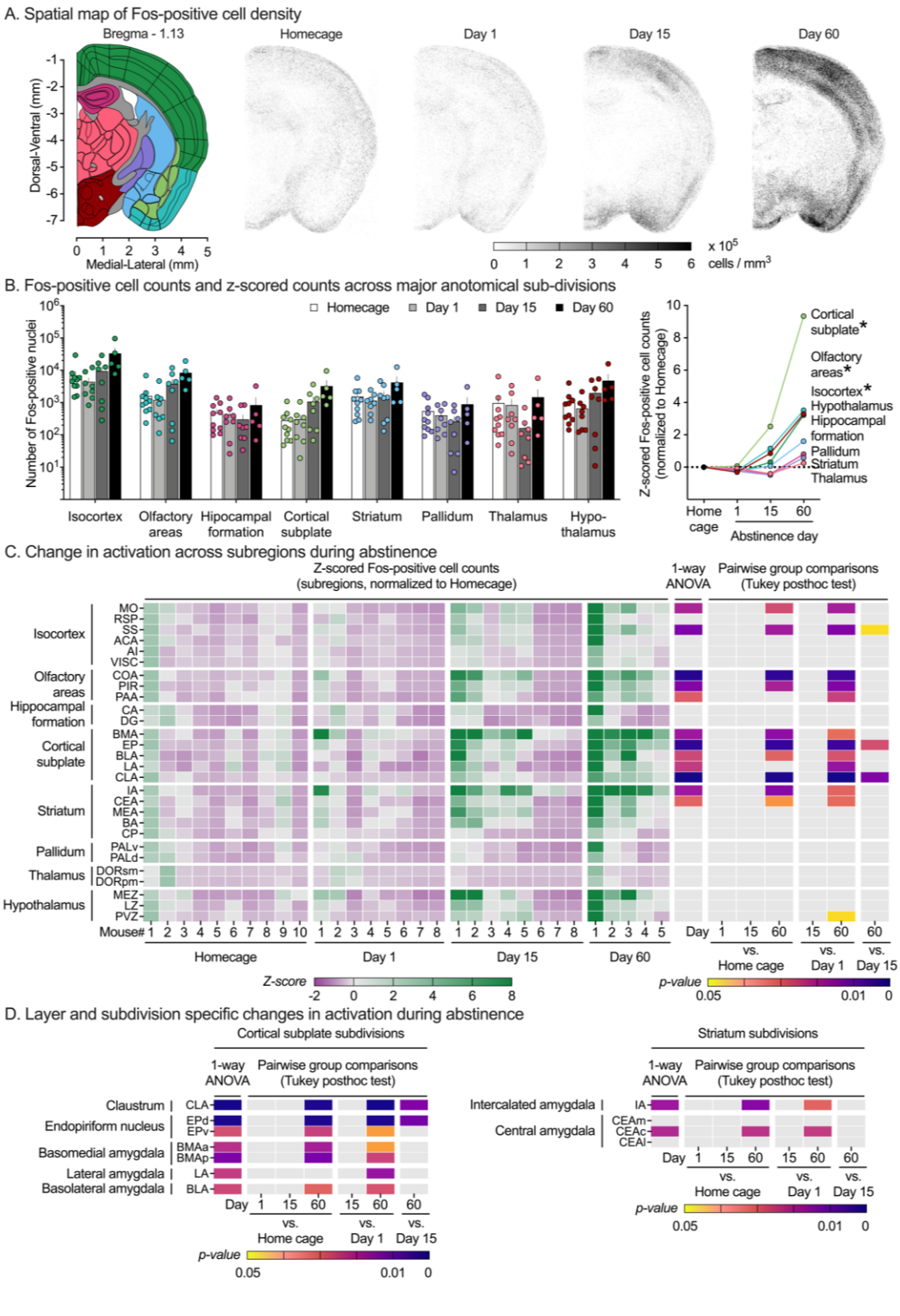


783

Figure 3. Targeted analysis of corticostriatal coronal subvolume (AP +1.55 to AP +1.75 relative to Bregma) using SMART2

(**A**) Spatial map of Fos+ cell density. Grayscale intensity of individual points (20 μm x 20 μm x 200 μm voxel) represents mean cell density in cells/mm^3 within AP +1.55 to AP +1.75 coronal subvolume. (**B, left**) Fos+ cell counts for 5 major anatomical regions within the subvolume. (**B, right**) Z-score normalized counts (normalized to home cage group distribution) for 5 major anatomical regions within the subvolume. * Significant differences ($p<0.05$) 1-way ANOVAs. (**C**) Changes in activation across the subvolume during abstinence. Fos+ cell counts for each region are z-score normalized to homecage group distribution for statistical analysis. Heatmap of individual z-scored Fos+ cell counts (**left**), 1-way ANOVA p-value (**middle**) and Tukey HSD pairwise group comparisons p-values (**right**) for 18 sub-regions within the analyzed coronal subvolume. Subregions are organized by 5 parent anatomical sub-divisions and ranked in descending order of mean day 60 group activation level. (**D**) Layer and subdivision specific changes in activation. Heatmap of 1-way ANOVA p-value and Tukey HSD pairwise group comparison p-values for selected sub-region layers or subdivisions within Isocortex (**left**) or Olfactory areas (**right**). See Supplementary Tables S6-S8 for a detailed listing of all statistical results associated with this figure.

Figure 4



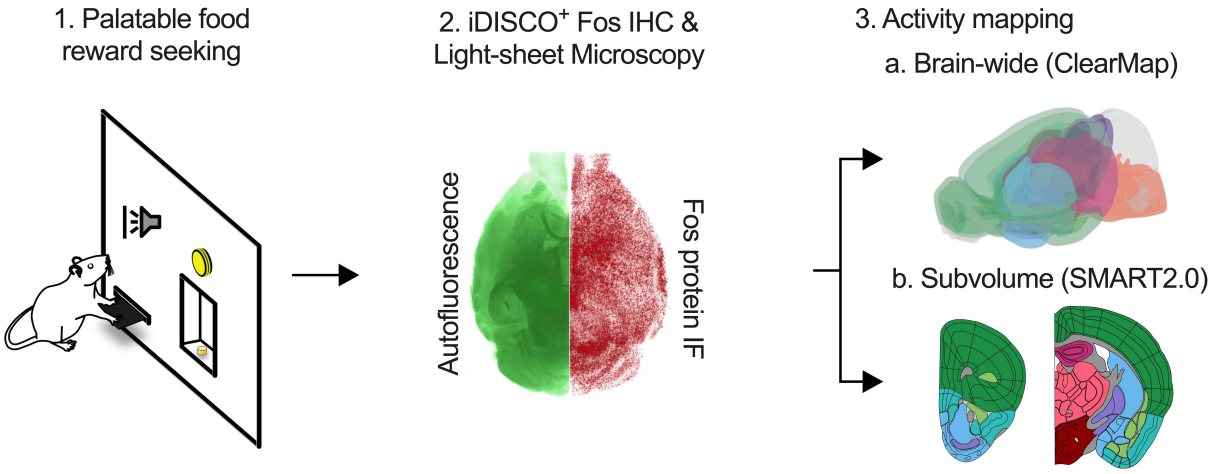
801

Figure 4. Targeted analysis of thalamocortical coronal subvolume (AP -1.08 to AP -1.28 relative to Bregma) using SMART2

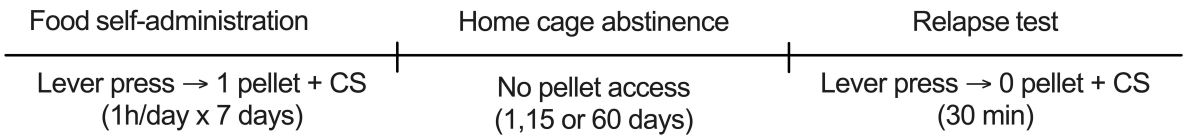
(**A**) Spatial map of Fos+ cell density. Grayscale intensity of individual points (20 μm x 20 μm x 200 μm voxel) represents mean cell density in cells/mm^3 within AP -1.08 to AP -1.28 coronal subvolume. (**B, left**) Fos+ cell counts for 8 major anatomical regions within the subvolume. (**B, right**) Z-score normalized counts (normalized to home cage group distribution) for 5 major anatomical regions within the subvolume. * Significant differences ($p<0.05$) 1-way ANOVAs. (**C**) Changes in activation across the subvolume. Fos+ cell counts for each region are z-score normalized to home cage group distribution for statistical analysis. Heatmap of individual z-scored Fos+ cell counts (left), 1-way ANOVAs p-values (middle) and Tukey HSD pairwise group comparisons p-values (**right**) for 28 sub-regions within the analyzed coronal subvolume. Subregions are organized by 8 parent anatomical subdivisions and ranked in descending order of mean day 60 group activation level. (**D**) Layer and subdivision specific changes in activation. Heatmap of 1-way ANOVAs p-values and Tukey HSD pairwise group comparison p-values for selected subregion layers or subdivisions within Cortical subplate (**left**) or Striatum (**right**). See Supplementary Tables S9-S11 for a detailed listing of all statistical results related to this figure.

Figure 1

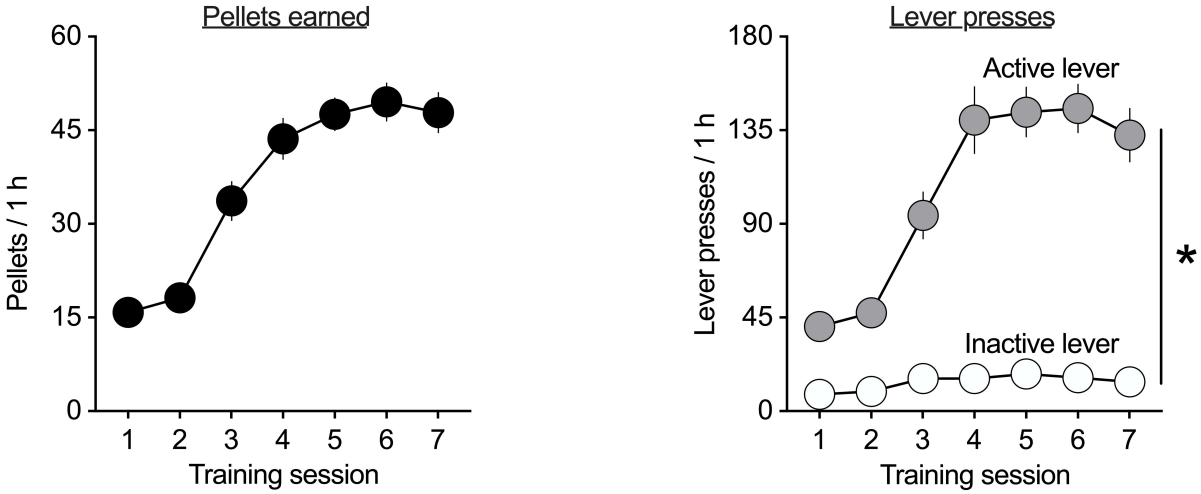
A. Experimental overview



B. Behavioral timeline



C. Food self-administration



D. Relapse (incubation) test

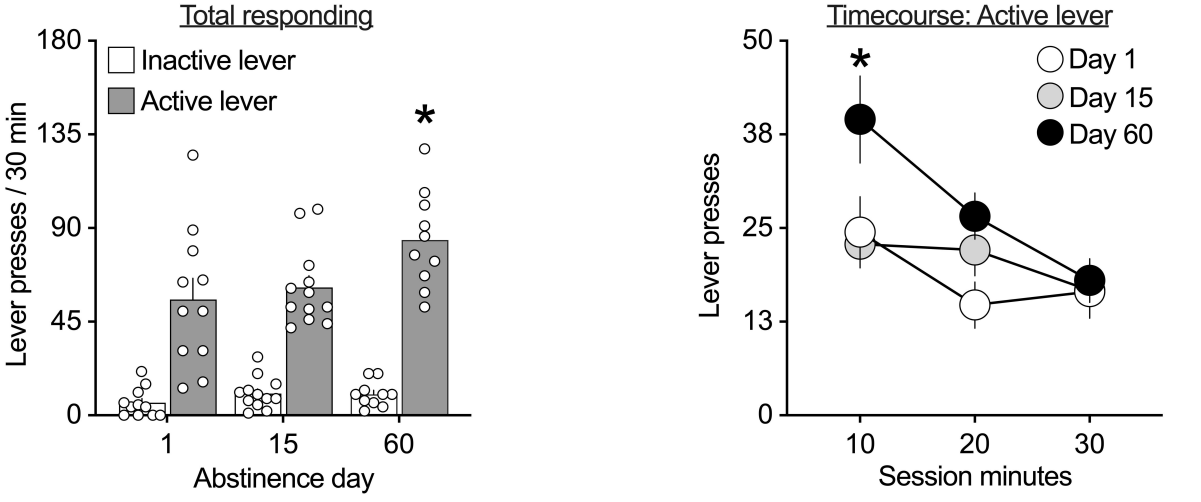
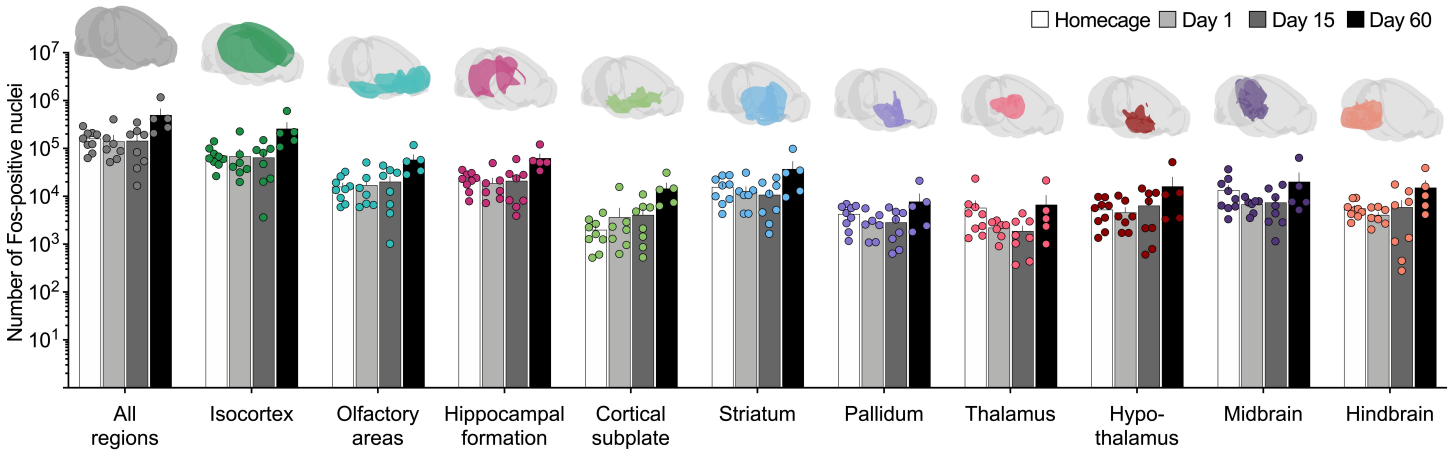


Figure 2

A. Fos-positive cell counts across major anotomical sub-divisions



B. Brain-wide changes in activation during abstinence (counts z-scored normalized to Homecage distribution)

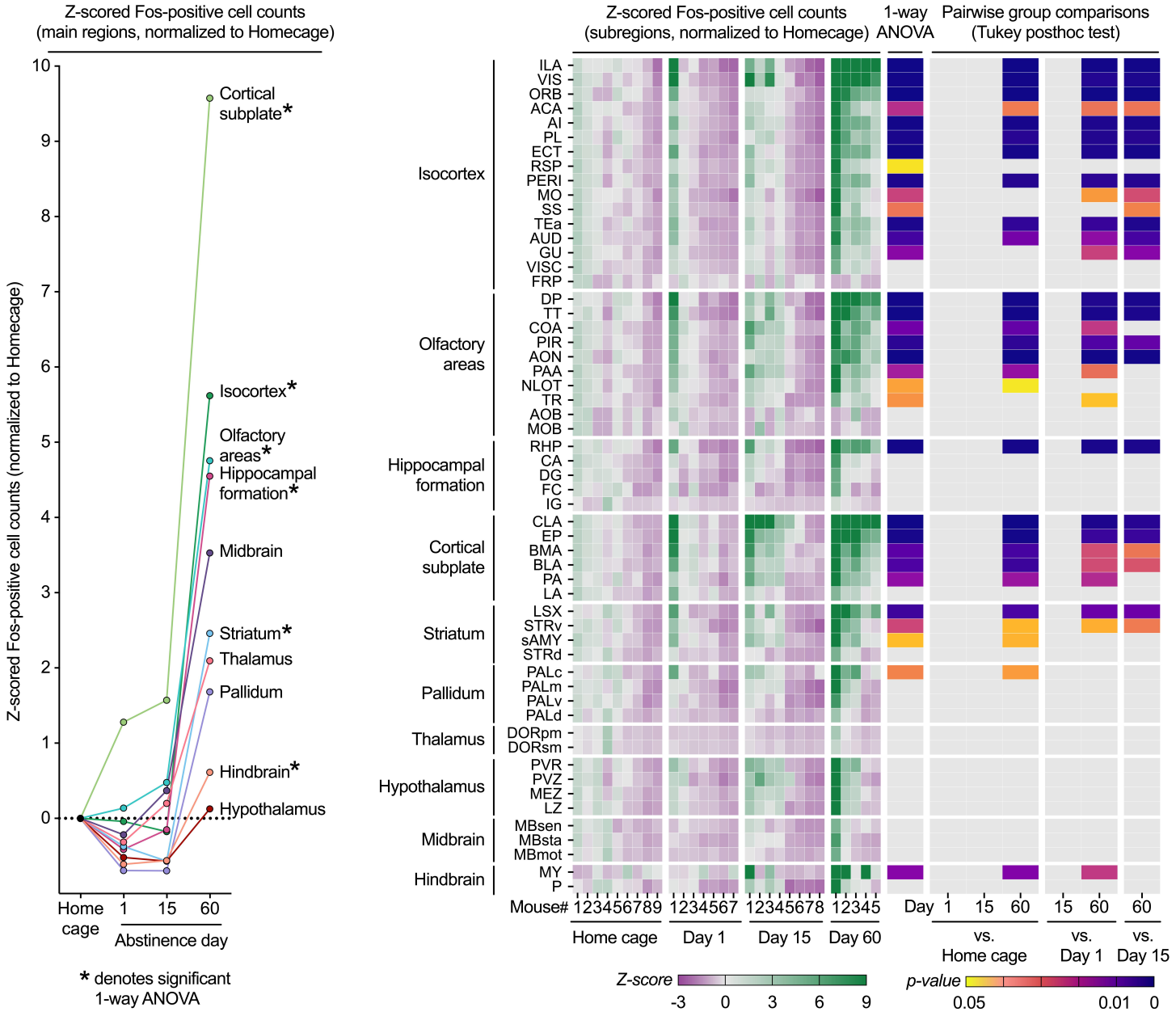


Figure 3

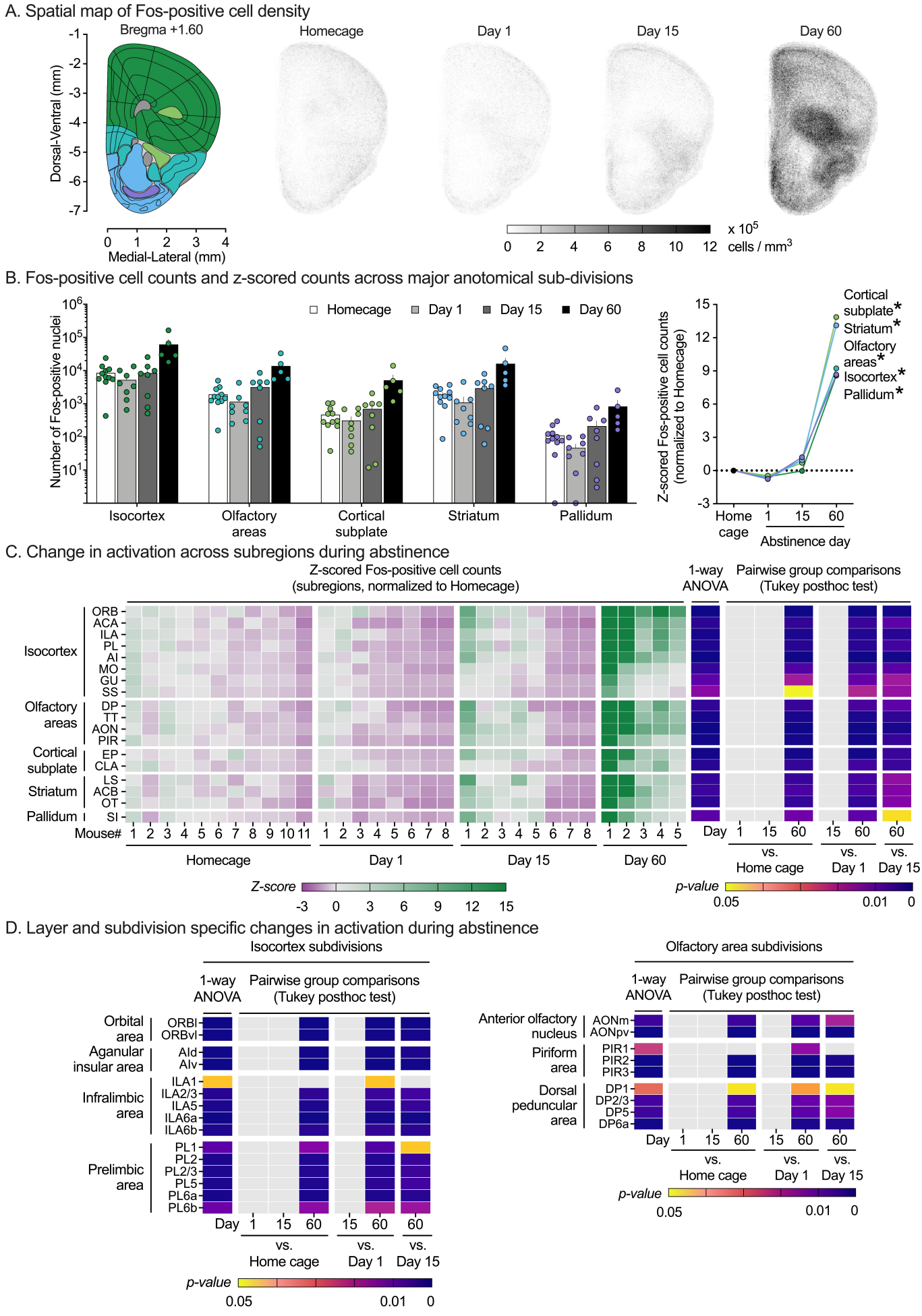


Figure 4

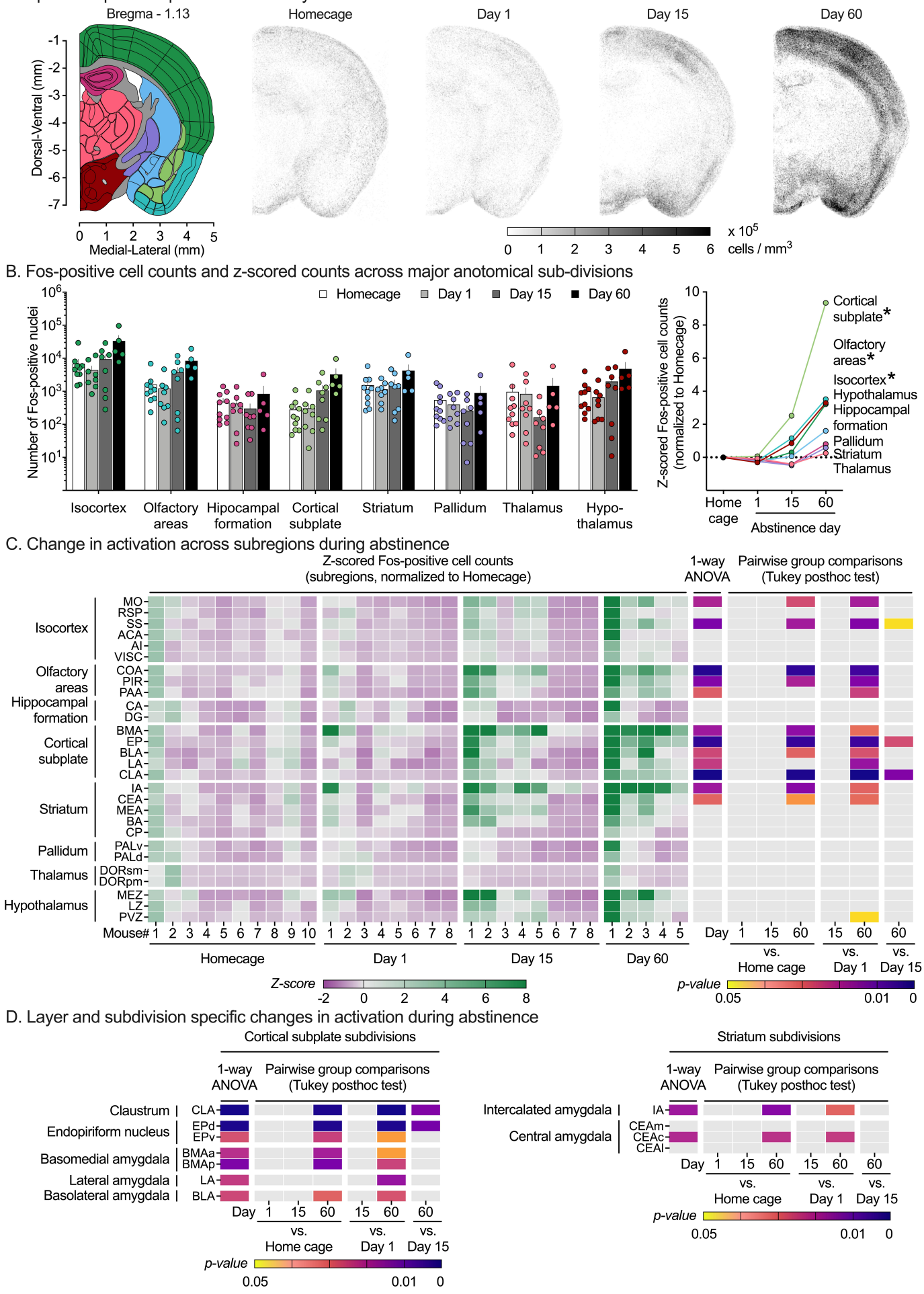
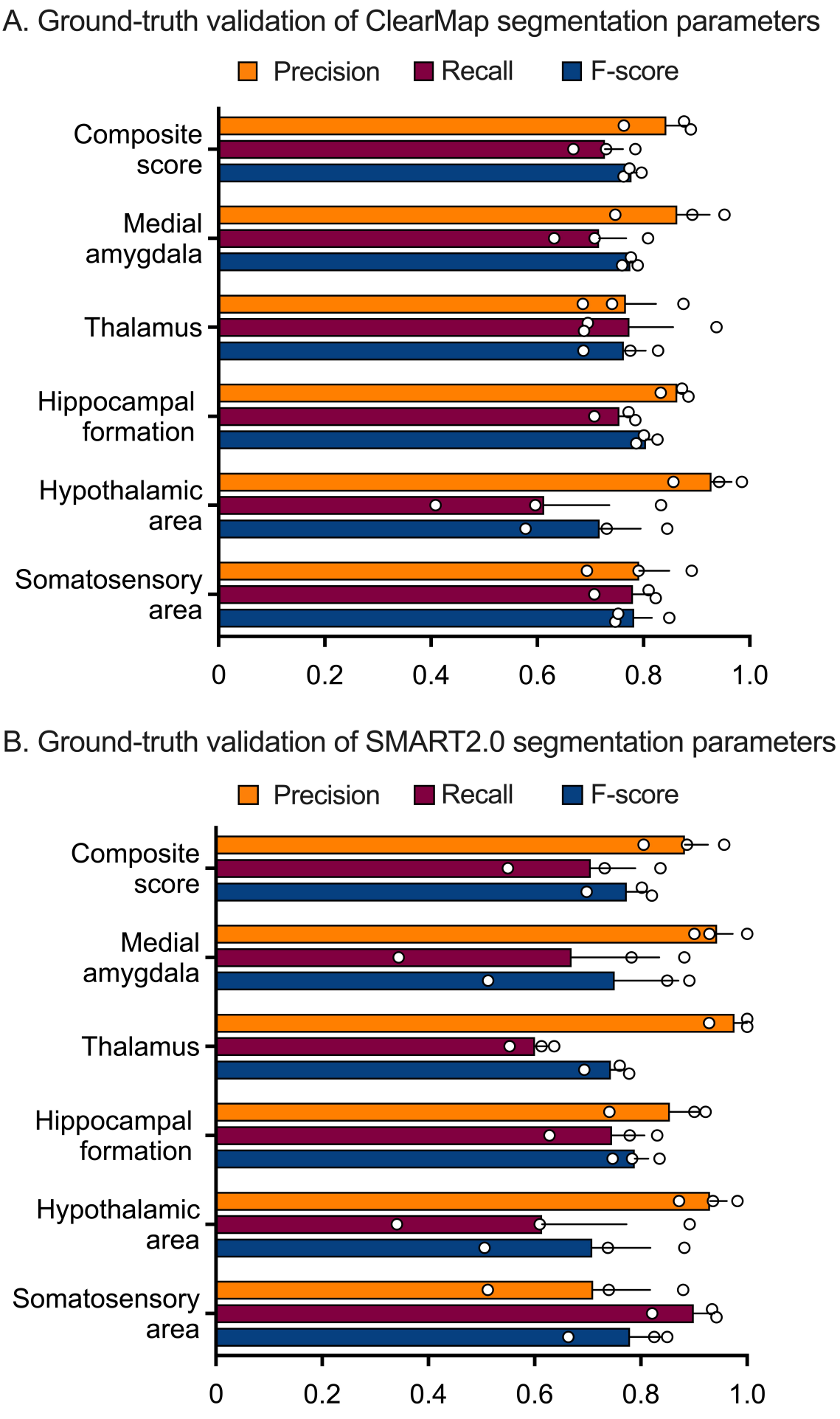


Figure S1



Supplementary Information for

Incubation of palatable food craving is associated with brain-wide neuronal activation in mice

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This PDF file includes:

Figure S1

Tables S1 to S11

SI References

Figure S1

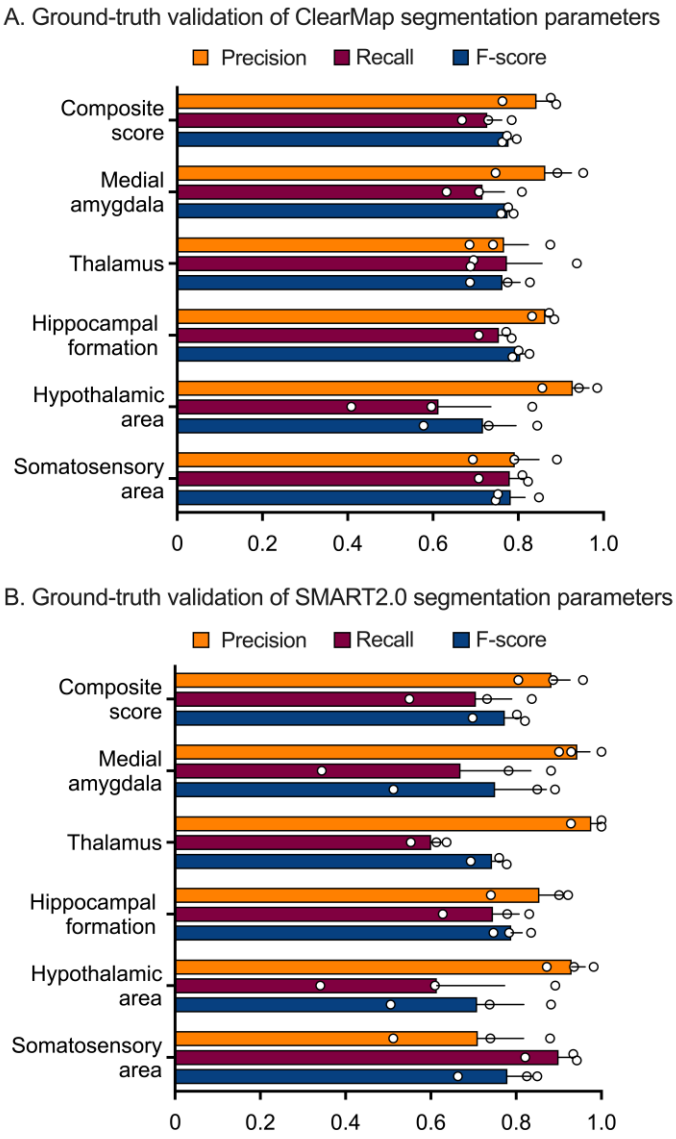

Fig. S1. Validation of ClearMap (A) and SMART2 (B) segmentation parameters against expert Fos+ cell annotation

Table S1. Incubation-relevant regions identified using targeted section-based activity mapping approaches

						Change in activation (Fos)			
Brain Region	Abbreviation	Reward	Animal	Model	Abstinence duration (days)	Incubation	Seeking	Inactivation	Citation
Ventral medial prefrontal cortex	vmPFC	Nicotine	Rat	SA	1, 7, 14, 28	–			(1)
	vmPFC	Methamphetamine	Rat	SA	1, 15	↑	↑		(2)
	vmPFC	Methamphetamine	Rat	SA	1, 30		↑		(3)
Prelimbic cortex	PL	Sucrose	Rat	SA	1, 30	↑			(4)
	PL	Pallatable food	Mouse	SA	1, 15, 60	↑(CM), ↑(SMART)	↑(CM), ↑(SMART)		Current study
Infralimbic cortex	IL	Sucrose	Rat	SA	1, 30	↑			(4)
	IL	Pallatable food	Mouse	SA	1, 15, 60	↑(CM), ↑(SMART)	↑(CM), ↑(SMART)		Current study
Dorsal medial prefrontal cortex	dmPFC	Nicotine	Rat	SA	1, 7, 14, 28	↑			(1)
	dmPFC	Methamphetamine	Rat	SA	1, 15	↑	↑		(2)
	dmPFC	Methamphetamine	Rat	SA	1, 30		↑		(3)
Orbitofrontal cortex	OFC	Oxycodone	Rat	SA	1, 15		↑	↓	(5)
	OFC	Methamphetamine	Rat	SA	1, 15	↑	↑		(2)
	OFC	Methamphetamine	Rat	SA	1, 26		↑		(6)
	OFC	Heroin	Rat	SA	1, 14		↑	↓	(7)
	OFC	Nicotine	Rat	SA	1, 7, 14, 28	↑		–	(1)
	OFC	Sucrose	Rat	SA	1, 30	–			(4)
	OFC	Pallatable food	Mouse	SA	1, 15, 60	↑(CM), ↑(SMART)	↑(CM), ↑(SMART)		Current study
Anterior cingulate cortex	ACC	Methamphetamine	Rat	SA	1, 26		↑		(6)
	ACC	Methamphetamine	Rat	SA	1, 15	↑	↑		(2)
	ACC	Sucrose	Rat	SA	1, 30	↑			(4)
	ACC	Pallatable food	Mouse	SA	1, 15, 60	↑(CM), ↑(SMART)	↑(CM), ↑(SMART)		Current study
Dorsal striatum	DS	Oxycodone	Rat	SA	5, 31		↑		(8)
	DS	Methamphetamine	Rat	SA	2, 35		↑		(9)
	DS	Pallatable food	Mouse	SA	1, 15, 60	–(CM)	–(CM)		Current study
Dorsomedial striatum	DMS	Methamphetamine	Rat	SA	1, 21	↑	↑	↓	(10)
Dorsolateral striatum	DLS	Methamphetamine	Rat	SA	1, 21	–	–		(10)
	DLS	Methamphetamine	Rat	SA	1, 26		↑		(6)
	DLS	Sucrose	Rat	SA	1, 30	↑			(4)
	AI	Methamphetamine	Rat	SA	1, 26		↑		(6)

Anterior insula cortex	AIV	Methamphetamine	Rat	SA	1, 15	↑	↑		(2)
	AI	Methamphetamine	Rat	SA	1, 30		↑		(3)
	AI	Pallatable food	Mouse	SA	1, 15, 60	↑(CM), ↑(SMART)	↑(CM), ↑(SMART)		Current study
Nucleus accumbens	NAc	Oxycodone	Rat	SA	5, 31		↓		(8)
	NAc	Pallatable food	Mouse	SA	1, 15, 60	↑(SMART)	↑(SMART)		Current study
Nucleus accumbens core	NAcC	Nicotine	Rat	SA	1, 7, 14, 28	↑			(1)
	NAcC	Sucrose	Rat	SA	1, 30	↑			(4)
	NAcC	Methamphetamine	Rat	SA	1, 15		↑	↓	(11)
Nucleus accumbens shell	NAcS	Nicotine	Rat	SA	1, 7, 14, 28	–			(1)
	NAcS	Sucrose	Rat	SA	1, 30	↑			(4)
	NAcS	Methamphetamine	Rat	SA	1, 15		–	–	(11)
Somatosensory cortex	SSC	Sucrose	Rat	SA	1, 30	↑			(4)
	SSC	Pallatable food	Mouse	SA	1, 15, 60	–(CM), ↑(SMART)	–(CM), ↑(SMART)		Current study
Medial anterior intralaminar nuclei of thalamus	AIT-M	Methamphetamine	Rat	SA	1, 26		↑		(6)
	AIT-M	Methamphetamine	Rat	SA	1, 30		–	–	(3)
Lateral anterior intralaminar nuclei of thalamus	AIT-L	Methamphetamine	Rat	SA	1, 26		↑		(6)
	AIT-L	Methamphetamine	Rat	SA	1, 30		↑	↓	(3)
Parafascicular nucleus	Pf	Methamphetamine	Rat	SA	1, 26		↑		(6)
	Pf	Methamphetamine	Rat	SA	1, 30		–		(3)
Basolateral amygdala	BLA	Methamphetamine	Rat	SA	1, 26		–	–	(6)
	BLA	Methamphetamine	Rat	SA	1,15	↑	↑		(2)
	BLA	Nicotine	Rat	SA	1, 7, 14, 28	↑			(1)
	BLA	Sucrose	Rat	SA	1, 30	–			(4)
	BLA	Methamphetamine	Rat	SA	1, 30		↑		(3)
	BLA	Pallatable food	Mouse	SA	1, 15, 60	↑(CM), ↑(SMART)	↑(CM), ↑(SMART)		Current study
Central amygdala	CeA	Nicotine	Rat	SA	1, 7, 14, 28	↑		↓	(1)
		Methamphetamine	Rat	SA	1, 15	↑	↑		(2, 12)
	CeA	Sucrose	Rat	SA	1, 30	↑			(4)
	CeA	Pallatable food	Mouse	SA	1, 15, 60	↑(SMART)	↑(SMART)		Current study
Dentate Gyrus of hippocampus	DG	Sucrose	Rat	SA	1, 30	–			(4)
	DG	Pallatable food	Mouse	SA	1, 15, 60	–(CM), –(SMART)	–(CM), –(SMART)		Current study

Table S2. Number of subjects that completed each phase of the study

	Experimental group					
Experimental phase	**Homecage**	**Day 1**	**Day 15**	**Day 60**	**Total**	**Figure**
Naïve (before start of training)	-	-	-	-	60	-
Food self-administration	-	-	-	-	46	1C
Food seeking & tissue collection	13	11	12	10	46	1D
iDISCO+ Fos IHC	11	9	9	8	37	-
Light sheet microscopy	11	8	8	5	32	-
ClearMap pipeline	9	7	8	5	29	2
SMART2.0 subvolume 1	11	8	8	5	32	3
SMART2.0 subvolume 2	10	8	8	5	31	4

Table S3. Statistical output for Figure 1: (analyses shown in figure 1 are highlighted in grey)

Experimental phase	Behavioral measure	Analysis method & factors	Statistical output			Figure
Food self-administration training	Pellets earned over 1 h (n=46)	RM-ANOVA (Session x Group)	F-value	P-value	Partial η2	1C, left
		Session (within-subjects)	F(3.079,129.330) = 70.576	<0.001	0.627	
		Group (between-subjects)	F(3,42) = 1.165	0.334	0.077	
		Session x Group	F(9.238,129.330) = 1.785	0.075	0.133	
		RM-ANOVA (Session)	F-value	P-value	Partial η2	
		Session (within-subjects)	F(3.215,144.687) = 67.173	<0.001	0.599	
Food self-administration training	Lever presses over 1 h (n=46)	RM-ANOVA (Lever x Session x Group)	F-value	P-value	Partial η2	1C, right
		Lever (within-subjects)	F(1,42) = 136.117	<0.001	0.764	
		Session (within-subjects)	F(3.606,151.460) = 38.849	<0.001	0.481	
		Group (between-subjects)	F(3,42) = 1.263	0.299	0.083	
		Lever x Group	F(3,42) = 0.564	0.642	0.039	
		Session x Group	F(10.819, 151.460) = 1.765	0.066	0.112	
		Lever x Session	F(3.5,147.017) = 29.225	<0.001	0.410	
		Lever x Session x Group	F(10.501, 147.017) = 1.558	0.121	0.100	
		RM-ANOVA (Lever x Session)	F-value	P-value	Partial η2	
		Lever (within-subjects)	F(1,45) = 142.935	<0.001	0.761	
		Session (within-subjects)	F(3.670,165.134) = 37.188	<0.001	0.452	
		Lever x Session	F(3.561,160.248) = 28.849	<0.001	0.391	
Relapse (incubation) tests	Lever presses over 30 min (n=33)	RM-ANOVA (Lever x Day)	F-value	P-value	Partial η2	1D, left
		Lever (within-subjects)	F(1,30) = 195.173	<0.001	0.867	
		Day (between-subjects)	F(2,30) = 3.300	0.051	0.180	
		Lever x Day	F(2,30) = 3.438	0.045	0.186	
		1-way-ANOVA (Lever)	F-value	P-value	η2	
		Active lever	F(2,30) = 3.491	0.043	0.189	
		Inactive lever	F(2,30) = 1.458	0.249	0.089	
		Tukey HSD (Active lever)	Mean Difference	Std. Error	P-value	
		Day 1 vs. Day 15	-5.856	10.830	0.852	
		Day 1 vs. Day 60	-28.473	11.336	0.045	
		Day 15 vs. Day 60	-22.617	11.109	0.121	
Relapse (incubation) tests	Binned (3 x 10 min) lever presses (n=33)	RM-ANOVA (Lever x Bin x Day)	F-value	P-value	Partial η2	1D, right
		Lever (within-subjects)	F(1,30) = 198.809	<0.001	0.867	
		Bin (within-subjects)	F(1.701,51.033) = 16.618	<0.001	0.356	
		Day (between-subjects)	F(2,30) = 3.252	0.053	0.178	
		Lever x Day	F(2,30) = 3.432	0.045	0.186	
		Bin x Day	F(3.401,51.033) = 2.230	0.088	0.129	
		Lever x Bin	F(1.882,56.464) = 5.967	0.005	0.166	
		Lever x Bin x Day	F(3.764,56.464) = 2.512	0.055	0.143	
	Lever presses in first 10 min bin (n=33)	RM-ANOVA (Lever x Day)	F-value	P-value	Partial η2	
		Lever (within-subjects)	F(1,30) = 104.334	<0.001	0.777	
		Day (between-subjects)	F(2,30) = 4.215	0.024	0.219	
		Lever x Day	F(2,30) = 4.892	0.014	0.246	
		1-way-ANOVA (Lever)	F-value	P-value	η2	
		Active lever	F(2,30) = 4.578	0.018	0.234	
		Inactive lever	F(2,30) = 3.624	0.039	0.195	

		Tukey HSD (Active lever)	Mean Difference	Std. Error	P-value
		Day 1 vs. Day 15	1.621	5.812	0.958
		Day 1 vs. Day 60	-15.045	6.084	0.049
		Day 15 vs. Day 60	-16.667	5.962	0.024
		Tukey HSD (Inactive lever)	Mean Difference	Std. Error	P-value
		Day 1 vs. Day 15	-3.962	1.491	0.032
		Day 1 vs. Day 60	-2.645	1.560	0.223
		Day 15 vs. Day 60	1.317	1.529	0.668

Table S4. Statistical output for Figure 2B, left panel : Analysis of Z-scored counts from 10 major anatomical regions (n=29, analyses shown in figure 2B are highlighted in grey)

Factors in analysis	**Statistical output**			
RM-ANOVA (Region x Group)	F-value	P-value	Partial η2	
Region (within-subjects)	F(1.908,47.688) = 18.157	<0.001	0.421	
Group (between-subjects)	F(3,25) = 4.414	0.013	0.346	
Region x Group	F(5.723,47.688) = 6.099	<0.001	0.423	
1-way-ANOVA (Region)	F-value (ndf=3,ddf=25)	P-value	η2	
Isocortex	5.690	0.004	0.406	
Olfactory areas	6.337	0.002	0.432	
Hippocampal formation	6.719	0.002	0.446	
Cortical subplate	7.040	0.001	0.458	
Striatum	3.102	0.045	0.271	
Pallidum	2.389	0.093	0.223	
Thalamus	1.501	0.238	0.153	
Hypothalamus	2.053	0.132	0.198	
Midbrain	1.568	0.222	0.158	
Hindbrain	3.051	0.047	0.268	
Tukey HSD	Comparison	Mean Difference	Std. Error	P-value
Isocortex	Home cage vs. Day 1	0.041	1.412	1.000
	Home cage vs. Day 15	0.175	1.362	0.999
	Homecage vs. Day 60	-5.619	1.563	0.007
	Day 1 vs. Day 15	0.133	1.451	1.000
	Day 1 vs. Day 60	-5.661	1.641	0.010
	Day 15 vs. Day 60	-5.794	1.598	0.007
Olfactory areas	Home cage vs. Day 1	-0.138	1.077	0.999
	Home cage vs. Day 15	-0.476	1.039	0.967
	Homecage vs. Day 60	-4.755	1.193	0.003
	Day 1 vs. Day 15	-0.338	1.107	0.990
	Day 1 vs. Day 60	-4.617	1.252	0.006
	Day 15 vs. Day 60	-4.279	1.219	0.009
Hippocampal formation	Home cage vs. Day 1	0.412	1.082	0.981
	Home cage vs. Day 15	0.151	1.043	0.999
	Homecage vs. Day 60	-4.551	1.197	0.004
	Day 1 vs. Day 15	-0.261	1.111	0.995
	Day 1 vs. Day 60	-4.963	1.257	0.003
	Day 15 vs. Day 60	-4.702	1.224	0.004
Cortical subplate	Home cage vs. Day 1	-1.279	1.972	0.915
	Home cage vs. Day 15	-1.571	1.902	0.842

	Homecage vs. Day 60	-9.575	2.183	0.001
	Day 1 vs. Day 15	-0.291	2.026	0.999
	Day 1 vs. Day 60	-8.296	2.292	0.007
	Day 15 vs. Day 60	-8.005	2.231	0.007
Striatum	Home cage vs. Day 1	0.374	0.948	0.979
	Home cage vs. Day 15	0.569	0.914	0.924
	Homecage vs. Day 60	-2.460	1.049	0.115
	Day 1 vs. Day 15	0.194	0.973	0.997
	Day 1 vs. Day 60	-2.834	1.101	0.073
	Day 15 vs. Day 60	-3.028	1.072	0.043
Pallidum	Home cage vs. Day 1	0.692	0.867	0.854
	Home cage vs. Day 15	0.695	0.836	0.839
	Homecage vs. Day 60	-1.681	0.959	0.319
	Day 1 vs. Day 15	0.002	0.890	1.000
	Day 1 vs. Day 60	-2.374	1.007	0.112
	Day 15 vs. Day 60	-2.376	0.981	0.099
Thalamus	Home cage vs. Day 1	0.520	0.382	0.533
	Home cage vs. Day 15	0.566	0.368	0.430
	Homecage vs. Day 60	-0.128	0.422	0.990
	Day 1 vs. Day 15	0.046	0.392	0.999
	Day 1 vs. Day 60	-0.648	0.443	0.475
	Day 15 vs. Day 60	-0.694	0.432	0.393
Hypothalamus	Home cage vs. Day 1	0.218	1.453	0.999
	Home cage vs. Day 15	-0.370	1.401	0.993
	Homecage vs. Day 60	-3.531	1.608	0.152
	Day 1 vs. Day 15	-0.587	1.492	0.979
	Day 1 vs. Day 60	-3.749	1.688	0.145
	Day 15 vs. Day 60	-3.161	1.644	0.244
Midbrain	Home cage vs. Day 1	0.611	0.563	0.701
	Home cage vs. Day 15	0.559	0.542	0.733
	Homecage vs. Day 60	-0.611	0.623	0.761
	Day 1 vs. Day 15	-0.051	0.578	1.000
	Day 1 vs. Day 60	-1.222	0.654	0.266
	Day 15 vs. Day 60	-1.171	0.636	0.279
Hindbrain	Home cage vs. Day 1	0.667	1.505	0.970
	Home cage vs. Day 15	-0.162	1.451	0.999
	Homecage vs. Day 60	-4.222	1.665	0.079
	Day 1 vs. Day 15	-0.829	1.545	0.949
	Day 1 vs. Day 60	-4.889	1.748	0.045
	Day 15 vs. Day 60	-4.060	1.702	0.106

Table S5. Statistical output for Figure 2B, right panel: Analysis of Z-scored counts from 56 subdivisions of 10 major anatomical regions (n=29)

Factors in analysis	**Statistical Output**			
RM-ANOVA (Region x Group)	F-value	P-value	Partial η2	
Region (within-subjects)	F(1.813,45.313) = 18.664	<0.001	0.427	
Group (between-subjects)	F(3,25) = 5.962	0.003	0.417	
Region x Group	F(5.438,45.313) = 7.981	<0.001	0.489	
1-way-ANOVA (Region)	F-value (ndf=3,ddf=25)	P-value	η2	
Infralimbic area (ILA)	10.835	<0.001	0.565	
Visual areas (VIS)	9.141	<0.001	0.523	
Orbital area (ORB)	12.209	<0.001	0.594	
Anterior cingulate area (ACA)	3.884	0.021	0.318	
Agranular insular area (AI)	8.880	<0.001	0.516	
Prelimbic area (PL)	7.764	<0.001	0.482	
Ectorhinal area (ECT)	9.158	<0.001	0.524	
Retrosplenial area (RSP)	3.044	0.047	0.268	
Perirhinal area (PERI)	7.569	<0.001	0.476	
Somatomotor areas (MO)	3.731	0.024	0.309	
Somatosensory areas (SS)	3.419	0.033	0.291	
Temporal association areas (TEa)	7.361	0.001	0.469	
Auditory areas (AUD)	5.471	0.005	0.396	
Gustatory areas (GU)	4.325	0.014	0.342	
Visceral area (VISC)	2.905	0.055	0.259	
Frontal pole, cerebral cortex (FRP)	0.574	0.638	0.064	
Dorsal peduncular area (DP)	9.128	<0.001	0.523	
Taenia tecta (TT)	9.403	<0.001	0.530	
Cortical amygdalar area (COA)	4.597	0.011	0.355	
Piriform area (PIR)	6.227	0.003	0.428	
Anterior olfactory nucleus (AON)	11.283	<0.001	0.575	
Piriform-amygdalar area (PAA)	4.054	0.018	0.327	
Nucleus of the lateral olfactory tract (NLOT)	3.233	0.039	0.280	
Postpiriform transition area (TR)	3.296	0.037	0.283	
Accessory olfactory bulb (AOB)	0.089	0.966	0.011	
Main olfactory bulb (MOB)	0.271	0.846	0.031	
Retrohippocampal region (RHP)	9.113	<0.001	0.522	
Ammon's horn (CA)	2.356	0.096	0.220	
Dentate gyrus (DG)	2.470	0.085	0.229	
Fasciola cinerea (FC)	1.094	0.370	0.116	
Induseum griseum (IG)	1.124	0.358	0.119	
Claustrum (CLA)	10.132	<0.001	0.549	
Endopiriform nucleus (EP)	7.998	<0.001	0.490	
Basomedial amygdalar nucleus (BMA)	4.967	0.008	0.373	
Basolateral amygdalar nucleus (BLA)	5.178	0.006	0.383	
Posterior amygdalar nucleus (PA)	4.254	0.015	0.338	
Lateral amygdalar nucleus (LA)	2.122	0.123	0.203	
Lateral septal complex (LSX)	5.580	0.005	0.401	
Striatum ventral region (STRv)	3.698	0.025	0.307	
Striatum-like amygdalar nuclei (sAMY)	3.146	0.043	0.274	
Striatum dorsal region (STRd)	2.341	0.098	0.219	
Pallidum, caudal region (PALc)	3.350	0.035	0.287	
Pallidum, medial region (PALm)	2.585	0.076	0.237	
Pallidum, ventral region (PALv)	2.382	0.093	0.222	
Pallidum, dorsal region (PALd)	1.924	0.152	0.188	
Thalamus, polymodal association cortex related (DORpm)	1.728	0.187	0.172	
Thalamus, sensory-motor cortex related (DORsm)	1.236	0.318	0.129	
Periventricular region (PVR)	2.619	0.073	0.239	
Periventricular zone (PVZ)	1.849	0.164	0.182	
Hypothalamic medial zone (MEZ)	2.273	0.105	0.214	
Hypothalamic lateral zone (LZ)	2.033	0.135	0.196	

Midbrain, sensory related (MBsen)	2.101	0.126	0.201	
Midbrain, behavioral state related (MBsta)	1.308	0.294	0.136	
Midbrain, motor related (MBmot)	1.414	0.262	0.145	
Medulla (MY)	4.341	0.014	0.343	
Pons (P)	2.064	0.131	0.199	
Tukey HSD	Comparison	Mean Difference	Std. Error	P-value
Infralimbic area (ILA)	Home cage vs. Day 1	-0.389	3.823	1.000
	Home cage vs. Day 15	-0.507	3.686	0.999
	Homecage vs. Day 60	-21.537	4.231	<0.001
	Day 1 vs. Day 15	-0.117	3.926	1.000
	Day 1 vs. Day 60	-21.147	4.442	<0.001
	Day 15 vs. Day 60	-21.030	4.325	<0.001
Visual areas (VIS)	Home cage vs. Day 1	-1.796	3.236	0.944
	Home cage vs. Day 15	-1.781	3.120	0.940
	Homecage vs. Day 60	-17.515	3.581	<0.001
	Day 1 vs. Day 15	0.015	3.323	1.000
	Day 1 vs. Day 60	-15.719	3.760	0.002
	Day 15 vs. Day 60	-15.734	3.661	0.001
Orbital area (ORB)	Home cage vs. Day 1	-0.058	1.362	1.000
	Home cage vs. Day 15	0.290	1.313	0.996
	Homecage vs. Day 60	-7.954	1.508	<0.001
	Day 1 vs. Day 15	0.348	1.399	0.994
	Day 1 vs. Day 60	-7.896	1.583	<0.001
	Day 15 vs. Day 60	-8.244	1.541	<0.001
Anterior cingulate area (ACA)	Home cage vs. Day 1	0.405	2.254	0.998
	Home cage vs. Day 15	0.195	2.173	1.000
	Homecage vs. Day 60	-7.311	2.494	0.034
	Day 1 vs. Day 15	-0.210	2.315	1.000
	Day 1 vs. Day 60	-7.716	2.619	0.033
	Day 15 vs. Day 60	-7.506	2.550	0.033
Agranular insular area (AI)	Home cage vs. Day 1	-0.102	1.216	1.000
	Home cage vs. Day 15	-0.017	1.172	1.000
	Homecage vs. Day 60	-6.155	1.345	<0.001
	Day 1 vs. Day 15	0.085	1.248	1.000
	Day 1 vs. Day 60	-6.052	1.412	0.001
	Day 15 vs. Day 60	-6.138	1.375	<0.001
Prelimbic area (PL)	Home cage vs. Day 1	0.471	1.318	0.984
	Home cage vs. Day 15	0.231	1.271	0.998
	Homecage vs. Day 60	-5.976	1.459	0.002
	Day 1 vs. Day 15	-0.240	1.354	0.998
	Day 1 vs. Day 60	-6.447	1.532	0.002
	Day 15 vs. Day 60	-6.207	1.491	0.002
Ectorhinal area (ECT)	Home cage vs. Day 1	-0.108	0.946	0.999
	Home cage vs. Day 15	0.114	0.912	0.999
	Homecage vs. Day 60	-4.827	1.047	<0.001
	Day 1 vs. Day 15	0.223	0.972	0.996
	Day 1 vs. Day 60	-4.719	1.099	0.001
	Day 15 vs. Day 60	-4.942	1.070	<0.001
Retrosplenial area (RSP)	Home cage vs. Day 1	0.302	1.546	0.997
	Home cage vs. Day 15	0.000	1.491	1.000
	Homecage vs. Day 60	-4.458	1.711	0.068
	Day 1 vs. Day 15	-0.302	1.588	0.998
	Day 1 vs. Day 60	-4.760	1.797	0.062
	Day 15 vs. Day 60	-4.458	1.749	0.076
Perirhinal area (PERI)	Home cage vs. Day 1	0.053	0.873	1.000
	Home cage vs. Day 15	0.038	0.842	1.000
	Homecage vs. Day 60	-4.031	0.966	0.002
	Day 1 vs. Day 15	-0.015	0.897	1.000
	Day 1 vs. Day 60	-4.084	1.015	0.002

	Day 15 vs. Day 60	-4.068	0.988	0.002
Somatomotor areas (MO)	Home cage vs. Day 1	0.565	1.308	0.972
	Home cage vs. Day 15	0.695	1.261	0.945
	Homecage vs. Day 60	-3.806	1.448	0.065
	Day 1 vs. Day 15	0.130	1.343	1.000
	Day 1 vs. Day 60	-4.371	1.520	0.038
	Day 15 vs. Day 60	-4.501	1.480	0.026
Somatosensory areas (SS)	Home cage vs. Day 1	0.211	1.155	0.998
	Home cage vs. Day 15	0.436	1.114	0.979
	Homecage vs. Day 60	-3.374	1.278	0.063
	Day 1 vs. Day 15	0.225	1.186	0.998
	Day 1 vs. Day 60	-3.586	1.342	0.059
	Day 15 vs. Day 60	-3.811	1.307	0.035
Temporal association areas (TEa)	Home cage vs. Day 1	0.088	0.767	0.999
	Home cage vs. Day 15	0.356	0.740	0.963
	Homecage vs. Day 60	-3.354	0.849	0.003
	Day 1 vs. Day 15	0.268	0.788	0.986
	Day 1 vs. Day 60	-3.442	0.892	0.004
	Day 15 vs. Day 60	-3.710	0.868	0.001
Auditory areas (AUD)	Home cage vs. Day 1	0.051	0.846	1.000
	Home cage vs. Day 15	0.361	0.816	0.970
	Homecage vs. Day 60	-3.186	0.936	0.011
	Day 1 vs. Day 15	0.310	0.869	0.984
	Day 1 vs. Day 60	-3.237	0.983	0.015
	Day 15 vs. Day 60	-3.547	0.957	0.005
Gustatory areas (GU)	Home cage vs. Day 1	0.422	0.643	0.912
	Home cage vs. Day 15	0.541	0.620	0.818
	Homecage vs. Day 60	-1.880	0.712	0.063
	Day 1 vs. Day 15	0.119	0.660	0.998
	Day 1 vs. Day 60	-2.303	0.747	0.024
	Day 15 vs. Day 60	-2.422	0.727	0.013
Visceral area (VISC)	Home cage vs. Day 1	0.212	0.578	0.983
	Home cage vs. Day 15	0.329	0.557	0.934
	Homecage vs. Day 60	-1.458	0.640	0.130
	Day 1 vs. Day 15	0.117	0.594	0.997
	Day 1 vs. Day 60	-1.671	0.672	0.087
	Day 15 vs. Day 60	-1.787	0.654	0.052
Frontal pole, cerebral cortex (FRP)	Home cage vs. Day 1	0.037	0.461	1.000
	Home cage vs. Day 15	-0.058	0.445	0.999
	Homecage vs. Day 60	0.574	0.510	0.678
	Day 1 vs. Day 15	-0.095	0.474	0.997
	Day 1 vs. Day 60	0.537	0.536	0.749
	Day 15 vs. Day 60	0.633	0.522	0.625
Dorsal peduncular area (DP)	Home cage vs. Day 1	-0.922	3.029	0.990
	Home cage vs. Day 15	-0.511	2.920	0.998
	Homecage vs. Day 60	-15.873	3.352	<0.001
	Day 1 vs. Day 15	0.412	3.110	0.999
	Day 1 vs. Day 60	-14.950	3.519	0.001
	Day 15 vs. Day 60	-15.362	3.426	<0.001
Taenia tecta (TT)	Home cage vs. Day 1	-0.156	1.645	1.000
	Home cage vs. Day 15	-0.524	1.586	0.987
	Homecage vs. Day 60	-8.727	1.821	<0.001
	Day 1 vs. Day 15	-0.368	1.690	0.996
	Day 1 vs. Day 60	-8.571	1.912	<0.001
	Day 15 vs. Day 60	-8.203	1.861	<0.001
Cortical amygdalar area (COA)	Home cage vs. Day 1	-0.463	1.844	0.994
	Home cage vs. Day 15	-1.448	1.778	0.847
	Homecage vs. Day 60	-7.131	2.041	0.009
	Day 1 vs. Day 15	-0.985	1.894	0.953
	Day 1 vs. Day 60	-6.668	2.143	0.022

	Day 15 vs. Day 60	-5.683	2.086	0.053
Piriform area (PIR)	Home cage vs. Day 1	-0.240	1.305	0.998
	Home cage vs. Day 15	-0.574	1.259	0.968
	Homecage vs. Day 60	-5.734	1.445	0.003
	Day 1 vs. Day 15	-0.334	1.341	0.994
	Day 1 vs. Day 60	-5.494	1.517	0.007
	Day 15 vs. Day 60	-5.160	1.477	0.009
Anterior olfactory nucleus (AON)	Home cage vs. Day 1	-0.095	0.860	0.999
	Home cage vs. Day 15	-0.280	0.829	0.986
	Homecage vs. Day 60	-4.991	0.951	<0.001
	Day 1 vs. Day 15	-0.185	0.883	0.997
	Day 1 vs. Day 60	-4.896	0.999	<0.001
	Day 15 vs. Day 60	-4.711	0.972	<0.001
Piriform-amygdalar area (PAA)	Home cage vs. Day 1	-0.233	1.280	0.998
	Home cage vs. Day 15	-1.101	1.234	0.809
	Homecage vs. Day 60	-4.634	1.417	0.015
	Day 1 vs. Day 15	-0.868	1.315	0.911
	Day 1 vs. Day 60	-4.401	1.487	0.032
	Day 15 vs. Day 60	-3.533	1.448	0.095
Nucleus of the lateral olfactory tract (NLOT)	Home cage vs. Day 1	0.006	1.259	1.000
	Home cage vs. Day 15	-0.120	1.214	1.000
	Homecage vs. Day 60	-3.862	1.394	0.048
	Day 1 vs. Day 15	-0.126	1.293	1.000
	Day 1 vs. Day 60	-3.868	1.463	0.063
	Day 15 vs. Day 60	-3.742	1.425	0.065
Postpiriform transition area (TR)	Home cage vs. Day 1	0.304	0.613	0.959
	Home cage vs. Day 15	0.195	0.591	0.987
	Homecage vs. Day 60	-1.702	0.679	0.083
	Day 1 vs. Day 15	-0.109	0.630	0.998
	Day 1 vs. Day 60	-2.006	0.712	0.043
	Day 15 vs. Day 60	-1.897	0.694	0.052
Accessory olfactory bulb (AOB)	Home cage vs. Day 1	0.027	0.588	1.000
	Home cage vs. Day 15	0.260	0.567	0.967
	Homecage vs. Day 60	0.010	0.651	1.000
	Day 1 vs. Day 15	0.233	0.604	0.980
	Day 1 vs. Day 60	-0.017	0.683	1.000
	Day 15 vs. Day 60	-0.250	0.665	0.982
Main olfactory bulb (MOB)	Home cage vs. Day 1	0.215	0.560	0.980
	Home cage vs. Day 15	0.049	0.540	1.000
	Homecage vs. Day 60	0.522	0.620	0.834
	Day 1 vs. Day 15	-0.167	0.575	0.991
	Day 1 vs. Day 60	0.306	0.651	0.965
	Day 15 vs. Day 60	0.473	0.634	0.877
Retrohippocampal region (RHP)	Home cage vs. Day 1	0.146	1.372	1.000
	Home cage vs. Day 15	-0.129	1.323	1.000
	Homecage vs. Day 60	-6.992	1.518	<0.001
	Day 1 vs. Day 15	-0.274	1.409	0.997
	Day 1 vs. Day 60	-7.138	1.594	<0.001
	Day 15 vs. Day 60	-6.863	1.552	<0.001
Ammon's horn (CA)	Home cage vs. Day 1	0.449	0.753	0.932
	Home cage vs. Day 15	0.400	0.726	0.945
	Homecage vs. Day 60	-1.625	0.834	0.234
	Day 1 vs. Day 15	-0.049	0.774	1.000
	Day 1 vs. Day 60	-2.075	0.875	0.109
	Day 15 vs. Day 60	-2.025	0.852	0.108
Dentate gyrus (DG)	Home cage vs. Day 1	0.914	0.658	0.518
	Home cage vs. Day 15	0.589	0.635	0.790
	Homecage vs. Day 60	-1.025	0.729	0.507
	Day 1 vs. Day 15	-0.325	0.676	0.963
	Day 1 vs. Day 60	-1.939	0.765	0.079

	Day 15 vs. Day 60	-1.614	0.745	0.160
Fasciola cinerea (FC)	Home cage vs. Day 1	0.424	0.713	0.933
	Home cage vs. Day 15	0.814	0.688	0.643
	Homecage vs. Day 60	-0.558	0.789	0.893
	Day 1 vs. Day 15	0.390	0.732	0.950
	Day 1 vs. Day 60	-0.982	0.829	0.641
	Day 15 vs. Day 60	-1.372	0.807	0.344
Induseum griseum (IG)	Home cage vs. Day 1	0.425	0.313	0.537
	Home cage vs. Day 15	0.475	0.302	0.412
	Homecage vs. Day 60	0.095	0.347	0.993
	Day 1 vs. Day 15	0.050	0.322	0.999
	Day 1 vs. Day 60	-0.331	0.364	0.801
	Day 15 vs. Day 60	-0.381	0.354	0.708
Claustrum (CLA)	Home cage vs. Day 1	-4.323	4.982	0.821
	Home cage vs. Day 15	-6.156	4.804	0.583
	Homecage vs. Day 60	-29.328	5.515	<0.001
	Day 1 vs. Day 15	-1.833	5.117	0.984
	Day 1 vs. Day 60	-25.006	5.789	0.001
	Day 15 vs. Day 60	-23.173	5.636	0.002
Endopiriform nucleus (EP)	Home cage vs. Day 1	-1.798	2.489	0.887
	Home cage vs. Day 15	-2.106	2.399	0.816
	Homecage vs. Day 60	-12.891	2.754	<0.001
	Day 1 vs. Day 15	-0.308	2.556	0.999
	Day 1 vs. Day 60	-11.093	2.891	0.004
	Day 15 vs. Day 60	-10.785	2.815	0.004
Basomedial amygdalar nucleus (BMA)	Home cage vs. Day 1	-1.148	2.059	0.944
	Home cage vs. Day 15	-1.584	1.986	0.855
	Homecage vs. Day 60	-8.437	2.279	0.005
	Day 1 vs. Day 15	-0.436	2.115	0.997
	Day 1 vs. Day 60	-7.289	2.393	0.026
	Day 15 vs. Day 60	-6.853	2.330	0.033
Basolateral amygdalar nucleus (BLA)	Home cage vs. Day 1	-0.778	1.181	0.911
	Home cage vs. Day 15	-0.924	1.139	0.849
	Homecage vs. Day 60	-4.961	1.307	0.004
	Day 1 vs. Day 15	-0.146	1.213	0.999
	Day 1 vs. Day 60	-4.183	1.372	0.026
	Day 15 vs. Day 60	-4.038	1.336	0.027
Posterior amygdalar nucleus (PA)	Home cage vs. Day 1	0.103	1.353	1.000
	Home cage vs. Day 15	-1.104	1.304	0.832
	Homecage vs. Day 60	-4.866	1.497	0.016
	Day 1 vs. Day 15	-1.207	1.389	0.821
	Day 1 vs. Day 60	-4.969	1.572	0.020
	Day 15 vs. Day 60	-3.762	1.530	0.092
Lateral amygdalar nucleus (LA)	Home cage vs. Day 1	-0.428	0.678	0.921
	Home cage vs. Day 15	0.302	0.653	0.966
	Homecage vs. Day 60	-1.540	0.750	0.196
	Day 1 vs. Day 15	0.731	0.696	0.722
	Day 1 vs. Day 60	-1.112	0.787	0.504
	Day 15 vs. Day 60	-1.842	0.767	0.102
Lateral septal complex (LSX)	Home cage vs. Day 1	-0.136	2.983	1.000
	Home cage vs. Day 15	-0.490	2.877	0.998
	Homecage vs. Day 60	-12.099	3.302	0.006
	Day 1 vs. Day 15	-0.353	3.064	0.999
	Day 1 vs. Day 60	-11.963	3.466	0.010
	Day 15 vs. Day 60	-11.610	3.375	0.010
Striatum ventral region (STRv)	Home cage vs. Day 1	0.246	1.452	0.998
	Home cage vs. Day 15	0.265	1.400	0.998
	Homecage vs. Day 60	-4.546	1.607	0.042
	Day 1 vs. Day 15	0.019	1.491	1.000
	Day 1 vs. Day 60	-4.793	1.687	0.041

	Day 15 vs. Day 60	-4.811	1.642	0.034
Striatum-like amygdalar nuclei (sAMY)	Home cage vs. Day 1	-0.193	1.447	0.999
	Home cage vs. Day 15	-0.558	1.395	0.978
	Homecage vs. Day 60	-4.541	1.601	0.042
	Day 1 vs. Day 15	-0.365	1.486	0.995
	Day 1 vs. Day 60	-4.347	1.681	0.071
	Day 15 vs. Day 60	-3.983	1.637	0.096
Striatum dorsal region (STRd)	Home cage vs. Day 1	0.457	0.566	0.851
	Home cage vs. Day 15	0.757	0.546	0.519
	Homecage vs. Day 60	-0.856	0.626	0.531
	Day 1 vs. Day 15	0.300	0.581	0.954
	Day 1 vs. Day 60	-1.312	0.657	0.216
	Day 15 vs. Day 60	-1.613	0.640	0.081
Pallidum, caudal region (PALc)	Home cage vs. Day 1	-0.198	2.328	1.000
	Home cage vs. Day 15	-0.487	2.245	0.996
	Homecage vs. Day 60	-7.402	2.577	0.038
	Day 1 vs. Day 15	-0.289	2.391	0.999
	Day 1 vs. Day 60	-7.204	2.705	0.060
	Day 15 vs. Day 60	-6.915	2.634	0.065
Pallidum, medial region (PALm)	Home cage vs. Day 1	0.720	1.136	0.920
	Home cage vs. Day 15	0.450	1.095	0.976
	Homecage vs. Day 60	-2.640	1.257	0.180
	Day 1 vs. Day 15	-0.270	1.166	0.995
	Day 1 vs. Day 60	-3.360	1.319	0.077
	Day 15 vs. Day 60	-3.090	1.285	0.102
Pallidum, ventral region (PALv)	Home cage vs. Day 1	0.656	0.796	0.842
	Home cage vs. Day 15	0.755	0.767	0.760
	Homecage vs. Day 60	-1.458	0.881	0.367
	Day 1 vs. Day 15	0.099	0.817	0.999
	Day 1 vs. Day 60	-2.114	0.924	0.128
	Day 15 vs. Day 60	-2.213	0.900	0.092
Pallidum, dorsal region (PALd)	Home cage vs. Day 1	0.683	0.453	0.449
	Home cage vs. Day 15	0.754	0.437	0.332
	Homecage vs. Day 60	-0.195	0.502	0.980
	Day 1 vs. Day 15	0.071	0.465	0.999
	Day 1 vs. Day 60	-0.878	0.526	0.361
	Day 15 vs. Day 60	-0.949	0.513	0.274
Thalamus, polymodal association cortex related (DORpm)	Home cage vs. Day 1	0.575	0.405	0.500
	Home cage vs. Day 15	0.651	0.390	0.360
	Homecage vs. Day 60	-0.152	0.448	0.986
	Day 1 vs. Day 15	0.077	0.416	0.998
	Day 1 vs. Day 60	-0.727	0.470	0.427
	Day 15 vs. Day 60	-0.804	0.458	0.318
Thalamus, sensory-motor cortex related (DORsm)	Home cage vs. Day 1	0.479	0.334	0.491
	Home cage vs. Day 15	0.494	0.323	0.435
	Homecage vs. Day 60	0.036	0.370	1.000
	Day 1 vs. Day 15	0.015	0.343	1.000
	Day 1 vs. Day 60	-0.443	0.389	0.669
	Day 15 vs. Day 60	-0.458	0.378	0.627
Periventricular region (PVR)	Home cage vs. Day 1	-0.035	1.965	1.000
	Home cage vs. Day 15	-0.922	1.895	0.961
	Homecage vs. Day 60	-5.591	2.175	0.073
	Day 1 vs. Day 15	-0.887	2.018	0.971
	Day 1 vs. Day 60	-5.556	2.283	0.096
	Day 15 vs. Day 60	-4.669	2.223	0.181
Periventricular zone (PVZ)	Home cage vs. Day 1	0.386	1.838	0.997
	Home cage vs. Day 15	-1.274	1.772	0.889
	Homecage vs. Day 60	-4.186	2.034	0.195
	Day 1 vs. Day 15	-1.660	1.888	0.816
	Day 1 vs. Day 60	-4.571	2.136	0.168

	Day 15 vs. Day 60	-2.912	2.079	0.511
Hypothalamic medial zone (MEZ)	Home cage vs. Day 1	0.318	1.568	0.997
	Home cage vs. Day 15	-0.477	1.512	0.989
	Homecage vs. Day 60	-3.987	1.736	0.126
	Day 1 vs. Day 15	-0.796	1.610	0.960
	Day 1 vs. Day 60	-4.305	1.822	0.111
	Day 15 vs. Day 60	-3.509	1.774	0.223
Hypothalamic lateral zone (LZ)	Home cage vs. Day 1	0.279	1.176	0.995
	Home cage vs. Day 15	-0.058	1.134	1.000
	Homecage vs. Day 60	-2.751	1.302	0.177
	Day 1 vs. Day 15	-0.337	1.208	0.992
	Day 1 vs. Day 60	-3.030	1.367	0.146
	Day 15 vs. Day 60	-2.694	1.331	0.206
Midbrain, sensory related (MBsen)	Home cage vs. Day 1	0.542	0.575	0.782
	Home cage vs. Day 15	0.508	0.554	0.796
	Homecage vs. Day 60	-0.939	0.636	0.466
	Day 1 vs. Day 15	-0.034	0.590	1.000
	Day 1 vs. Day 60	-1.482	0.668	0.146
	Day 15 vs. Day 60	-1.448	0.650	0.143
Midbrain, behavioral state related (MBsta)	Home cage vs. Day 1	0.756	0.750	0.746
	Home cage vs. Day 15	0.467	0.723	0.916
	Homecage vs. Day 60	-0.862	0.830	0.729
	Day 1 vs. Day 15	-0.289	0.770	0.982
	Day 1 vs. Day 60	-1.618	0.872	0.272
	Day 15 vs. Day 60	-1.329	0.849	0.415
Midbrain, motor related (MBmot)	Home cage vs. Day 1	0.640	0.588	0.700
	Home cage vs. Day 15	0.590	0.567	0.728
	Homecage vs. Day 60	-0.557	0.651	0.828
	Day 1 vs. Day 15	-0.050	0.604	1.000
	Day 1 vs. Day 60	-1.197	0.684	0.320
	Day 15 vs. Day 60	-1.147	0.666	0.334
Medulla (MY)	Home cage vs. Day 1	-0.203	2.017	1.000
	Home cage vs. Day 15	-1.732	1.945	0.810
	Homecage vs. Day 60	-7.485	2.233	0.013
	Day 1 vs. Day 15	-1.528	2.072	0.881
	Day 1 vs. Day 60	-7.282	2.344	0.023
	Day 15 vs. Day 60	-5.753	2.282	0.081
Pons (P)	Home cage vs. Day 1	0.692	1.280	0.948
	Home cage vs. Day 15	0.158	1.234	0.999
	Homecage vs. Day 60	-2.773	1.416	0.231
	Day 1 vs. Day 15	-0.534	1.314	0.977
	Day 1 vs. Day 60	-3.465	1.487	0.118
	Day 15 vs. Day 60	-2.931	1.447	0.206

Table S6. Statistical output for Figure 3B, right panel : Analysis of Z-scored counts from 5 major anatomical regions within AP +1.55 to AP +1.75 coronal subvolume (n=31, analyses shown in figure 3B are highlighted in grey)

Factors in analysis	**Statistical output**			
RM-ANOVA (Region x Group)	F-value	P-value	Partial η2	
Region (within-subjects)	F(1.573,4.720) = 6.533	0.006	0.189	
Group (between-subjects)	F(3,28) = 6.853	0.001	0.423	
Region x Group	F(4.720,44.049) = 4.689	0.002	0.334	
1-way-ANOVA (Region)	F-value (ndf=3,ddf=28)	P-value	η2	
Isocortex	7.388	0.001	0.442	
Olfactory areas	8.949	<0.001	0.489	
Cortical subplate	7.689	<0.001	0.452	
Striatum	5.953	0.003	0.389	
Pallidum	4.790	0.008	0.339	
Tukey HSD	Comparison	Mean Difference	Std. Error	P-value
Isocortex	Home cage vs. Day 1	0.545	1.778	0.990
	Home cage vs. Day 15	0.031	1.778	1.000
	Homecage vs. Day 60	-8.578	2.064	0.001
	Day 1 vs. Day 15	-0.514	1.913	0.993
	Day 1 vs. Day 60	-9.123	2.181	0.001
	Day 15 vs. Day 60	-8.609	2.181	0.003
Olfactory areas	Home cage vs. Day 1	0.595	1.703	0.985
	Home cage vs. Day 15	-0.957	1.703	0.942
	Homecage vs. Day 60	-9.228	1.977	0.000
	Day 1 vs. Day 15	-1.552	1.833	0.832
	Day 1 vs. Day 60	-9.822	2.090	0.000
	Day 15 vs. Day 60	-8.271	2.090	0.003
Cortical subplate	Home cage vs. Day 1	0.459	2.752	0.998
	Home cage vs. Day 15	-0.700	2.752	0.994
	Homecage vs. Day 60	-13.872	3.194	0.001
	Day 1 vs. Day 15	-1.159	2.961	0.979
	Day 1 vs. Day 60	-14.331	3.376	0.001
	Day 15 vs. Day 60	-13.172	3.376	0.003
Striatum	Home cage vs. Day 1	0.754	2.972	0.994
	Home cage vs. Day 15	-0.941	2.972	0.989
	Homecage vs. Day 60	-13.111	3.450	0.004
	Day 1 vs. Day 15	-1.696	3.198	0.951
	Day 1 vs. Day 60	-13.866	3.646	0.004
	Day 15 vs. Day 60	-12.170	3.646	0.012
Pallidum	Home cage vs. Day 1	0.750	2.204	0.986
	Home cage vs. Day 15	-1.217	2.204	0.945
	Homecage vs. Day 60	-8.677	2.559	0.011
	Day 1 vs. Day 15	-1.966	2.372	0.840
	Day 1 vs. Day 60	-9.427	2.704	0.008
	Day 15 vs. Day 60	-7.460	2.704	0.047

Table S7. Statistical output for Figure 3C: Analysis of Z-scored counts from 18 subregions within AP +1.55 to AP +1.75 coronal subvolume (n=31)

Factors in analysis	**Statistical Output**			
RM-ANOVA (Region x Group)	F-value	P-value	Partial η2	
Region (within-subjects)	F(1.168,32.698) = 9.502	0.003	0.253	
Group (between-subjects)	F(3,28) = 7.240	0.001	0.437	
Region x Group	F(3.503,32.698) = 6.321	0.001	0.404	
1-way-ANOVA (Region)	F-value (ndf=3,ddf=28)	P-value	η2	
Orbital area (ORB)	11.349	<0.001	0.549	
Anterior cingulate area (ACA)	6.074	0.003	0.394	
Infralimbic area (ILA)	7.624	<0.001	0.450	
Prelimbic area (PL)	6.868	0.001	0.424	
Agranular insular area (AI)	11.030	<0.001	0.542	
Somatomotor areas (MO)	5.737	0.003	0.381	
Gustatory areas (GU)	4.764	0.008	0.338	
Somatosensory areas (SS)	4.215	0.014	0.311	
Dorsal peduncular area (DP)	5.876	0.003	0.386	
Taenia tecta (TT)	7.782	<0.001	0.455	
Anterior olfactory nucleus (AON)	10.154	<0.001	0.521	
Piriform area (PIR)	8.909	<0.001	0.488	
Endopiriform nucleus (EP)	8.681	<0.001	0.482	
Claustrum (CLA)	6.220	0.002	0.400	
Lateral septum (LS)	5.714	0.004	0.380	
Nucleus accumbens (ACB)	5.509	0.004	0.371	
Olfactory tubercle (OT)	6.070	0.003	0.394	
Substantia innominata (SI)	4.790	0.008	0.339	
Tukey HSD	Comparison	Mean Difference	Std. Error	P-value
Orbital area (ORB)	Home cage vs. Day 1	0.018	4.269	1.000
	Home cage vs. Day 15	-1.379	4.269	0.988
	Homecage vs. Day 60	-26.455	4.956	<0.001
	Day 1 vs. Day 15	-1.397	4.594	0.990
	Day 1 vs. Day 60	-26.473	5.238	<0.001
	Day 15 vs. Day 60	-25.076	5.238	<0.001
Anterior cingulate area (ACA)	Home cage vs. Day 1	0.885	5.763	0.999
	Home cage vs. Day 15	-1.098	5.763	0.997
	Homecage vs. Day 60	-25.764	6.689	0.003
	Day 1 vs. Day 15	-1.982	6.201	0.988
	Day 1 vs. Day 60	-26.648	7.070	0.004
	Day 15 vs. Day 60	-24.666	7.070	0.008
Infralimbic area (ILA)	Home cage vs. Day 1	0.147	4.793	1.000
	Home cage vs. Day 15	-0.367	4.793	1.000
	Homecage vs. Day 60	-24.079	5.564	<0.001
	Day 1 vs. Day 15	-0.514	5.158	1.000
	Day 1 vs. Day 60	-24.226	5.881	0.002
	Day 15 vs. Day 60	-23.713	5.881	0.002
Prelimbic area (PL)	Home cage vs. Day 1	0.578	4.348	0.999
	Home cage vs. Day 15	-1.044	4.348	0.995
	Homecage vs. Day 60	-20.753	5.047	0.002
	Day 1 vs. Day 15	-1.623	4.678	0.985
	Day 1 vs. Day 60	-21.332	5.334	0.002
	Day 15 vs. Day 60	-19.709	5.334	0.005
Agranular insular area (AI)	Home cage vs. Day 1	0.349	1.931	0.998
	Home cage vs. Day 15	-0.879	1.931	0.968
	Homecage vs. Day 60	-11.727	2.241	<0.001
	Day 1 vs. Day 15	-1.228	2.077	0.934
	Day 1 vs. Day 60	-12.076	2.369	<0.001
	Day 15 vs. Day 60	-10.848	2.369	<0.001
Somatomotor areas (MO)	Home cage vs. Day 1	0.662	1.516	0.972

	Home cage vs. Day 15	0.255	1.516	0.998
	Homecage vs. Day 60	-6.283	1.760	0.007
	Day 1 vs. Day 15	-0.406	1.632	0.994
	Day 1 vs. Day 60	-6.945	1.860	0.004
	Day 15 vs. Day 60	-6.539	1.860	0.008
Gustatory areas (GU)	Home cage vs. Day 1	0.481	0.908	0.951
	Home cage vs. Day 15	0.201	0.908	0.996
	Homecage vs. Day 60	-3.361	1.054	0.017
	Day 1 vs. Day 15	-0.281	0.977	0.992
	Day 1 vs. Day 60	-3.842	1.115	0.009
	Day 15 vs. Day 60	-3.561	1.115	0.017
Somatosensory areas (SS)	Home cage vs. Day 1	0.503	0.744	0.905
	Home cage vs. Day 15	0.587	0.744	0.859
	Homecage vs. Day 60	-2.363	0.863	0.049
	Day 1 vs. Day 15	0.084	0.800	1.000
	Day 1 vs. Day 60	-2.866	0.912	0.019
	Day 15 vs. Day 60	-2.950	0.912	0.016
Dorsal peduncular area (DP)	Home cage vs. Day 1	0.309	6.916	1.000
	Home cage vs. Day 15	-1.005	6.916	0.999
	Homecage vs. Day 60	-30.601	8.027	0.004
	Day 1 vs. Day 15	-1.314	7.442	0.998
	Day 1 vs. Day 60	-30.910	8.485	0.006
	Day 15 vs. Day 60	-29.596	8.485	0.008
Taenia tecta (TT)	Home cage vs. Day 1	0.297	4.496	1.000
	Home cage vs. Day 15	-1.455	4.496	0.988
	Homecage vs. Day 60	-23.030	5.219	<0.001
	Day 1 vs. Day 15	-1.752	4.838	0.983
	Day 1 vs. Day 60	-23.327	5.516	0.001
	Day 15 vs. Day 60	-21.575	5.516	0.003
Anterior olfactory nucleus (AON)	Home cage vs. Day 1	0.447	2.981	0.999
	Home cage vs. Day 15	-1.445	2.981	0.962
	Homecage vs. Day 60	-17.425	3.460	<0.001
	Day 1 vs. Day 15	-1.892	3.208	0.934
	Day 1 vs. Day 60	-17.872	3.657	<0.001
	Day 15 vs. Day 60	-15.980	3.657	<0.001
Piriform area (PIR)	Home cage vs. Day 1	0.608	1.229	0.960
	Home cage vs. Day 15	-0.835	1.229	0.904
	Homecage vs. Day 60	-6.574	1.427	<0.001
	Day 1 vs. Day 15	-1.442	1.323	0.698
	Day 1 vs. Day 60	-7.182	1.508	<0.001
	Day 15 vs. Day 60	-5.739	1.508	0.004
Endopiriform nucleus (EP)	Home cage vs. Day 1	0.502	1.817	0.992
	Home cage vs. Day 15	-1.348	1.817	0.879
	Homecage vs. Day 60	-9.782	2.109	<0.001
	Day 1 vs. Day 15	-1.850	1.955	0.780
	Day 1 vs. Day 60	-10.284	2.229	<0.001
	Day 15 vs. Day 60	-8.434	2.229	0.004
Claustrum (CLA)	Home cage vs. Day 1	0.145	2.131	1.000
	Home cage vs. Day 15	0.332	2.131	0.999
	Homecage vs. Day 60	-9.499	2.474	0.003
	Day 1 vs. Day 15	0.187	2.293	1.000
	Day 1 vs. Day 60	-9.644	2.615	0.005
	Day 15 vs. Day 60	-9.831	2.615	0.004
Lateral septum (LS)	Home cage vs. Day 1	0.373	4.426	1.000
	Home cage vs. Day 15	-1.852	4.426	0.975
	Homecage vs. Day 60	-19.497	5.138	0.004
	Day 1 vs. Day 15	-2.225	4.763	0.966
	Day 1 vs. Day 60	-19.869	5.431	0.005
	Day 15 vs. Day 60	-17.644	5.431	0.015
Nucleus accumbens (ACB)	Home cage vs. Day 1	0.920	4.006	0.996

	Home cage vs. Day 15	-0.662	4.006	0.998
	Homecage vs. Day 60	-16.919	4.650	0.006
	Day 1 vs. Day 15	-1.582	4.311	0.983
	Day 1 vs. Day 60	-17.839	4.915	0.006
	Day 15 vs. Day 60	-16.257	4.915	0.013
Olfactory tubercle (OT)	Home cage vs. Day 1	0.638	2.081	0.990
	Home cage vs. Day 15	-0.817	2.081	0.979
	Homecage vs. Day 60	-9.247	2.415	0.004
	Day 1 vs. Day 15	-1.455	2.239	0.915
	Day 1 vs. Day 60	-9.885	2.553	0.003
	Day 15 vs. Day 60	-8.430	2.553	0.013
Substantia innominata (SI)	Home cage vs. Day 1	0.750	2.204	0.986
	Home cage vs. Day 15	-1.217	2.204	0.945
	Homecage vs. Day 60	-8.677	2.559	0.011
	Day 1 vs. Day 15	-1.966	2.372	0.840
	Day 1 vs. Day 60	-9.427	2.704	0.008
	Day 15 vs. Day 60	-7.460	2.704	0.047

Table S8. Statistical output for Figure 3D: Analysis of Z-scored counts from 43 subdivisions within AP +1.55 to AP +1.75 coronal subvolume (n=31)

Factors in analysis	**Statistical Output**			
RM-ANOVA (Region x Group)	F-value	P-value	Partial η2	
Region (within-subjects)	F(1.151,32.235) = 10.299	0.002	0.269	
Group (between-subjects)	F(3,28) = 7.124	0.001	0.433	
Region x Group	F(3.454,32.235) = 6.518	0.001	0.411	
1-way-ANOVA (Region)	F-value (ndf=3,ddf=28)	P-value	η2	
Prelimbic area, layer 5 (PL5)	6.890	0.001	0.425	
Infralimbic area, layer 6a (ILA6a)	9.712	<0.001	0.510	
Infralimbic area, layer 5 (ILA5)	7.170	0.001	0.434	
Orbital area, lateral part (ORBl)	12.128	<0.001	0.565	
Prelimbic area, layer 6a (PL6a)	7.815	<0.001	0.456	
Anterior cingulate area (ACA)	6.074	0.003	0.394	
Orbital area, ventrolateral part (ORBvl)	9.708	<0.001	0.510	
Prelimbic area, layer 2/3 (PL2/3)	6.974	0.001	0.428	
Agranular insular area, ventral part (AIv)	12.000	<0.001	0.563	
Prelimbic area, layer 2 (PL2)	7.211	<0.001	0.436	
Infralimbic area, layer 2/3 (ILA2/3)	6.352	0.002	0.405	
Gustatory areas, layer 6a (GU6a)	5.163	0.006	0.356	
Secondary motor area (MOs)	6.852	0.001	0.423	
Agranular insular area, dorsal part (AId)	9.063	<0.001	0.493	
Infralimbic area, layer 6b (ILA6b)	6.884	0.001	0.424	
Prelimbic area, layer 1 (PL1)	4.814	0.008	0.340	
Prelimbic area, layer 6b (PL6b)	4.534	0.010	0.327	
Gustatory areas, layer 5 (GU5)	4.795	0.008	0.339	
Primary motor area (MOp)	4.837	0.008	0.341	
Infralimbic area, layer 1 (ILA1)	3.078	0.044	0.248	
Gustatory areas, layer 2/3 (GU2/3)	4.394	0.012	0.320	
Gustatory areas, layer 4 (GU4)	5.697	0.004	0.379	
Somatosensory areas (SS)	4.215	0.014	0.311	
Gustatory areas, layer 1 (GU1)	1.780	0.174	0.160	
Dorsal peduncular area, layer 6a (DP6a)	8.272	<0.001	0.470	
Anterior olfactory nucleus, medial part (AONm)	5.459	0.004	0.369	
Dorsal peduncular area, layer 5 (DP5)	5.389	0.005	0.366	
Dorsal peduncular area, layer 2/3 (DP2/3)	5.265	0.005	0.361	
Taenia tecta (TT)	7.782	<0.001	0.455	
Anterior olfactory nucleus, posteroventral part (AONpv)	14.286	<0.001	0.605	
Piriform area, pyramidal layer (PIR2)	11.287	<0.001	0.547	
Piriform area, polymorph layer (PIR3)	13.079	<0.001	0.584	
Dorsal peduncular area, layer 1 (DP1)	3.411	0.031	0.268	
Piriform area, molecular layer (PIR1)	3.680	0.024	0.283	
Endopiriform nucleus (EP)	8.681	<0.001	0.482	
Claustrum (CLA)	6.220	0.002	0.400	
Lateral septal nucleus (LS)	5.714	0.004	0.380	
Nucleus accumbens (ACB)	5.509	0.004	0.371	
Olfactory tubercle, pyramidal layer (OT2)	6.123	0.002	0.396	
Olfactory tubercle, polymorph layer (OT3)	5.647	0.004	0.377	
Islands of Calleja (isl)	5.863	0.003	0.386	
Olfactory tubercle, molecular layer (OT1)	5.797	0.003	0.383	
Substantia innominata (SI)	4.790	0.008	0.339	
Tukey HSD	Comparison	Mean Difference	Std. Error	P-value
Prelimbic area, layer 5 (PL5)	Home cage vs. Day 1	0.173	8.195	1.000
	Home cage vs. Day 15	-2.553	8.195	0.989
	Homecage vs. Day 60	-39.626	9.512	0.001
	Day 1 vs. Day 15	-2.726	8.818	0.990
	Day 1 vs. Day 60	-39.799	10.054	0.003
	Day 15 vs. Day 60	-37.073	10.054	0.005

Infralimbic area, layer 6a (ILA6a)	Home cage vs. Day 1	0.006	6.467	1.000
	Home cage vs. Day 15	-0.628	6.467	1.000
	Homecage vs. Day 60	-36.751	7.506	<0.001
	Day 1 vs. Day 15	-0.635	6.959	1.000
	Day 1 vs. Day 60	-36.758	7.934	<0.001
	Day 15 vs. Day 60	-36.123	7.934	<0.001
Infralimbic area, layer 5 (ILA5)	Home cage vs. Day 1	-0.164	7.212	1.000
	Home cage vs. Day 15	-1.514	7.212	0.997
	Homecage vs. Day 60	-35.505	8.372	0.001
	Day 1 vs. Day 15	-1.350	7.761	0.998
	Day 1 vs. Day 60	-35.341	8.849	0.002
	Day 15 vs. Day 60	-33.992	8.849	0.003
Orbital area, lateral part (ORBl)	Home cage vs. Day 1	-0.033	4.174	1.000
	Home cage vs. Day 15	-1.622	4.174	0.980
	Homecage vs. Day 60	-26.803	4.845	<0.001
	Day 1 vs. Day 15	-1.589	4.491	0.984
	Day 1 vs. Day 60	-26.770	5.121	<0.001
	Day 15 vs. Day 60	-25.181	5.121	<0.001
Prelimbic area, layer 6a (PL6a)	Home cage vs. Day 1	0.289	5.125	1.000
	Home cage vs. Day 15	-1.004	5.125	0.997
	Homecage vs. Day 60	-26.178	5.949	<0.001
	Day 1 vs. Day 15	-1.293	5.515	0.995
	Day 1 vs. Day 60	-26.467	6.288	0.001
	Day 15 vs. Day 60	-25.174	6.288	0.002
Anterior cingulate area (ACA)	Home cage vs. Day 1	0.885	5.763	0.999
	Home cage vs. Day 15	-1.098	5.763	0.997
	Homecage vs. Day 60	-25.764	6.689	0.003
	Day 1 vs. Day 15	-1.982	6.201	0.988
	Day 1 vs. Day 60	-26.648	7.070	0.004
	Day 15 vs. Day 60	-24.666	7.070	0.008
Orbital area, ventrolateral part (ORBvl)	Home cage vs. Day 1	0.108	3.902	1.000
	Home cage vs. Day 15	-0.749	3.902	0.997
	Homecage vs. Day 60	-22.237	4.530	<0.001
	Day 1 vs. Day 15	-0.857	4.199	0.997
	Day 1 vs. Day 60	-22.345	4.788	<0.001
	Day 15 vs. Day 60	-21.488	4.788	<0.001
Prelimbic area, layer 2/3 (PL2/3)	Home cage vs. Day 1	0.436	3.311	0.999
	Home cage vs. Day 15	-0.640	3.311	0.997
	Homecage vs. Day 60	-15.893	3.843	0.002
	Day 1 vs. Day 15	-1.076	3.563	0.990
	Day 1 vs. Day 60	-16.329	4.062	0.002
	Day 15 vs. Day 60	-15.253	4.062	0.004
Agranular insular area, ventral part (AIv)	Home cage vs. Day 1	0.259	2.367	1.000
	Home cage vs. Day 15	-1.416	2.367	0.932
	Homecage vs. Day 60	-15.115	2.747	<0.001
	Day 1 vs. Day 15	-1.674	2.547	0.912
	Day 1 vs. Day 60	-15.374	2.904	<0.001
	Day 15 vs. Day 60	-13.699	2.904	<0.001
Prelimbic area, layer 2 (PL2)	Home cage vs. Day 1	0.641	3.082	0.997
	Home cage vs. Day 15	-0.639	3.082	0.997
	Homecage vs. Day 60	-14.970	3.578	0.001
	Day 1 vs. Day 15	-1.280	3.317	0.980
	Day 1 vs. Day 60	-15.611	3.782	0.002
	Day 15 vs. Day 60	-14.331	3.782	0.004
Infralimbic area, layer 2/3 (ILA2/3)	Home cage vs. Day 1	0.140	3.022	1.000
	Home cage vs. Day 15	-0.099	3.022	1.000
	Homecage vs. Day 60	-13.806	3.507	0.003
	Day 1 vs. Day 15	-0.239	3.251	1.000
	Day 1 vs. Day 60	-13.947	3.707	0.004
	Day 15 vs. Day 60	-13.708	3.707	0.005

Gustatory areas, layer 6a (GU6a)	Home cage vs. Day 1	-0.031	3.305	1.000
	Home cage vs. Day 15	0.316	3.305	1.000
	Homecage vs. Day 60	-13.541	3.837	0.008
	Day 1 vs. Day 15	0.346	3.557	1.000
	Day 1 vs. Day 60	-13.510	4.055	0.012
	Day 15 vs. Day 60	-13.856	4.055	0.010
Secondary motor area (MOs)	Home cage vs. Day 1	0.804	2.360	0.986
	Home cage vs. Day 15	-0.225	2.360	1.000
	Homecage vs. Day 60	-10.987	2.739	0.002
	Day 1 vs. Day 15	-1.029	2.539	0.977
	Day 1 vs. Day 60	-11.791	2.895	0.002
	Day 15 vs. Day 60	-10.762	2.895	0.005
Agranular insular area, dorsal part (AId)	Home cage vs. Day 1	0.444	1.445	0.990
	Home cage vs. Day 15	-0.273	1.445	0.998
	Homecage vs. Day 60	-7.813	1.677	<0.001
	Day 1 vs. Day 15	-0.717	1.555	0.967
	Day 1 vs. Day 60	-8.257	1.773	<0.001
	Day 15 vs. Day 60	-7.540	1.773	0.001
Infralimbic area, layer 6b (ILA6b)	Home cage vs. Day 1	0.115	1.549	1.000
	Home cage vs. Day 15	0.345	1.549	0.996
	Homecage vs. Day 60	-7.230	1.798	0.002
	Day 1 vs. Day 15	0.230	1.667	0.999
	Day 1 vs. Day 60	-7.345	1.900	0.003
	Day 15 vs. Day 60	-7.575	1.900	0.002
Prelimbic area, layer 1 (PL1)	Home cage vs. Day 1	0.904	1.622	0.944
	Home cage vs. Day 15	-0.659	1.622	0.977
	Homecage vs. Day 60	-6.202	1.883	0.013
	Day 1 vs. Day 15	-1.563	1.745	0.807
	Day 1 vs. Day 60	-7.107	1.990	0.007
	Day 15 vs. Day 60	-5.544	1.990	0.044
Prelimbic area, layer 6b (PL6b)	Home cage vs. Day 1	0.139	1.618	1.000
	Home cage vs. Day 15	0.253	1.618	0.999
	Homecage vs. Day 60	-6.130	1.878	0.014
	Day 1 vs. Day 15	0.115	1.741	1.000
	Day 1 vs. Day 60	-6.268	1.985	0.019
	Day 15 vs. Day 60	-6.383	1.985	0.016
Gustatory areas, layer 5 (GU5)	Home cage vs. Day 1	0.482	1.274	0.981
	Home cage vs. Day 15	0.475	1.274	0.982
	Homecage vs. Day 60	-4.746	1.479	0.017
	Day 1 vs. Day 15	-0.007	1.371	1.000
	Day 1 vs. Day 60	-5.228	1.564	0.012
	Day 15 vs. Day 60	-5.221	1.564	0.012
Primary motor area (MOp)	Home cage vs. Day 1	0.569	1.115	0.956
	Home cage vs. Day 15	0.427	1.115	0.981
	Homecage vs. Day 60	-4.109	1.294	0.018
	Day 1 vs. Day 15	-0.143	1.199	0.999
	Day 1 vs. Day 60	-4.678	1.368	0.010
	Day 15 vs. Day 60	-4.535	1.368	0.013
Infralimbic area, layer 1 (ILA1)	Home cage vs. Day 1	0.595	1.348	0.971
	Home cage vs. Day 15	0.167	1.348	0.999
	Homecage vs. Day 60	-4.021	1.565	0.071
	Day 1 vs. Day 15	-0.428	1.451	0.991
	Day 1 vs. Day 60	-4.616	1.654	0.044
	Day 15 vs. Day 60	-4.188	1.654	0.077
Gustatory areas, layer 2/3 (GU2/3)	Home cage vs. Day 1	0.450	0.933	0.962
	Home cage vs. Day 15	-0.060	0.933	1.000
	Homecage vs. Day 60	-3.391	1.083	0.020
	Day 1 vs. Day 15	-0.510	1.004	0.957
	Day 1 vs. Day 60	-3.841	1.145	0.012
	Day 15 vs. Day 60	-3.331	1.145	0.033

Gustatory areas, layer 4 (GU4)	Home cage vs. Day 1	0.424	0.612	0.899
	Home cage vs. Day 15	0.307	0.612	0.958
	Homecage vs. Day 60	-2.392	0.710	0.011
	Day 1 vs. Day 15	-0.118	0.658	0.998
	Day 1 vs. Day 60	-2.817	0.751	0.004
	Day 15 vs. Day 60	-2.699	0.751	0.006
Somatosensory areas (SS)	Home cage vs. Day 1	0.503	0.744	0.905
	Home cage vs. Day 15	0.587	0.744	0.859
	Homecage vs. Day 60	-2.363	0.863	0.049
	Day 1 vs. Day 15	0.084	0.800	1.000
	Day 1 vs. Day 60	-2.866	0.912	0.019
	Day 15 vs. Day 60	-2.950	0.912	0.016
Gustatory areas, layer 1 (GU1)	Home cage vs. Day 1	0.600	0.407	0.466
	Home cage vs. Day 15	0.199	0.407	0.961
	Homecage vs. Day 60	-0.520	0.472	0.693
	Day 1 vs. Day 15	-0.401	0.438	0.797
	Day 1 vs. Day 60	-1.120	0.499	0.137
	Day 15 vs. Day 60	-0.719	0.499	0.487
Dorsal peduncular area, layer 6a (DP6a)	Home cage vs. Day 1	-0.241	8.398	1.000
	Home cage vs. Day 15	-1.628	8.398	0.997
	Homecage vs. Day 60	-44.350	9.748	<0.001
	Day 1 vs. Day 15	-1.388	9.037	0.999
	Day 1 vs. Day 60	-44.110	10.303	0.001
	Day 15 vs. Day 60	-42.722	10.303	0.002
Anterior olfactory nucleus, medial part (AONm)	Home cage vs. Day 1	-0.032	10.103	1.000
	Home cage vs. Day 15	-4.662	10.103	0.967
	Homecage vs. Day 60	-43.890	11.727	0.004
	Day 1 vs. Day 15	-4.630	10.871	0.974
	Day 1 vs. Day 60	-43.858	12.395	0.007
	Day 15 vs. Day 60	-39.228	12.395	0.018
Dorsal peduncular area, layer 5 (DP5)	Home cage vs. Day 1	0.457	8.203	1.000
	Home cage vs. Day 15	-1.710	8.203	0.997
	Homecage vs. Day 60	-34.858	9.522	0.005
	Day 1 vs. Day 15	-2.167	8.827	0.995
	Day 1 vs. Day 60	-35.315	10.064	0.008
	Day 15 vs. Day 60	-33.147	10.064	0.013
Dorsal peduncular area, layer 2/3 (DP2/3)	Home cage vs. Day 1	0.106	6.575	1.000
	Home cage vs. Day 15	-0.988	6.575	0.999
	Homecage vs. Day 60	-27.615	7.632	0.006
	Day 1 vs. Day 15	-1.095	7.075	0.999
	Day 1 vs. Day 60	-27.722	8.067	0.009
	Day 15 vs. Day 60	-26.627	8.067	0.013
Taenia tecta (TT)	Home cage vs. Day 1	0.297	4.496	1.000
	Home cage vs. Day 15	-1.455	4.496	0.988
	Homecage vs. Day 60	-23.030	5.219	<0.001
	Day 1 vs. Day 15	-1.752	4.838	0.983
	Day 1 vs. Day 60	-23.327	5.516	0.001
	Day 15 vs. Day 60	-21.575	5.516	0.003
Anterior olfactory nucleus, posteroventral part (AONpv)	Home cage vs. Day 1	0.505	1.855	0.993
	Home cage vs. Day 15	-0.902	1.855	0.962
	Homecage vs. Day 60	-12.766	2.153	<0.001
	Day 1 vs. Day 15	-1.407	1.996	0.894
	Day 1 vs. Day 60	-13.271	2.276	<0.001
	Day 15 vs. Day 60	-11.864	2.276	<0.001
Piriform area, pyramidal layer (PIR2)	Home cage vs. Day 1	0.300	1.761	0.998
	Home cage vs. Day 15	-1.671	1.761	0.779
	Homecage vs. Day 60	-10.933	2.044	<0.001
	Day 1 vs. Day 15	-1.971	1.894	0.727
	Day 1 vs. Day 60	-11.233	2.160	<0.001
	Day 15 vs. Day 60	-9.262	2.160	0.001

Piriform area, polymorph layer (PIR3)	Home cage vs. Day 1	0.455	1.583	0.992
	Home cage vs. Day 15	-1.758	1.583	0.686
	Homecage vs. Day 60	-10.521	1.838	<0.001
	Day 1 vs. Day 15	-2.213	1.704	0.571
	Day 1 vs. Day 60	-10.976	1.943	<0.001
	Day 15 vs. Day 60	-8.762	1.943	<0.001
Dorsal peduncular area, layer 1 (DP1)	Home cage vs. Day 1	0.736	2.473	0.991
	Home cage vs. Day 15	0.445	2.473	0.998
	Homecage vs. Day 60	-7.903	2.871	0.048
	Day 1 vs. Day 15	-0.291	2.661	1.000
	Day 1 vs. Day 60	-8.640	3.035	0.039
	Day 15 vs. Day 60	-8.348	3.035	0.048
Piriform area, molecular layer (PIR1)	Home cage vs. Day 1	0.785	0.754	0.726
	Home cage vs. Day 15	0.020	0.754	1.000
	Homecage vs. Day 60	-2.226	0.875	0.074
	Day 1 vs. Day 15	-0.765	0.811	0.782
	Day 1 vs. Day 60	-3.012	0.925	0.015
	Day 15 vs. Day 60	-2.247	0.925	0.094
Endopiriform nucleus (EP)	Home cage vs. Day 1	0.502	1.817	0.992
	Home cage vs. Day 15	-1.348	1.817	0.879
	Homecage vs. Day 60	-9.782	2.109	<0.001
	Day 1 vs. Day 15	-1.850	1.955	0.780
	Day 1 vs. Day 60	-10.284	2.229	<0.001
	Day 15 vs. Day 60	-8.434	2.229	0.004
Claustrum (CLA)	Home cage vs. Day 1	0.145	2.131	1.000
	Home cage vs. Day 15	0.332	2.131	0.999
	Homecage vs. Day 60	-9.499	2.474	0.003
	Day 1 vs. Day 15	0.187	2.293	1.000
	Day 1 vs. Day 60	-9.644	2.615	0.005
	Day 15 vs. Day 60	-9.831	2.615	0.004
Lateral septal nucleus (LS)	Home cage vs. Day 1	0.373	4.426	1.000
	Home cage vs. Day 15	-1.852	4.426	0.975
	Homecage vs. Day 60	-19.497	5.138	0.004
	Day 1 vs. Day 15	-2.225	4.763	0.966
	Day 1 vs. Day 60	-19.869	5.431	0.005
	Day 15 vs. Day 60	-17.644	5.431	0.015
Nucleus accumbens (ACB)	Home cage vs. Day 1	0.920	4.006	0.996
	Home cage vs. Day 15	-0.662	4.006	0.998
	Homecage vs. Day 60	-16.919	4.650	0.006
	Day 1 vs. Day 15	-1.582	4.311	0.983
	Day 1 vs. Day 60	-17.839	4.915	0.006
	Day 15 vs. Day 60	-16.257	4.915	0.013
Olfactory tubercle, pyramidal layer (OT2)	Home cage vs. Day 1	0.594	3.094	0.997
	Home cage vs. Day 15	-1.463	3.094	0.964
	Homecage vs. Day 60	-13.995	3.591	0.003
	Day 1 vs. Day 15	-2.057	3.329	0.925
	Day 1 vs. Day 60	-14.589	3.796	0.003
	Day 15 vs. Day 60	-12.532	3.796	0.013
Olfactory tubercle, polymorph layer (OT3)	Home cage vs. Day 1	0.689	3.130	0.996
	Home cage vs. Day 15	-1.222	3.130	0.979
	Homecage vs. Day 60	-13.525	3.634	0.005
	Day 1 vs. Day 15	-1.912	3.368	0.941
	Day 1 vs. Day 60	-14.215	3.841	0.005
	Day 15 vs. Day 60	-12.303	3.841	0.017
Islands of Calleja (isl)	Home cage vs. Day 1	0.637	1.457	0.971
	Home cage vs. Day 15	-0.384	1.457	0.993
	Homecage vs. Day 60	-6.247	1.691	0.005
	Day 1 vs. Day 15	-1.022	1.568	0.914
	Day 1 vs. Day 60	-6.884	1.787	0.003
	Day 15 vs. Day 60	-5.862	1.787	0.014

Olfactory tubercle, molecular layer (OT1)	Home cage vs. Day 1	0.589	1.366	0.973
	Home cage vs. Day 15	-0.442	1.366	0.988
	Homecage vs. Day 60	-5.842	1.586	0.005
	Day 1 vs. Day 15	-1.030	1.470	0.896
	Day 1 vs. Day 60	-6.430	1.676	0.003
	Day 15 vs. Day 60	-5.400	1.676	0.016
Substantia innominata (SI)	Home cage vs. Day 1	0.750	2.204	0.986
	Home cage vs. Day 15	-1.217	2.204	0.945
	Homecage vs. Day 60	-8.677	2.559	0.011
	Day 1 vs. Day 15	-1.966	2.372	0.840
	Day 1 vs. Day 60	-9.427	2.704	0.008
	Day 15 vs. Day 60	-7.460	2.704	0.047

Table S9. Statistical output for Figure 4B, right panel : Analysis of Z-scored counts from 8 major anatomical regions within AP -1.08 to AP -1.28 coronal subvolume (n=30, analyses shown in figure 3B are highlighted in grey)

Factors in analysis	**Statistical output**			
RM-ANOVA (Region x Group)	F-value	P-value	Partial η2	
Region (within-subjects)	F(1.198,32.349) = 14.028	<0.001	0.342	
Group (between-subjects)	F(3,27) = 3.189	0.040	0.262	
Region x Group	F(3.594,32.349) = 5.476	0.002	0.378	
1-way-ANOVA (Region)	F-value (ndf=3,ddf=27)	P-value	η2	
Isocortex	4.078	0.016	0.312	
Olfactory areas	5.049	0.007	0.359	
Hippocampal formation	0.754	0.530	0.077	
Cortical subplate	4.772	0.009	0.346	
Striatum	2.085	0.126	0.188	
Pallidum	1.098	0.367	0.109	
Thalamus	0.761	0.526	0.078	
Hypothalamus	2.723	0.064	0.232	
Tukey HSD	Comparison	Mean Difference	Std. Error	P-value
Isocortex	Home cage vs. Day 1	0.309	0.910	0.986
	Home cage vs. Day 15	-0.304	0.910	0.987
	Homecage vs. Day 60	-3.219	1.051	0.024
	Day 1 vs. Day 15	-0.613	0.959	0.918
	Day 1 vs. Day 60	-3.528	1.093	0.016
	Day 15 vs. Day 60	-2.915	1.093	0.058
Olfactory areas	Home cage vs. Day 1	0.215	0.883	0.995
	Home cage vs. Day 15	-1.152	0.883	0.568
	Homecage vs. Day 60	-3.513	1.019	0.010
	Day 1 vs. Day 15	-1.367	0.930	0.469
	Day 1 vs. Day 60	-3.728	1.061	0.008
	Day 15 vs. Day 60	-2.361	1.061	0.142
Hippocampal formation	Home cage vs. Day 1	0.143	0.692	0.997
	Home cage vs. Day 15	0.417	0.692	0.930
	Homecage vs. Day 60	-0.808	0.799	0.744
	Day 1 vs. Day 15	0.273	0.729	0.982
	Day 1 vs. Day 60	-0.951	0.831	0.666
	Day 15 vs. Day 60	-1.224	0.831	0.467
Cortical subplate	Home cage vs. Day 1	-0.071	2.315	1.000
	Home cage vs. Day 15	-2.517	2.315	0.700
	Homecage vs. Day 60	-9.349	2.673	0.008
	Day 1 vs. Day 15	-2.446	2.440	0.749
	Day 1 vs. Day 60	-9.278	2.782	0.013
	Day 15 vs. Day 60	-6.832	2.782	0.090
Striatum	Home cage vs. Day 1	0.245	0.655	0.982
	Home cage vs. Day 15	-0.075	0.655	0.999
	Homecage vs. Day 60	-1.602	0.757	0.173
	Day 1 vs. Day 15	-0.320	0.691	0.966
	Day 1 vs. Day 60	-1.847	0.787	0.113
	Day 15 vs. Day 60	-1.527	0.787	0.236

Pallidum	Home cage vs. Day 1	0.248	0.507	0.961
	Home cage vs. Day 15	0.478	0.507	0.782
	Homecage vs. Day 60	-0.585	0.586	0.751
	Day 1 vs. Day 15	0.231	0.535	0.973
	Day 1 vs. Day 60	-0.833	0.610	0.531
	Day 15 vs. Day 60	-1.064	0.610	0.321
Thalamus	Home cage vs. Day 1	0.071	0.385	0.998
	Home cage vs. Day 15	0.412	0.385	0.710
	Homecage vs. Day 60	-0.253	0.444	0.940
	Day 1 vs. Day 15	0.341	0.405	0.834
	Day 1 vs. Day 60	-0.324	0.462	0.896
	Day 15 vs. Day 60	-0.665	0.462	0.487
Hypothalamus	Home cage vs. Day 1	0.301	1.133	0.993
	Home cage vs. Day 15	-0.862	1.133	0.871
	Homecage vs. Day 60	-3.289	1.308	0.080
	Day 1 vs. Day 15	-1.162	1.194	0.766
	Day 1 vs. Day 60	-3.589	1.362	0.062
	Day 15 vs. Day 60	-2.427	1.362	0.303

Table S10. Statistical output for Figure 4C: Analysis of Z-scored counts from 28 subregions within AP -1.08 to AP -1.28 coronal subvolume (n=30)

Factors in analysis	**Statistical Output**			
RM-ANOVA (Region x Group)	F-value	P-value	Partial η2	
Region (within-subjects)	F(1.140,30.793) = 9.528	0.003	0.261	
Group (between-subjects)	F(3,27) = 3.373	0.033	0.273	
Region x Group	F(3.421,30.793) = 3.453	0.024	0.277	
1-way-ANOVA (Region)	F-value (ndf=3,ddf=27)	P-value	η2	
Somatomotor areas (MO)	3.936	0.019	0.304	
Retrosplenial area (RSP)	1.904	0.153	0.175	
Somatosensory areas (SS)	4.377	0.012	0.327	
Anterior cingulate area (ACA)	1.580	0.217	0.149	
Agranular insular area (AI)	2.042	0.132	0.185	
Visceral area (VISC)	1.943	0.146	0.178	
Cortical amygdalar area (COA)	6.456	0.002	0.418	
Piriform area (PIR)	4.306	0.013	0.324	
Piriform-amygdalar area (PAA)	3.508	0.029	0.280	
Ammon's horn (CA)	0.832	0.488	0.085	
Dentate gyrus (DG)	0.683	0.570	0.071	
Basomedial amygdalar nucleus (BMA)	4.061	0.017	0.311	
Endopiriform nucleus (EP)	5.828	0.003	0.393	
Basolateral amygdalar nucleus (BLA)	3.638	0.025	0.288	
Lateral amygdalar nucleus (LA)	3.728	0.023	0.293	
Claustrum (CLA)	7.956	<0.001	0.469	
Intercalated amygdalar nucleus (IA)	4.051	0.017	0.310	
Central amygdalar nucleus (CEA)	3.465	0.030	0.278	
Medial amygdalar nucleus (MEA)	2.867	0.055	0.242	
Bed nucleus of the accessory olfactory tract (BA)	1.560	0.222	0.148	
Caudoputamen (CP)	1.126	0.356	0.111	
Pallidum, ventral region (PALv)	1.535	0.228	0.146	
Pallidum, dorsal region (PALd)	0.835	0.487	0.085	
Thalamus, sensory-motor cortex related (DORsm)	0.726	0.545	0.075	
Thalamus, polymodal association cortex related (DORpm)	0.786	0.512	0.080	
Hypothalamic medial zone (MEZ)	2.509	0.080	0.218	
Hypothalamic lateral zone (LZ)	2.677	0.067	0.229	
Periventricular zone (PVZ)	2.933	0.051	0.246	
Tukey HSD	Comparison	Mean Difference	Std. Error	P-value
Somatomotor areas (MO)	Home cage vs. Day 1	0.523	1.555	0.987
	Home cage vs. Day 15	-0.803	1.555	0.954
	Homecage vs. Day 60	-5.428	1.795	0.026
	Day 1 vs. Day 15	-1.326	1.639	0.849
	Day 1 vs. Day 60	-5.951	1.868	0.018
	Day 15 vs. Day 60	-4.625	1.868	0.087
Retrosplenial area (RSP)	Home cage vs. Day 1	0.264	1.817	0.999
	Home cage vs. Day 15	-0.350	1.817	0.997
	Homecage vs. Day 60	-4.456	2.098	0.171
	Day 1 vs. Day 15	-0.614	1.915	0.988
	Day 1 vs. Day 60	-4.720	2.184	0.160
	Day 15 vs. Day 60	-4.106	2.184	0.260
Somatosensory areas (SS)	Home cage vs. Day 1	0.274	0.928	0.991
	Home cage vs. Day 15	-0.343	0.928	0.982
	Homecage vs. Day 60	-3.431	1.072	0.017
	Day 1 vs. Day 15	-0.617	0.978	0.921
	Day 1 vs. Day 60	-3.705	1.116	0.013
	Day 15 vs. Day 60	-3.088	1.116	0.047
Anterior cingulate area (ACA)	Home cage vs. Day 1	0.238	1.115	0.996
	Home cage vs. Day 15	-0.347	1.115	0.989

	Homecage vs. Day 60	-2.465	1.287	0.246
	Day 1 vs. Day 15	-0.585	1.175	0.959
	Day 1 vs. Day 60	-2.703	1.340	0.207
	Day 15 vs. Day 60	-2.118	1.340	0.406
Agranular insular area (AI)	Home cage vs. Day 1	0.354	0.425	0.838
	Home cage vs. Day 15	0.148	0.425	0.985
	Homecage vs. Day 60	-0.864	0.490	0.313
	Day 1 vs. Day 15	-0.206	0.448	0.967
	Day 1 vs. Day 60	-1.218	0.510	0.104
	Day 15 vs. Day 60	-1.012	0.510	0.219
Visceral area (VISC)	Home cage vs. Day 1	0.342	0.400	0.828
	Home cage vs. Day 15	0.162	0.400	0.977
	Homecage vs. Day 60	-0.776	0.462	0.355
	Day 1 vs. Day 15	-0.181	0.422	0.973
	Day 1 vs. Day 60	-1.118	0.481	0.118
	Day 15 vs. Day 60	-0.937	0.481	0.233
Cortical amygdalar area (COA)	Home cage vs. Day 1	-0.030	1.080	1.000
	Home cage vs. Day 15	-2.262	1.080	0.180
	Homecage vs. Day 60	-4.858	1.247	0.003
	Day 1 vs. Day 15	-2.232	1.138	0.227
	Day 1 vs. Day 60	-4.828	1.297	0.005
	Day 15 vs. Day 60	-2.596	1.297	0.213
Piriform area (PIR)	Home cage vs. Day 1	0.278	0.852	0.988
	Home cage vs. Day 15	-0.735	0.852	0.824
	Homecage vs. Day 60	-3.122	0.984	0.018
	Day 1 vs. Day 15	-1.013	0.898	0.676
	Day 1 vs. Day 60	-3.400	1.024	0.013
	Day 15 vs. Day 60	-2.387	1.024	0.116
Piriform-amygdalar area (PAA)	Home cage vs. Day 1	0.434	0.709	0.927
	Home cage vs. Day 15	-0.660	0.709	0.789
	Homecage vs. Day 60	-2.178	0.819	0.059
	Day 1 vs. Day 15	-1.094	0.747	0.472
	Day 1 vs. Day 60	-2.612	0.852	0.024
	Day 15 vs. Day 60	-1.518	0.852	0.304
Ammon's horn (CA)	Home cage vs. Day 1	0.068	0.820	1.000
	Home cage vs. Day 15	0.345	0.820	0.975
	Homecage vs. Day 60	-1.155	0.947	0.620
	Day 1 vs. Day 15	0.277	0.865	0.988
	Day 1 vs. Day 60	-1.223	0.986	0.607
	Day 15 vs. Day 60	-1.500	0.986	0.439
Dentate gyrus (DG)	Home cage vs. Day 1	0.288	0.453	0.920
	Home cage vs. Day 15	0.547	0.453	0.628
	Homecage vs. Day 60	-0.098	0.523	0.998
	Day 1 vs. Day 15	0.259	0.478	0.948
	Day 1 vs. Day 60	-0.386	0.545	0.893
	Day 15 vs. Day 60	-0.645	0.545	0.641
Basomedial amygdalar nucleus (BMA)	Home cage vs. Day 1	-1.346	5.743	0.995
	Home cage vs. Day 15	-7.829	5.743	0.532
	Homecage vs. Day 60	-21.664	6.632	0.015
	Day 1 vs. Day 15	-6.483	6.054	0.710
	Day 1 vs. Day 60	-20.318	6.903	0.031
	Day 15 vs. Day 60	-13.835	6.903	0.211
Endopiriform nucleus (EP)	Home cage vs. Day 1	0.136	1.601	1.000
	Home cage vs. Day 15	-1.252	1.601	0.862
	Homecage vs. Day 60	-7.064	1.848	0.004
	Day 1 vs. Day 15	-1.388	1.687	0.843
	Day 1 vs. Day 60	-7.200	1.924	0.005
	Day 15 vs. Day 60	-5.812	1.924	0.026

Basolateral amygdalar nucleus (BLA)	Home cage vs. Day 1	0.386	2.051	0.998
	Home cage vs. Day 15	-1.199	2.051	0.936
	Homecage vs. Day 60	-7.035	2.369	0.030
	Day 1 vs. Day 15	-1.585	2.162	0.883
	Day 1 vs. Day 60	-7.421	2.465	0.027
	Day 15 vs. Day 60	-5.836	2.465	0.108
Lateral amygdalar nucleus (LA)	Home cage vs. Day 1	0.642	0.867	0.880
	Home cage vs. Day 15	-0.399	0.867	0.967
	Homecage vs. Day 60	-2.729	1.002	0.051
	Day 1 vs. Day 15	-1.041	0.914	0.670
	Day 1 vs. Day 60	-3.371	1.043	0.016
	Day 15 vs. Day 60	-2.330	1.043	0.139
Claustrum (CLA)	Home cage vs. Day 1	0.332	0.529	0.922
	Home cage vs. Day 15	-0.473	0.529	0.808
	Homecage vs. Day 60	-2.585	0.611	0.001
	Day 1 vs. Day 15	-0.805	0.558	0.485
	Day 1 vs. Day 60	-2.917	0.636	<0.001
	Day 15 vs. Day 60	-2.112	0.636	0.013
Intercalated amygdalar nucleus (IA)	Home cage vs. Day 1	-1.038	4.303	0.995
	Home cage vs. Day 15	-4.797	4.303	0.684
	Homecage vs. Day 60	-16.372	4.968	0.014
	Day 1 vs. Day 15	-3.758	4.535	0.840
	Day 1 vs. Day 60	-15.334	5.171	0.030
	Day 15 vs. Day 60	-11.576	5.171	0.138
Central amygdalar nucleus (CEA)	Home cage vs. Day 1	0.306	1.325	0.996
	Home cage vs. Day 15	-0.553	1.325	0.975
	Homecage vs. Day 60	-4.391	1.530	0.037
	Day 1 vs. Day 15	-0.859	1.397	0.926
	Day 1 vs. Day 60	-4.698	1.593	0.031
	Day 15 vs. Day 60	-3.838	1.593	0.099
Medial amygdalar nucleus (MEA)	Home cage vs. Day 1	0.156	1.389	0.999
	Home cage vs. Day 15	-1.515	1.389	0.698
	Homecage vs. Day 60	-4.193	1.604	0.065
	Day 1 vs. Day 15	-1.671	1.464	0.668
	Day 1 vs. Day 60	-4.349	1.669	0.066
	Day 15 vs. Day 60	-2.677	1.669	0.393
Bed nucleus of the accessory olfactory tract (BA)	Home cage vs. Day 1	0.077	0.695	0.999
	Home cage vs. Day 15	-0.702	0.695	0.745
	Homecage vs. Day 60	-1.485	0.802	0.272
	Day 1 vs. Day 15	-0.779	0.732	0.714
	Day 1 vs. Day 60	-1.562	0.835	0.264
	Day 15 vs. Day 60	-0.783	0.835	0.785
Caudoputamen (CP)	Home cage vs. Day 1	0.277	0.369	0.875
	Home cage vs. Day 15	0.447	0.369	0.625
	Homecage vs. Day 60	-0.294	0.426	0.900
	Day 1 vs. Day 15	0.170	0.389	0.971
	Day 1 vs. Day 60	-0.571	0.443	0.578
	Day 15 vs. Day 60	-0.741	0.443	0.358
Pallidum, ventral region (PALv)	Home cage vs. Day 1	0.452	0.706	0.918
	Home cage vs. Day 15	0.546	0.706	0.865
	Homecage vs. Day 60	-1.127	0.815	0.520
	Day 1 vs. Day 15	0.095	0.744	0.999
	Day 1 vs. Day 60	-1.579	0.848	0.268
	Day 15 vs. Day 60	-1.673	0.848	0.223
Pallidum, dorsal region (PALd)	Home cage vs. Day 1	0.167	0.443	0.981
	Home cage vs. Day 15	0.441	0.443	0.753
	Homecage vs. Day 60	-0.373	0.512	0.885
	Day 1 vs. Day 15	0.275	0.467	0.935
	Day 1 vs. Day 60	-0.540	0.533	0.743
	Day 15 vs. Day 60	-0.814	0.533	0.435

Thalamus, sensory-motor cortex related (DORsm)	Home cage vs. Day 1	-0.044	0.433	1.000
	Home cage vs. Day 15	0.367	0.433	0.831
	Homecage vs. Day 60	-0.384	0.500	0.868
	Day 1 vs. Day 15	0.411	0.456	0.805
	Day 1 vs. Day 60	-0.340	0.520	0.914
	Day 15 vs. Day 60	-0.750	0.520	0.485
Thalamus, polymodal association cortex related (DORpm)	Home cage vs. Day 1	0.115	0.368	0.989
	Home cage vs. Day 15	0.429	0.368	0.654
	Homecage vs. Day 60	-0.203	0.425	0.964
	Day 1 vs. Day 15	0.314	0.388	0.850
	Day 1 vs. Day 60	-0.317	0.443	0.890
	Day 15 vs. Day 60	-0.631	0.443	0.495
Hypothalamic medial zone (MEZ)	Home cage vs. Day 1	0.011	5.940	1.000
	Home cage vs. Day 15	-4.111	5.940	0.899
	Homecage vs. Day 60	-17.359	6.859	0.078
	Day 1 vs. Day 15	-4.122	6.261	0.912
	Day 1 vs. Day 60	-17.369	7.139	0.095
	Day 15 vs. Day 60	-13.248	7.139	0.271
Hypothalamic lateral zone (LZ)	Home cage vs. Day 1	0.303	0.979	0.989
	Home cage vs. Day 15	-0.737	0.979	0.875
	Homecage vs. Day 60	-2.790	1.131	0.088
	Day 1 vs. Day 15	-1.040	1.032	0.746
	Day 1 vs. Day 60	-3.093	1.177	0.063
	Day 15 vs. Day 60	-2.053	1.177	0.321
Periventricular zone (PVZ)	Home cage vs. Day 1	0.329	0.763	0.973
	Home cage vs. Day 15	-0.667	0.763	0.818
	Homecage vs. Day 60	-2.211	0.881	0.081
	Day 1 vs. Day 15	-0.996	0.804	0.609
	Day 1 vs. Day 60	-2.540	0.917	0.046
	Day 15 vs. Day 60	-1.545	0.917	0.351

Table S11. Statistical output for Figure 4D: Analysis of Z-scored counts from 64 subdivisions within AP -1.08 to AP -1.28 coronal subvolume (n=30)

Factors in analysis	**Statistical Output**			
RM-ANOVA (Region x Group)	F-value	P-value	Partial η2	
Region (within-subjects)	F(1.157,31.231) = 7.762	0.007	0.223	
Group (between-subjects)	F(3,27) = 3.085	0.044	0.255	
Region x Group	F(3.470,31.231) = 3.030	0.038	0.252	
1-way-ANOVA (Region)	F-value (ndf=3,ddf=27)	P-value	η2	
Primary motor area (MOp)	4.339	0.013	0.325	
Secondary motor area (MOs)	3.221	0.038	0.264	
Retrosplenial area, dorsal part (RSPd)	2.218	0.109	0.198	
Primary somatosensory area (SSp)	4.814	0.008	0.348	
Retrosplenial area, ventral part (RSPv)	1.483	0.241	0.142	
Anterior cingulate area, dorsal part (ACAd)	1.843	0.163	0.170	
Lateral visual area, layer 6a (VISC6a)	4.728	0.009	0.344	
Supplemental somatosensory area (SSs)	2.948	0.051	0.247	
Lateral visual area, layer 5 (VISC5)	2.624	0.071	0.226	
Anterior cingulate area, ventral part (ACAv)	0.815	0.497	0.083	
Agranular insular area (AI)	2.042	0.132	0.185	
Lateral visual area, layer 4 (VISC4)	1.378	0.271	0.133	
Lateral visual area, layer 2/3 (VISC23)	0.994	0.411	0.099	
Lateral visual area, layer 1 (VISC1)	0.959	0.426	0.096	
Cortical amygdalar area, anterior part (COAa)	6.442	0.002	0.417	
Piriform area, pyramidal layer (PIR2)	6.051	0.003	0.402	
Piriform area, polymorph layer (PIR3)	3.758	0.022	0.295	
Cortical amygdalar area, posterior part (COAp)	5.892	0.003	0.396	
Piriform-amygdalar area, pyramidal layer (PAA2)	5.601	0.004	0.384	
Piriform-amygdalar area, polymorph layer (PAA3)	3.050	0.046	0.253	
Piriform-amygdalar area, molecular layer (PAA1)	2.201	0.111	0.197	
Piriform area, molecular layer (PIR1)	2.858	0.056	0.241	
Ammon's horn (CA)	0.832	0.488	0.085	
Dentate gyrus (DG)	0.683	0.570	0.071	
Basolateral amygdalar nucleus, anterior part (BMAa)	3.825	0.021	0.298	
Basolateral amygdalar nucleus, posterior part (BMAp)	4.353	0.013	0.326	
Endopiriform nucleus, ventral part (EPv)	3.584	0.027	0.285	
Basolateral amygdalar nucleus (BLA)	3.638	0.025	0.288	
Endopiriform nucleus, dorsal part (EPd)	7.269	0.001	0.447	
Lateral amygdalar nucleus (LA)	3.728	0.023	0.293	
Claustrum (CLA)	7.956	<0.001	0.469	
Intercalated amygdalar nucleus (IA)	4.051	0.017	0.310	
Medial amygdalar nucleus, anterodorsal part (MEAad)	2.909	0.053	0.244	
Central amygdalar nucleus, capsular part (CEAc)	3.893	0.020	0.302	
Central amygdalar nucleus, medial part (CEAm)	2.632	0.070	0.226	
Medial amygdalar nucleus, anteroventral part (MEAav)	2.158	0.116	0.193	
Bed nucleus of the accessory olfactory tract (BA)	1.560	0.222	0.148	
Central amygdalar nucleus, lateral part (CEAl)	2.627	0.071	0.226	
Caudoputamen (CP)	1.126	0.356	0.111	
Pallidum, ventral region (PALv)	1.535	0.228	0.146	
Globus pallidus, external segment (GPe)	0.890	0.459	0.090	
Globus pallidus, internal segment (GPi)	0.789	0.511	0.081	
Central medial nucleus of the thalamus (CM)	1.667	0.197	0.156	
Anteroventral nucleus of thalamus (AV)	1.514	0.233	0.144	
Ventral medial nucleus of the thalamus (VM)	1.298	0.295	0.126	
Lateral habenula (LH)	1.481	0.242	0.141	
Anteromedial nucleus (AM)	1.094	0.369	0.108	
Submedial nucleus of the thalamus (SMT)	0.806	0.501	0.082	
Paracentral nucleus (PCN)	0.990	0.412	0.099	
Mediodorsal nucleus of thalamus (MD)	0.739	0.538	0.076	
Ventral anterior-lateral complex of the thalamus (VAL)	0.686	0.568	0.071	

Central lateral nucleus of the thalamus (CL)	0.793	0.508	0.081	
Ventral posterior complex of the thalamus (VP)	0.581	0.633	0.061	
Medial habenula (MH)	1.102	0.366	0.109	
Reticular nucleus of the thalamus (RT)	0.572	0.638	0.060	
Anterodorsal nucleus (AD)	1.295	0.296	0.126	
Lateral dorsal nucleus of thalamus (LD)	0.829	0.490	0.084	
Anterior hypothalamic nucleus (AHN)	2.324	0.097	0.205	
Hypothalamic medial zone (MEZ)	2.509	0.080	0.218	
Paraventricular hypothalamic nucleus (PVH)	1.827	0.166	0.169	
Lateral hypothalamic area (LHA)	2.356	0.094	0.207	
Arcuate hypothalamic nucleus (ARH)	2.970	0.049	0.248	
Tuberal nucleus (TU)	2.406	0.089	0.211	
Supraoptic nucleus (SO)	0.554	0.650	0.058	

Tukey HSD	Comparison	Mean Difference	Std. Error	P-value
Primary motor area (MOp)	Home cage vs. Day 1	0.551	1.633	0.986
	Home cage vs. Day 15	-0.845	1.633	0.954
	Homecage vs. Day 60	-5.997	1.885	0.018
	Day 1 vs. Day 15	-1.397	1.721	0.848
	Day 1 vs. Day 60	-6.548	1.962	0.012
	Day 15 vs. Day 60	-5.151	1.962	0.064
Secondary motor area (MOs)	Home cage vs. Day 1	0.469	1.423	0.987
	Home cage vs. Day 15	-0.720	1.423	0.957
	Homecage vs. Day 60	-4.478	1.643	0.051
	Day 1 vs. Day 15	-1.189	1.500	0.857
	Day 1 vs. Day 60	-4.947	1.710	0.035
	Day 15 vs. Day 60	-3.757	1.710	0.150
Retrosplenial area, dorsal part (RSPd)	Home cage vs. Day 1	0.331	1.683	0.997
	Home cage vs. Day 15	-0.571	1.683	0.986
	Homecage vs. Day 60	-4.452	1.943	0.125
	Day 1 vs. Day 15	-0.902	1.774	0.956
	Day 1 vs. Day 60	-4.783	2.022	0.108
	Day 15 vs. Day 60	-3.880	2.022	0.244
Primary somatosensory area (SSp)	Home cage vs. Day 1	0.252	1.078	0.995
	Home cage vs. Day 15	-0.650	1.078	0.930
	Homecage vs. Day 60	-4.239	1.244	0.011
	Day 1 vs. Day 15	-0.903	1.136	0.856
	Day 1 vs. Day 60	-4.491	1.295	0.009
	Day 15 vs. Day 60	-3.588	1.295	0.046
Retrosplenial area, ventral part (RSPv)	Home cage vs. Day 1	0.130	1.963	1.000
	Home cage vs. Day 15	0.058	1.963	1.000
	Homecage vs. Day 60	-4.203	2.267	0.271
	Day 1 vs. Day 15	-0.072	2.069	1.000
	Day 1 vs. Day 60	-4.333	2.359	0.279
	Day 15 vs. Day 60	-4.261	2.359	0.292
Anterior cingulate area, dorsal part (ACAd)	Home cage vs. Day 1	0.349	1.428	0.995
	Home cage vs. Day 15	-0.656	1.428	0.967
	Homecage vs. Day 60	-3.402	1.649	0.191
	Day 1 vs. Day 15	-1.005	1.505	0.908
	Day 1 vs. Day 60	-3.752	1.716	0.153
	Day 15 vs. Day 60	-2.747	1.716	0.395
Lateral visual area, layer 6a (VISC6a)	Home cage vs. Day 1	0.282	0.604	0.966
	Home cage vs. Day 15	-0.003	0.604	1.000
	Homecage vs. Day 60	-2.233	0.697	0.017
	Day 1 vs. Day 15	-0.285	0.637	0.969
	Day 1 vs. Day 60	-2.515	0.726	0.009
	Day 15 vs. Day 60	-2.229	0.726	0.023
Supplemental somatosensory area (SSs)	Home cage vs. Day 1	0.317	0.651	0.961
	Home cage vs. Day 15	0.282	0.651	0.972
	Homecage vs. Day 60	-1.774	0.751	0.109
	Day 1 vs. Day 15	-0.035	0.686	1.000

	Day 1 vs. Day 60	-2.091	0.782	0.057
	Day 15 vs. Day 60	-2.056	0.782	0.063
Lateral visual area, layer 5 (VISC5)	Home cage vs. Day 1	0.252	0.579	0.972
	Home cage vs. Day 15	0.179	0.579	0.990
	Homecage vs. Day 60	-1.519	0.669	0.130
	Day 1 vs. Day 15	-0.073	0.611	0.999
	Day 1 vs. Day 60	-1.771	0.696	0.076
	Day 15 vs. Day 60	-1.698	0.696	0.094
Anterior cingulate area, ventral part (ACAv)	Home cage vs. Day 1	0.061	0.651	1.000
	Home cage vs. Day 15	0.141	0.651	0.996
	Homecage vs. Day 60	-0.975	0.752	0.572
	Day 1 vs. Day 15	0.080	0.686	0.999
	Day 1 vs. Day 60	-1.036	0.782	0.556
	Day 15 vs. Day 60	-1.116	0.782	0.494
Agranular insular area (AI)	Home cage vs. Day 1	0.354	0.425	0.838
	Home cage vs. Day 15	0.148	0.425	0.985
	Homecage vs. Day 60	-0.864	0.490	0.313
	Day 1 vs. Day 15	-0.206	0.448	0.967
	Day 1 vs. Day 60	-1.218	0.510	0.104
	Day 15 vs. Day 60	-1.012	0.510	0.219
Lateral visual area, layer 4 (VISC4)	Home cage vs. Day 1	0.274	0.370	0.880
	Home cage vs. Day 15	0.178	0.370	0.963
	Homecage vs. Day 60	-0.579	0.427	0.536
	Day 1 vs. Day 15	-0.096	0.390	0.995
	Day 1 vs. Day 60	-0.853	0.445	0.244
	Day 15 vs. Day 60	-0.757	0.445	0.342
Lateral visual area, layer 2/3 (VISC23)	Home cage vs. Day 1	0.369	0.365	0.744
	Home cage vs. Day 15	0.125	0.365	0.986
	Homecage vs. Day 60	-0.371	0.421	0.814
	Day 1 vs. Day 15	-0.244	0.384	0.919
	Day 1 vs. Day 60	-0.740	0.438	0.349
	Day 15 vs. Day 60	-0.496	0.438	0.674
Lateral visual area, layer 1 (VISC1)	Home cage vs. Day 1	0.426	0.312	0.532
	Home cage vs. Day 15	0.262	0.312	0.835
	Homecage vs. Day 60	-0.101	0.360	0.992
	Day 1 vs. Day 15	-0.163	0.329	0.959
	Day 1 vs. Day 60	-0.527	0.375	0.507
	Day 15 vs. Day 60	-0.364	0.375	0.768
Cortical amygdalar area, anterior part (COAa)	Home cage vs. Day 1	-0.106	1.128	1.000
	Home cage vs. Day 15	-2.405	1.128	0.169
	Homecage vs. Day 60	-5.100	1.303	0.003
	Day 1 vs. Day 15	-2.299	1.189	0.239
	Day 1 vs. Day 60	-4.994	1.356	0.005
	Day 15 vs. Day 60	-2.696	1.356	0.218
Piriform area, pyramidal layer (PIR2)	Home cage vs. Day 1	-0.037	1.015	1.000
	Home cage vs. Day 15	-1.509	1.015	0.459
	Homecage vs. Day 60	-4.583	1.172	0.003
	Day 1 vs. Day 15	-1.472	1.070	0.525
	Day 1 vs. Day 60	-4.546	1.220	0.005
	Day 15 vs. Day 60	-3.074	1.220	0.079
Piriform area, polymorph layer (PIR3)	Home cage vs. Day 1	0.257	1.240	0.997
	Home cage vs. Day 15	-1.021	1.240	0.843
	Homecage vs. Day 60	-4.309	1.432	0.027
	Day 1 vs. Day 15	-1.278	1.307	0.763
	Day 1 vs. Day 60	-4.566	1.490	0.024
	Day 15 vs. Day 60	-3.288	1.490	0.147
Cortical amygdalar area, posterior part (COAp)	Home cage vs. Day 1	0.357	0.851	0.975
	Home cage vs. Day 15	-1.475	0.851	0.326
	Homecage vs. Day 60	-3.489	0.982	0.007
	Day 1 vs. Day 15	-1.833	0.897	0.197
	Day 1 vs. Day 60	-3.847	1.022	0.004

	Day 15 vs. Day 60	-2.014	1.022	0.224
Piriform-amygdalar area, pyramidal layer (PAA2)	Home cage vs. Day 1	0.361	0.715	0.957
	Home cage vs. Day 15	-0.878	0.715	0.615
	Homecage vs. Day 60	-2.904	0.825	0.008
	Day 1 vs. Day 15	-1.239	0.753	0.371
	Day 1 vs. Day 60	-3.265	0.859	0.004
	Day 15 vs. Day 60	-2.025	0.859	0.110
Piriform-amygdalar area, polymorph layer (PAA3)	Home cage vs. Day 1	0.207	0.727	0.992
	Home cage vs. Day 15	-0.835	0.727	0.663
	Homecage vs. Day 60	-2.178	0.839	0.068
	Day 1 vs. Day 15	-1.042	0.766	0.534
	Day 1 vs. Day 60	-2.386	0.873	0.051
	Day 15 vs. Day 60	-1.343	0.873	0.430
Piriform-amygdalar area, molecular layer (PAA1)	Home cage vs. Day 1	0.614	0.694	0.813
	Home cage vs. Day 15	-0.322	0.694	0.966
	Homecage vs. Day 60	-1.493	0.801	0.267
	Day 1 vs. Day 15	-0.936	0.732	0.584
	Day 1 vs. Day 60	-2.107	0.834	0.078
	Day 15 vs. Day 60	-1.171	0.834	0.508
Piriform area, molecular layer (PIR1)	Home cage vs. Day 1	0.472	0.543	0.821
	Home cage vs. Day 15	-0.080	0.543	0.999
	Homecage vs. Day 60	-1.407	0.627	0.137
	Day 1 vs. Day 15	-0.552	0.573	0.771
	Day 1 vs. Day 60	-1.880	0.653	0.036
	Day 15 vs. Day 60	-1.328	0.653	0.201
Ammon's horn (CA)	Home cage vs. Day 1	0.068	0.820	1.000
	Home cage vs. Day 15	0.345	0.820	0.975
	Homecage vs. Day 60	-1.155	0.947	0.620
	Day 1 vs. Day 15	0.277	0.865	0.988
	Day 1 vs. Day 60	-1.223	0.986	0.607
	Day 15 vs. Day 60	-1.500	0.986	0.439
Dentate gyrus (DG)	Home cage vs. Day 1	0.288	0.453	0.920
	Home cage vs. Day 15	0.547	0.453	0.628
	Homecage vs. Day 60	-0.098	0.523	0.998
	Day 1 vs. Day 15	0.259	0.478	0.948
	Day 1 vs. Day 60	-0.386	0.545	0.893
	Day 15 vs. Day 60	-0.645	0.545	0.641
Basolateral amygdalar nucleus, anterior part (BMAa)	Home cage vs. Day 1	-1.473	5.942	0.995
	Home cage vs. Day 15	-7.660	5.942	0.577
	Homecage vs. Day 60	-21.841	6.861	0.018
	Day 1 vs. Day 15	-6.186	6.263	0.758
	Day 1 vs. Day 60	-20.368	7.141	0.039
	Day 15 vs. Day 60	-14.181	7.141	0.218
Basolateral amygdalar nucleus, posterior part (BMAp)	Home cage vs. Day 1	-1.094	5.286	0.997
	Home cage vs. Day 15	-7.763	5.286	0.470
	Homecage vs. Day 60	-20.491	6.104	0.012
	Day 1 vs. Day 15	-6.669	5.572	0.634
	Day 1 vs. Day 60	-19.396	6.353	0.024
	Day 15 vs. Day 60	-12.728	6.353	0.212
Endopiriform nucleus, ventral part (EPv)	Home cage vs. Day 1	-0.359	3.557	1.000
	Home cage vs. Day 15	-2.447	3.557	0.901
	Homecage vs. Day 60	-12.575	4.108	0.024
	Day 1 vs. Day 15	-2.088	3.750	0.944
	Day 1 vs. Day 60	-12.215	4.275	0.038
	Day 15 vs. Day 60	-10.127	4.275	0.108
Basolateral amygdalar nucleus (BLA)	Home cage vs. Day 1	0.386	2.051	0.998
	Home cage vs. Day 15	-1.199	2.051	0.936
	Homecage vs. Day 60	-7.035	2.369	0.030
	Day 1 vs. Day 15	-1.585	2.162	0.883
	Day 1 vs. Day 60	-7.421	2.465	0.027
	Day 15 vs. Day 60	-5.836	2.465	0.108

Endopiriform nucleus, dorsal part (EPd)	Home cage vs. Day 1	0.239	1.168	0.997
	Home cage vs. Day 15	-0.963	1.168	0.843
	Homecage vs. Day 60	-5.697	1.349	0.001
	Day 1 vs. Day 15	-1.201	1.231	0.764
	Day 1 vs. Day 60	-5.936	1.404	0.001
	Day 15 vs. Day 60	-4.734	1.404	0.011
Lateral amygdalar nucleus (LA)	Home cage vs. Day 1	0.642	0.867	0.880
	Home cage vs. Day 15	-0.399	0.867	0.967
	Homecage vs. Day 60	-2.729	1.002	0.051
	Day 1 vs. Day 15	-1.041	0.914	0.670
	Day 1 vs. Day 60	-3.371	1.043	0.016
	Day 15 vs. Day 60	-2.330	1.043	0.139
Claustrum (CLA)	Home cage vs. Day 1	0.332	0.529	0.922
	Home cage vs. Day 15	-0.473	0.529	0.808
	Homecage vs. Day 60	-2.585	0.611	0.001
	Day 1 vs. Day 15	-0.805	0.558	0.485
	Day 1 vs. Day 60	-2.917	0.636	<0.001
	Day 15 vs. Day 60	-2.112	0.636	0.013
Intercalated amygdalar nucleus (IA)	Home cage vs. Day 1	-1.038	4.303	0.995
	Home cage vs. Day 15	-4.797	4.303	0.684
	Homecage vs. Day 60	-16.372	4.968	0.014
	Day 1 vs. Day 15	-3.758	4.535	0.840
	Day 1 vs. Day 60	-15.334	5.171	0.030
	Day 15 vs. Day 60	-11.576	5.171	0.138
Medial amygdalar nucleus, anterodorsal part (MEAad)	Home cage vs. Day 1	0.407	2.621	0.999
	Home cage vs. Day 15	-1.729	2.621	0.911
	Homecage vs. Day 60	-8.060	3.027	0.059
	Day 1 vs. Day 15	-2.136	2.763	0.866
	Day 1 vs. Day 60	-8.466	3.150	0.056
	Day 15 vs. Day 60	-6.331	3.150	0.209
Central amygdalar nucleus, capsular part (CEAc)	Home cage vs. Day 1	0.192	1.745	1.000
	Home cage vs. Day 15	-1.233	1.745	0.894
	Homecage vs. Day 60	-6.266	2.015	0.021
	Day 1 vs. Day 15	-1.426	1.839	0.865
	Day 1 vs. Day 60	-6.459	2.097	0.023
	Day 15 vs. Day 60	-5.033	2.097	0.101
Central amygdalar nucleus, medial part (CEAm)	Home cage vs. Day 1	0.352	0.996	0.985
	Home cage vs. Day 15	0.062	0.996	1.000
	Homecage vs. Day 60	-2.730	1.150	0.107
	Day 1 vs. Day 15	-0.290	1.050	0.992
	Day 1 vs. Day 60	-3.081	1.197	0.071
	Day 15 vs. Day 60	-2.792	1.197	0.116
Medial amygdalar nucleus, anteroventral part (MEAav)	Home cage vs. Day 1	0.007	0.757	1.000
	Home cage vs. Day 15	-1.266	0.757	0.357
	Homecage vs. Day 60	-1.731	0.874	0.220
	Day 1 vs. Day 15	-1.273	0.798	0.398
	Day 1 vs. Day 60	-1.738	0.910	0.248
	Day 15 vs. Day 60	-0.465	0.910	0.956
Bed nucleus of the accessory olfactory tract (BA)	Home cage vs. Day 1	0.077	0.695	0.999
	Home cage vs. Day 15	-0.702	0.695	0.745
	Homecage vs. Day 60	-1.485	0.802	0.272
	Day 1 vs. Day 15	-0.779	0.732	0.714
	Day 1 vs. Day 60	-1.562	0.835	0.264
	Day 15 vs. Day 60	-0.783	0.835	0.785
Central amygdalar nucleus, lateral part (CEAl)	Home cage vs. Day 1	0.524	0.542	0.770
	Home cage vs. Day 15	0.391	0.542	0.888
	Homecage vs. Day 60	-1.179	0.626	0.259
	Day 1 vs. Day 15	-0.133	0.572	0.995
	Day 1 vs. Day 60	-1.703	0.652	0.065
	Day 15 vs. Day 60	-1.570	0.652	0.099
Caudoputamen (CP)	Home cage vs. Day 1	0.277	0.369	0.875

	Home cage vs. Day 15	0.447	0.369	0.625
	Homecage vs. Day 60	-0.294	0.426	0.900
	Day 1 vs. Day 15	0.170	0.389	0.971
	Day 1 vs. Day 60	-0.571	0.443	0.578
	Day 15 vs. Day 60	-0.741	0.443	0.358
Pallidum, ventral region (PALv)	Home cage vs. Day 1	0.452	0.706	0.918
	Home cage vs. Day 15	0.546	0.706	0.865
	Homecage vs. Day 60	-1.127	0.815	0.520
	Day 1 vs. Day 15	0.095	0.744	0.999
	Day 1 vs. Day 60	-1.579	0.848	0.268
	Day 15 vs. Day 60	-1.673	0.848	0.223
Globus pallidus, external segment (GPe)	Home cage vs. Day 1	0.136	0.441	0.990
	Home cage vs. Day 15	0.368	0.441	0.837
	Homecage vs. Day 60	-0.482	0.509	0.780
	Day 1 vs. Day 15	0.232	0.465	0.958
	Day 1 vs. Day 60	-0.618	0.530	0.653
	Day 15 vs. Day 60	-0.850	0.530	0.393
Globus pallidus, internal segment (GPi)	Home cage vs. Day 1	0.197	0.451	0.971
	Home cage vs. Day 15	0.513	0.451	0.669
	Homecage vs. Day 60	-0.260	0.521	0.958
	Day 1 vs. Day 15	0.316	0.475	0.909
	Day 1 vs. Day 60	-0.457	0.542	0.833
	Day 15 vs. Day 60	-0.774	0.542	0.494
Central medial nucleus of the thalamus (CM)	Home cage vs. Day 1	0.013	1.224	1.000
	Home cage vs. Day 15	0.288	1.224	0.995
	Homecage vs. Day 60	-2.706	1.413	0.246
	Day 1 vs. Day 15	0.275	1.290	0.996
	Day 1 vs. Day 60	-2.719	1.471	0.273
	Day 15 vs. Day 60	-2.995	1.471	0.200
Anteroventral nucleus of thalamus (AV)	Home cage vs. Day 1	-0.088	0.699	0.999
	Home cage vs. Day 15	0.486	0.699	0.898
	Homecage vs. Day 60	-1.284	0.807	0.400
	Day 1 vs. Day 15	0.574	0.736	0.863
	Day 1 vs. Day 60	-1.196	0.840	0.496
	Day 15 vs. Day 60	-1.770	0.840	0.176
Ventral medial nucleus of the thalamus (VM)	Home cage vs. Day 1	0.101	0.676	0.999
	Home cage vs. Day 15	0.354	0.676	0.953
	Homecage vs. Day 60	-1.182	0.780	0.443
	Day 1 vs. Day 15	0.253	0.712	0.984
	Day 1 vs. Day 60	-1.283	0.812	0.406
	Day 15 vs. Day 60	-1.536	0.812	0.256
Lateral habenula (LH)	Home cage vs. Day 1	0.279	0.454	0.927
	Home cage vs. Day 15	0.479	0.454	0.720
	Homecage vs. Day 60	-0.618	0.525	0.646
	Day 1 vs. Day 15	0.200	0.479	0.975
	Day 1 vs. Day 60	-0.896	0.546	0.374
	Day 15 vs. Day 60	-1.097	0.546	0.210
Anteromedial nucleus (AM)	Home cage vs. Day 1	-0.044	0.404	1.000
	Home cage vs. Day 15	0.347	0.404	0.826
	Homecage vs. Day 60	-0.530	0.466	0.671
	Day 1 vs. Day 15	0.391	0.426	0.795
	Day 1 vs. Day 60	-0.486	0.485	0.751
	Day 15 vs. Day 60	-0.877	0.485	0.293
Submedial nucleus of the thalamus (SMT)	Home cage vs. Day 1	0.161	0.447	0.984
	Home cage vs. Day 15	0.329	0.447	0.882
	Homecage vs. Day 60	-0.479	0.516	0.790
	Day 1 vs. Day 15	0.168	0.471	0.984
	Day 1 vs. Day 60	-0.640	0.537	0.637
	Day 15 vs. Day 60	-0.808	0.537	0.449
Paracentral nucleus (PCN)	Home cage vs. Day 1	0.076	0.364	0.997

	Home cage vs. Day 15	0.330	0.364	0.801
	Homecage vs. Day 60	-0.418	0.420	0.754
	Day 1 vs. Day 15	0.254	0.384	0.910
	Day 1 vs. Day 60	-0.494	0.438	0.675
	Day 15 vs. Day 60	-0.748	0.438	0.338
Mediodorsal nucleus of thalamus (MD)	Home cage vs. Day 1	0.070	0.410	0.998
	Home cage vs. Day 15	0.363	0.410	0.812
	Homecage vs. Day 60	-0.361	0.474	0.871
	Day 1 vs. Day 15	0.293	0.433	0.905
	Day 1 vs. Day 60	-0.432	0.493	0.818
	Day 15 vs. Day 60	-0.725	0.493	0.469
Ventral anterior-lateral complex of the thalamus (VAL)	Home cage vs. Day 1	0.085	0.356	0.995
	Home cage vs. Day 15	0.386	0.356	0.702
	Homecage vs. Day 60	-0.185	0.411	0.969
	Day 1 vs. Day 15	0.301	0.375	0.853
	Day 1 vs. Day 60	-0.270	0.428	0.921
	Day 15 vs. Day 60	-0.571	0.428	0.550
Central lateral nucleus of the thalamus (CL)	Home cage vs. Day 1	0.198	0.330	0.931
	Home cage vs. Day 15	0.405	0.330	0.616
	Homecage vs. Day 60	-0.132	0.381	0.985
	Day 1 vs. Day 15	0.206	0.348	0.933
	Day 1 vs. Day 60	-0.330	0.397	0.839
	Day 15 vs. Day 60	-0.537	0.397	0.539
Ventral posterior complex of the thalamus (VP)	Home cage vs. Day 1	-0.170	0.405	0.974
	Home cage vs. Day 15	0.364	0.405	0.806
	Homecage vs. Day 60	-0.073	0.468	0.999
	Day 1 vs. Day 15	0.534	0.427	0.601
	Day 1 vs. Day 60	0.097	0.487	0.997
	Day 15 vs. Day 60	-0.437	0.487	0.806
Medial habenula (MH)	Home cage vs. Day 1	0.271	0.367	0.880
	Home cage vs. Day 15	0.603	0.367	0.373
	Homecage vs. Day 60	-0.025	0.424	1.000
	Day 1 vs. Day 15	0.331	0.387	0.827
	Day 1 vs. Day 60	-0.297	0.441	0.906
	Day 15 vs. Day 60	-0.628	0.441	0.496
Reticular nucleus of the thalamus (RT)	Home cage vs. Day 1	-0.001	0.338	1.000
	Home cage vs. Day 15	0.391	0.338	0.659
	Homecage vs. Day 60	0.047	0.390	0.999
	Day 1 vs. Day 15	0.392	0.356	0.692
	Day 1 vs. Day 60	0.048	0.406	0.999
	Day 15 vs. Day 60	-0.344	0.406	0.831
Anterodorsal nucleus (AD)	Home cage vs. Day 1	0.314	0.313	0.749
	Home cage vs. Day 15	0.579	0.313	0.273
	Homecage vs. Day 60	0.066	0.362	0.998
	Day 1 vs. Day 15	0.265	0.330	0.852
	Day 1 vs. Day 60	-0.248	0.376	0.911
	Day 15 vs. Day 60	-0.514	0.376	0.531
Lateral dorsal nucleus of thalamus (LD)	Home cage vs. Day 1	0.211	0.300	0.895
	Home cage vs. Day 15	0.473	0.300	0.408
	Homecage vs. Day 60	0.222	0.346	0.918
	Day 1 vs. Day 15	0.262	0.316	0.841
	Day 1 vs. Day 60	0.011	0.360	1.000
	Day 15 vs. Day 60	-0.251	0.360	0.897
Anterior hypothalamic nucleus (AHN)	Home cage vs. Day 1	0.281	6.307	1.000
	Home cage vs. Day 15	-2.952	6.307	0.965
	Homecage vs. Day 60	-17.598	7.283	0.098
	Day 1 vs. Day 15	-3.232	6.649	0.962
	Day 1 vs. Day 60	-17.878	7.581	0.110
	Day 15 vs. Day 60	-14.646	7.581	0.239
Hypothalamic medial zone (MEZ)	Home cage vs. Day 1	0.011	5.940	1.000

	Home cage vs. Day 15	-4.111	5.940	0.899
	Homecage vs. Day 60	-17.359	6.859	0.078
	Day 1 vs. Day 15	-4.122	6.261	0.912
	Day 1 vs. Day 60	-17.369	7.139	0.095
	Day 15 vs. Day 60	-13.248	7.139	0.271
Paraventricular hypothalamic nucleus (PVH)	Home cage vs. Day 1	-0.083	5.886	1.000
	Home cage vs. Day 15	-1.804	5.886	0.990
	Homecage vs. Day 60	-14.620	6.797	0.163
	Day 1 vs. Day 15	-1.721	6.204	0.992
	Day 1 vs. Day 60	-14.537	7.074	0.194
	Day 15 vs. Day 60	-12.816	7.074	0.290
Lateral hypothalamic area (LHA)	Home cage vs. Day 1	0.337	1.425	0.995
	Home cage vs. Day 15	-0.964	1.425	0.905
	Homecage vs. Day 60	-3.861	1.646	0.112
	Day 1 vs. Day 15	-1.300	1.502	0.822
	Day 1 vs. Day 60	-4.198	1.713	0.091
	Day 15 vs. Day 60	-2.898	1.713	0.347
Arcuate hypothalamic nucleus (ARH)	Home cage vs. Day 1	0.323	0.509	0.920
	Home cage vs. Day 15	-0.423	0.509	0.839
	Homecage vs. Day 60	-1.414	0.588	0.100
	Day 1 vs. Day 15	-0.746	0.537	0.516
	Day 1 vs. Day 60	-1.737	0.612	0.040
	Day 15 vs. Day 60	-0.991	0.612	0.385
Tuberal nucleus (TU)	Home cage vs. Day 1	0.256	0.533	0.963
	Home cage vs. Day 15	-0.563	0.533	0.718
	Homecage vs. Day 60	-1.319	0.615	0.165
	Day 1 vs. Day 15	-0.818	0.562	0.476
	Day 1 vs. Day 60	-1.574	0.640	0.090
	Day 15 vs. Day 60	-0.756	0.640	0.644
Supraoptic nucleus (SO)	Home cage vs. Day 1	0.302	0.662	0.968
	Home cage vs. Day 15	-0.103	0.662	0.999
	Homecage vs. Day 60	-0.713	0.764	0.787
	Day 1 vs. Day 15	-0.405	0.697	0.937
	Day 1 vs. Day 60	-1.015	0.795	0.585
	Day 15 vs. Day 60	-0.610	0.795	0.868

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