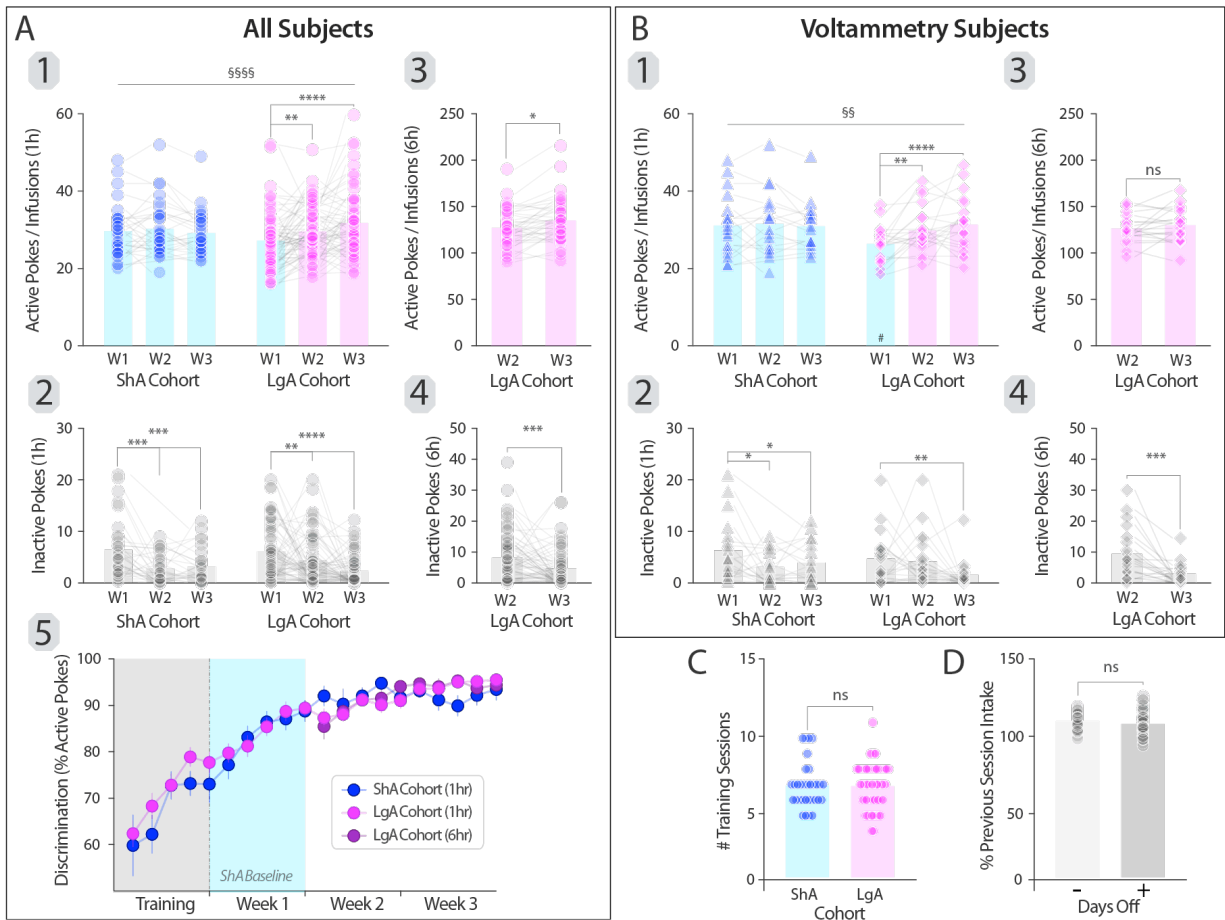


Supplementary Figure 1. Voltammetry recording sites

Histological verification of carbon fiber microelectrode placements from voltammetry subjects. Electrode placements from ShA cohort subjects are indicated by blue circles (left panel), and LgA cohort subjects by magenta circles (right panel). Coronal sections are labeled with anterior-posterior coordinates⁴⁶. Electrode placements for some subjects could not be determined because of headcap loss prior to perfusion, or the lesion site was undetectable post-fixation.



Supplementary Figure 2. Drug-Taking Behavior

A) Behavior and training data from all subjects

A1, A2) Average weekly active and inactive nose pokes in 1 hour of self-administration (ShA or 1st hr of LgA) from ShA (n = 32) and LgA (n = 66) subjects. Active pokes (equal to number of infusions received) significantly increased over weeks in the LgA cohort, but remained stable in the ShA cohort (Mixed-effects REML: significant main effect of week: $F(2, 187) = 4.93$, $p = 0.0082$, and significant week \times drug-access interaction: $F(2, 187) = 9.88$, $§§§§p < 0.0001$). Inactive pokes decreased over weeks in both groups (Mixed-effects REML- significant main effect of week: $F(2, 185) = 24.22$, $***p < 0.0001$; non-significant week \times drug-access group interaction: $F(2, 185) = 1.99$, $p = 0.139$).

A3, A4) Average weekly active and inactive nose pokes from six-hour sessions in LgA cohort. Active pokes increased significantly from week 2 to week 3 (Two-Tailed Wilcoxon matched-pairs signed rank: $W = 716$, $*p = 0.021$, $n = 66$ pairs), and inactive pokes decreased significantly (Two-Tailed Wilcoxon matched-pairs signed rank: $W = -1030$, $***p = 0.0006$, $n = 65$ pairs).

A5) Average daily %Active pokes (mean \pm SEM of Active/Total pokes \times 100%) aligned to session where the self-administration acquisition criteria was met (ShA cohort -1hr: n = 32 blue, LgA cohort-1hr: n = 66, magenta, LgA cohort-6hrs: n = 66, dark magenta). The percentage of active pokes increased over training in both ShA and LgA cohorts similarly (comparison of ShA vs. LgA first-hour with ME-REML: significant main effect of session: $F(19, 1556) = 34.62$, $****p < 0.0001$ and non-significant session \times drug-access group interaction: $F(19, 1556) = 0.847$, $p = 0.65$).

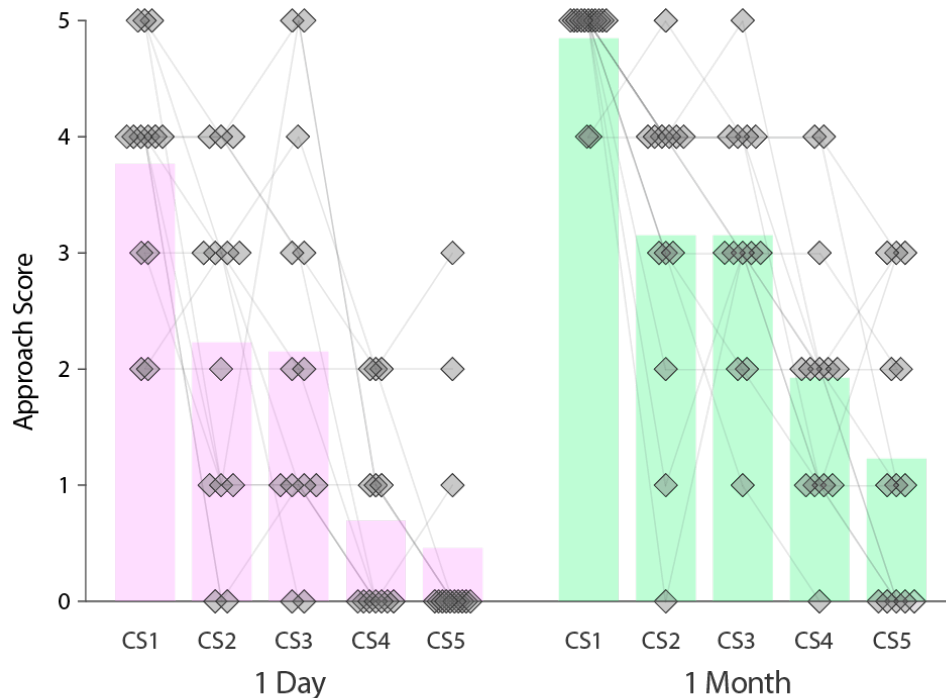
In all bar plots (A-D), individual points represent responses from individual subjects and bars represent the mean across subjects. For grouped barplots (A, B), asterisks above bars denote significant within-drug access group Šidák post hoc comparisons between weeks (Two-Tailed $*p < 0.05$, $p < 0.01$, etc.), and hash symbols at the base of bars denote significant between-drug access group comparisons at the same time point (Two-Tailed $\#p < 0.05$, $\#\#p < 0.01$, etc.).**

B) Behavior and training data as in A but only including the subset of subjects from which successful FSCV recordings were obtained.

In line with the analysis of all subjects, active pokes increased over weeks in LgA cohort (n=22) and remained stable in ShA cohort (n=19) voltammetry subjects ME-REML- significant main effect of week: $F(2,73) = 3.558$, $*p = 0.034$ and significant week \times drug-access group interaction: $F(2, 73) = 6.724$, $§§p = 0.0021$. Inactive pokes also decreased over weeks in voltammetry subjects from both groups similarly (ME-REML: significant main effect of week: $F(2,76) = 8.549$, $***p < 0.0004$; week \times drug-access group interaction was not significant: $F(2, 76) = 2.476$, $p = 0.09$).

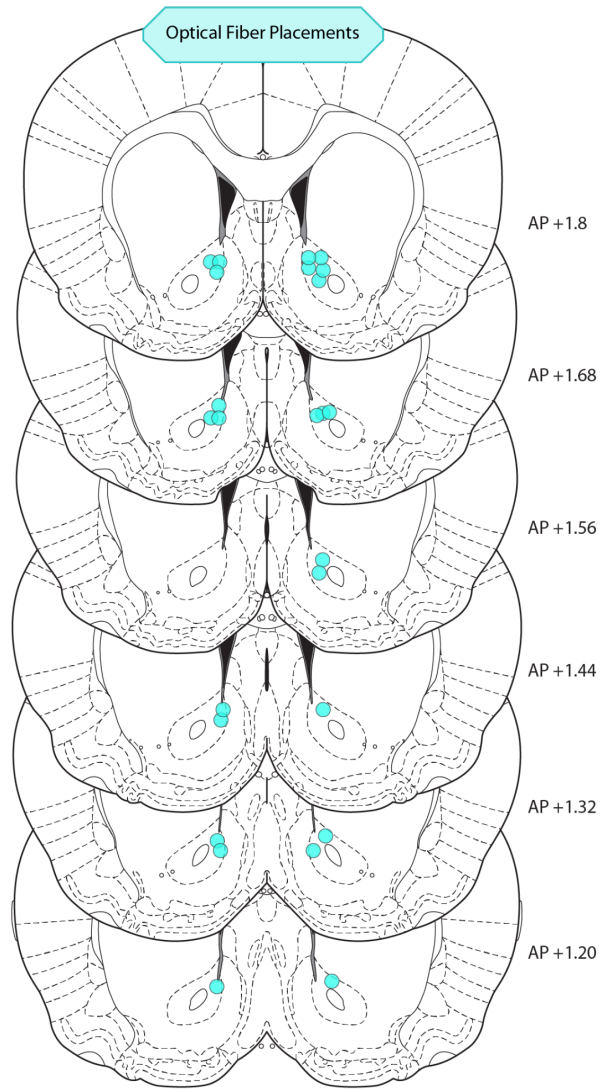
C) The number of training sessions required to reach self-administration acquisition criteria (< 10 active pokes in three training sessions) was not different for subjects in ShA (blue) & LgA cohorts (magenta; Two-Tailed Mann-Witney U = 1001, $n = 99$, $p = 0.67$).

D) Percent change in intake between sequential self-administration sessions occurring with or without days off in-between in LgA. Days off between sessions had no impact on subsequent drug intake (Two-Tailed Wilcoxon matched-pairs signed rank test, $W = -390$, $n = 60$ pairs, n.s. $p = 0.15$).



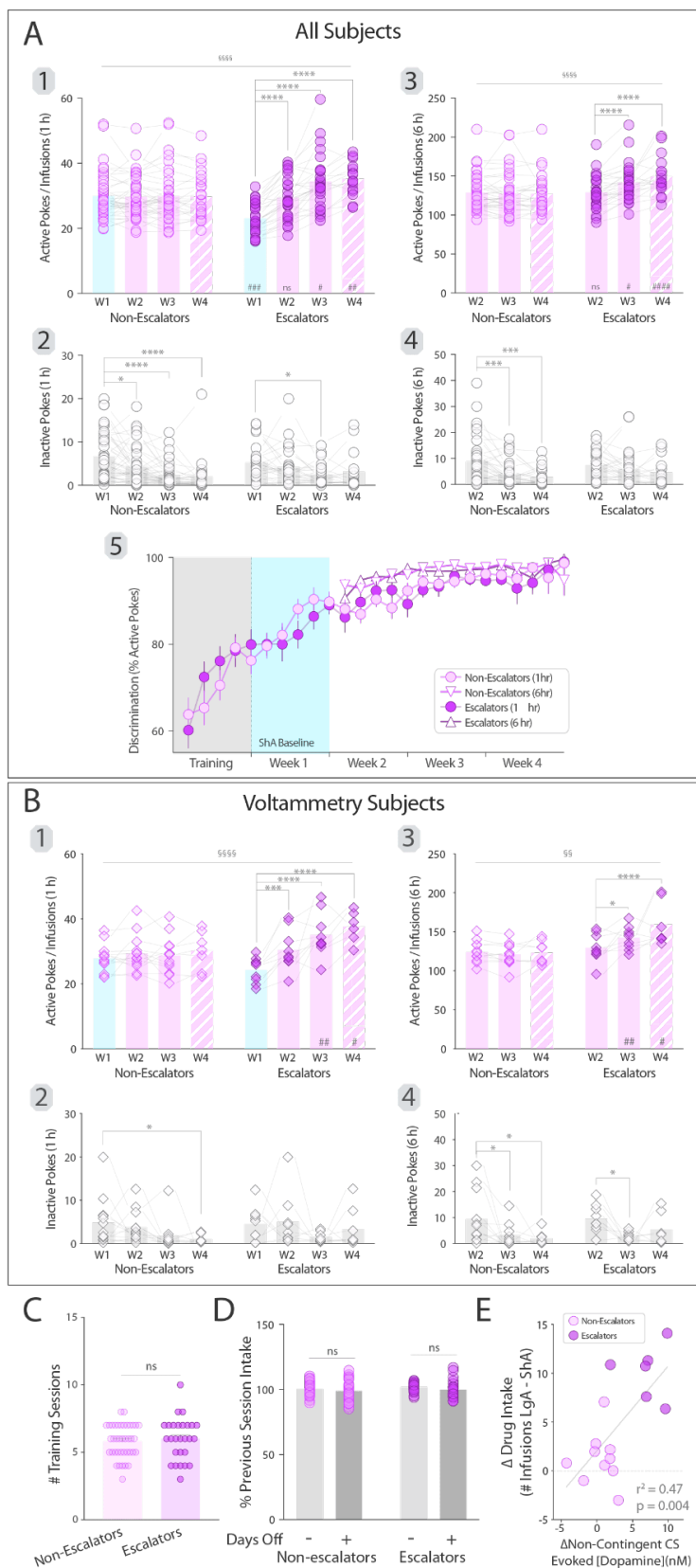
Supplementary Figure 3. Individual CS-elicited Approach Scores after 1 Day and 1 Month of Abstinence

Bars represent the average CS-elicited approach across 13 subjects for each of the series of 5 non-contingent CS presentations delivered at 1 day and 1 month of abstinence timepoints. Individual diamonds connected by lines reflect the approach scores from individual subjects. Conditioned approach to each CS presentation was scored on a 0-5 scale (0 = no response, and 5 = orient, approach and interact with active port, see methods for further details). Approach scores were analyzed using a Two-Way Repeated Measures ANOVA with Greenhouse-Geisser Correction for Sphericity with incubation time point (Day 1 vs. Day 30) and CS presentation number (CS#) as factors. There was a significant main effect of incubation time point ($F(1, 12) = 22.16$, $***p = 0.0005$, $\epsilon = 0.785$), with higher approach scores observed on Day 30, indicating an incubation-related enhancement in cue reactivity. A significant main effect of CS# was also observed ($F(3.14, 37.70) = 39.97$, $****p < 0.0001$, $\epsilon = 1.00$), reflecting reliable variation in approach behavior across CS trials within a session. However, there was no interaction between incubation timepoint and CS# ($F(3.133, 37.59) = 0.1980$, $p = 0.904$, $\epsilon = 0.783$), suggesting that although overall conditioned approach scores increased with incubation, the temporal pattern of responding across CS trials was preserved.



Supplementary Figure 4. Optical fiber placements

Histological verification of optic fiber placements are indicated by cyan circles. Coronal sections are labeled with anterior-posterior coordinates³³. In some cases, placements could not be obtained because the fiber track was undetectable, or brain tissue was damaged during processing.



Supplementary Figure 5. Escalators vs. Non-Escalator Drug-taking Behavior

A1-2: Average weekly active and inactive nose pokes in one hour of self-administration (ShA Week 1 or 1st hour LgA Weeks 2-4) from all non-escalators ($n=37$) and escalators ($n=29$). Active nose pokes (equivalent to the number of infusions received) significantly increased over weeks in escalators but remained stable in nonescalators. Mixed Effects REML (ME-REML)- significant main effect of week: $F(3, 166)=41.49$, $****p<0.0001$ and significant week \times escalation group interaction: $F(3, 166)=43.37$, $****p<0.0001$. Inactive pokes decreased over weeks in both groups. ME-REML- significant main effect of week: $F(3, 166)=14.12$, $****p<0.0001$, but week \times escalation group interaction was not significant: $F(3, 166)=1.143$, $p=0.33$. **A3-4:** Average weekly active and inactive nose poke data from all six-hours of LgA (Weeks 2-4) in nonescalators and escalators. Total active pokes in 6 hrs significantly increased over weeks in escalators, but remained stable in nonescalators. ME-REML- significant main effect of escalation group: $F(1, 64)=5.493$, $*p<0.022$, main effect of week: $F(2, 106)=12.89$, $****p<0.0001$, and week \times escalation group interaction: $F(2, 106)=18.84$, $****p<0.0001$. Inactive pokes decreased over weeks in both groups. ME-REML- significant main effect of week: $F(2, 100)=8.681$, $***p=0.0006$; but week \times escalation group interaction was not significant: $F(2, 100)=2.01$, $p=0.1391$. **A5:** Average daily %Active pokes (mean \pm SEM, Active / Total pokes \times 100%), aligned to meeting the acquisition criteria plotted separately for nonescalators (light magenta, 1 hr: circles, 6 hr: triangles) and escalators (dark magenta 1 hr: circles, 6 hr: triangles). The proportion of active pokes increased similarly over sessions in nonescalators and escalators (comparison of ShA vs. LgA first-hour; ME-REML- significant main effect of session: $F(24, 1198)=25.97$, $****p<0.0001$, session \times escalation group interaction was not significant: $F(24, 1198)=0.742$, $p=0.811$).

*In all bar plots (A-E), individual points represent responses from individual subjects and bars represent the mean across subjects. For grouped barplots (A, B), asterisks above bars denote significant within- escalation group Sidák post hoc comparisons between weeks (Two-Tailed $*p<0.05$, $**p<0.01$, etc.), and hash symbols at the base of bars denote significant between-escalation group comparisons at the same time point (Two-Tailed $\#p<0.05$, $\#\#p<0.01$, etc.).*

B) Bar plots are equivalent to those in A but only include data from subjects with successful voltammetry recordings ($n=13$ nonescalators, $n=9$ escalators). As in the analysis of all subjects, active pokes in 1st hour and all 6 hours increased over weeks in voltammetry escalators but not non-escalators. **B1)** 1st hr active pokes: ME-REML- significant main effect of week: $F(3, 51)=20.72$, $****p<0.0001$, and significant week \times escalation group interaction: $F(3, 51)=12.49$, $****p<0.0001$. **B3)** 6 hr active pokes: ME-REML- significant main effect of escalation group: $F(1, 19)=8.29$, $**p=0.0096$, main effect of week: $F(2, 32)=7.77$, $**p=0.0018$ and significant week \times escalation group interaction: $F(2, 32)=7.02$, $***p<0.003$. The number of inactive pokes also decreased over weeks in voltammetry subjects from both groups. **B2)** 1st hr inactive pokes: ME-REML- significant main effect of week: $F(3, 53)=4.587$, $**p=0.0063$, but week \times escalation group interaction was not significant: $F(3, 53)=0.7796$, $p=0.51$. **B4)** 6 hr inactive pokes: ME-REML- significant main effect of week: $F(2, 32)=8.995$, $***p=0.0008$ but week \times escalation group interaction was not significant: $F(2, 32)=0.6964$, $p=0.51$.

C) Number of training sessions to reach self-administration acquisition criteria (<10 active pokes in three sessions) for non-escalators ($n=39$, light magenta) and escalators ($n=27$, dark magenta). There was no difference in the number of training sessions required to reach the training acquisition criteria between groups (Two-Tailed Mann-Witney $U=495.5$, $p=0.68$).

D) Average change in intake between sequential self-administration sessions occurring with or without days off in-between (Mean % Change). Days off between sessions had no impact on subsequent drug intake. ME-REML- non-significant main effect of days off: $F(1, 121)=2.908$, $p=0.10$, non-significant main effect of escalation group: $F(1, 121)=1.04$, $p=0.3$.

E) Correlation between the change in non-contingent CS-evoked dopamine (Mean LgA CS-evoked DA- Mean ShA CS-evoked DA) and the change in drug intake (Mean final week LgA Intake- Mean ShA Intake; (best-fit line for all subjects, $n=16$, slope = 7.54, $R^2=0.46$; $F(1,14)=12.32$; $**p=0.004$).