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## Supplemental information

# Responses and functions of dopamine in nucleus accumbens core during social behaviors

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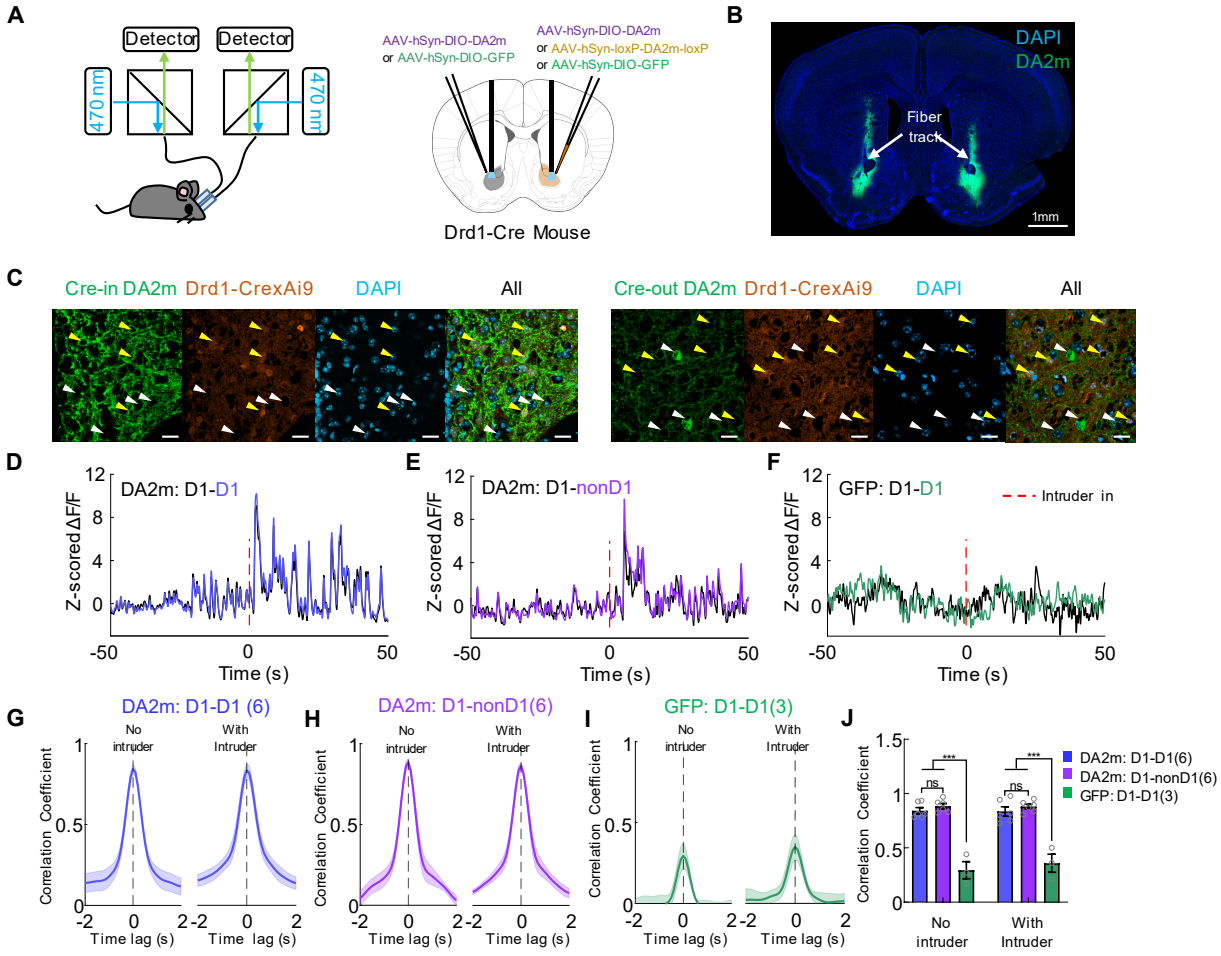


**Figure S1. DA release sensed by D1 cells and non-D1 cells are highly correlated, but not in the GFP expressing mice, and no increase in DA activity at the onset of locomotion. Related to Figure 1.**

(A) Schematic illustration of the recording setup and virus injection. Atlas image adopted from (Franklin and Paxinos, 2007)

(B) Representative image showing the expression of GRAB$_{DA2m}$ in both hemispheres and optic fiber tracks. (Scale bar, 1mm.)

(C) Representative images showing the expression of GRAB$_{DA2m}$ in D1 cells (left, yellow arrows) in an animal injected with Cre-in virus and in non-D1 cells (right, white arrows) in an animal injected with Cre-out virus. (Scale bar, 20μm.)

(D) Representative traces of GRAB$_{DA2m}$ from D1R cells in both hemispheres. Time 0 indicates when a conspecific intruder is introduced.

(E) Representative traces of GRAB$_{DA2m}$ from D1R cells in one hemisphere and that of non-D1R cells in the other hemisphere, following the conventions as in D.

(F) Representative traces of GFP from D1R cells in both hemispheres, following the conventions as in D.

(G-I) The time shifted correlation coefficient between the GRAB$_{DA2m}$ signals in D1R – D1R cells (G) and D1R – non-D1R cells (H), and GFP signals in D1R – D1R cells (I) from two hemispheres before and after the introduction of an intruder. (n=6 mice for each GRAB$_{DA2m}$ group, and n=3 for GFP group.)

(J) Group summary of peak correlation coefficients of GRAB$_{DA2m}$ or GFP signals between hemispheres. (n= 6 mice for each GRAB$_{DA2m}$ group and n=3 for the GFP group.)

Error bars and shaded areas in (G-J) represent ± SEM. Numbers in bar graphs represent number of test animals. (J) Two-way ANOVA followed by multiple comparison test with Bonferroni's correction. *p<0.05, **p<0.01, and ***p<0.001.

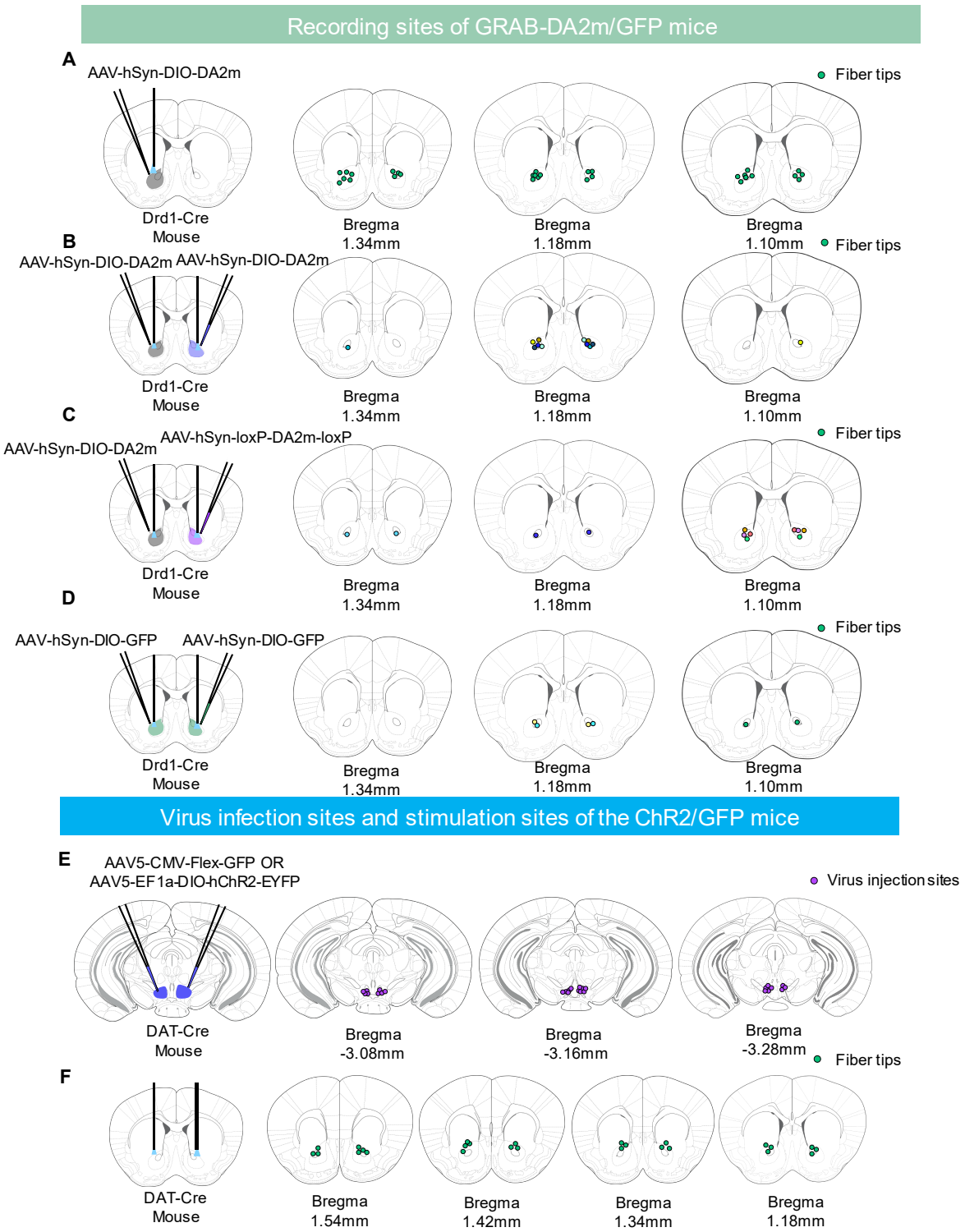


**Figure S2. Virus injection sites and optic fiber placements for recording and optogenetic activation. Related to Figures 1, 3, and 6.**

(A-F) Experimental design (left) and coronal brain sections (right) at the bregma level of NAc showing the optic fiber endings (A-D, F) and VTA showing the virus injection sites (E). Each dot represents one animal in (A), dots with same color in (B-D) represent the same animal. Brain atlas images are modified from (Franklin and Paxinos, 2013).

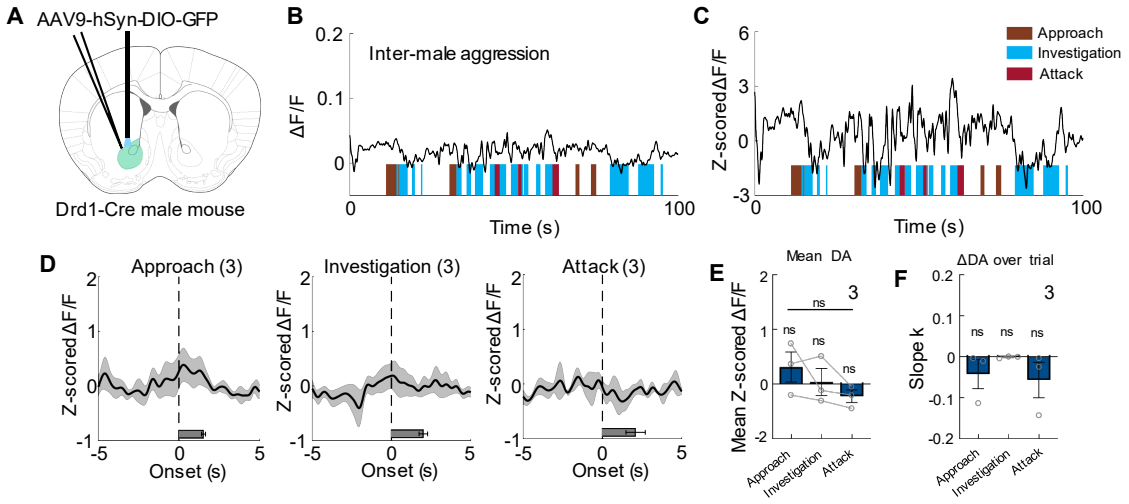


**Figure S3. No change in fluorescence signal during any behaviors in GFP expressing animals. Related to Figure 1, 2, and 5.**

(A) Schematics illustrating the experimental design. Atlas image adopted from Franklin and Paxinos (2007).
(B-C) Representative traces of ΔF/F (B) and Z scored ΔF/F (C) of GFP during inter-male aggression.
(D) Average PETHs aligned to the onset of approach, investigation, and attack. Horizontal bars indicate average duration of the behaviors. (n = 3 male mice.)
(E) Averaged GFP responses during approach, investigation, and attack. (n = 3 male mice.)
(F) Slopes of the best fitted lines of GFP responses over repeated behavioral events. (n=3 male mice.)

Error bars and shaded areas in (D-F) represent ± SEM. Numbers in bar graphs represent number of test animals. (E) One-way ANOVA; (E-F) One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR .

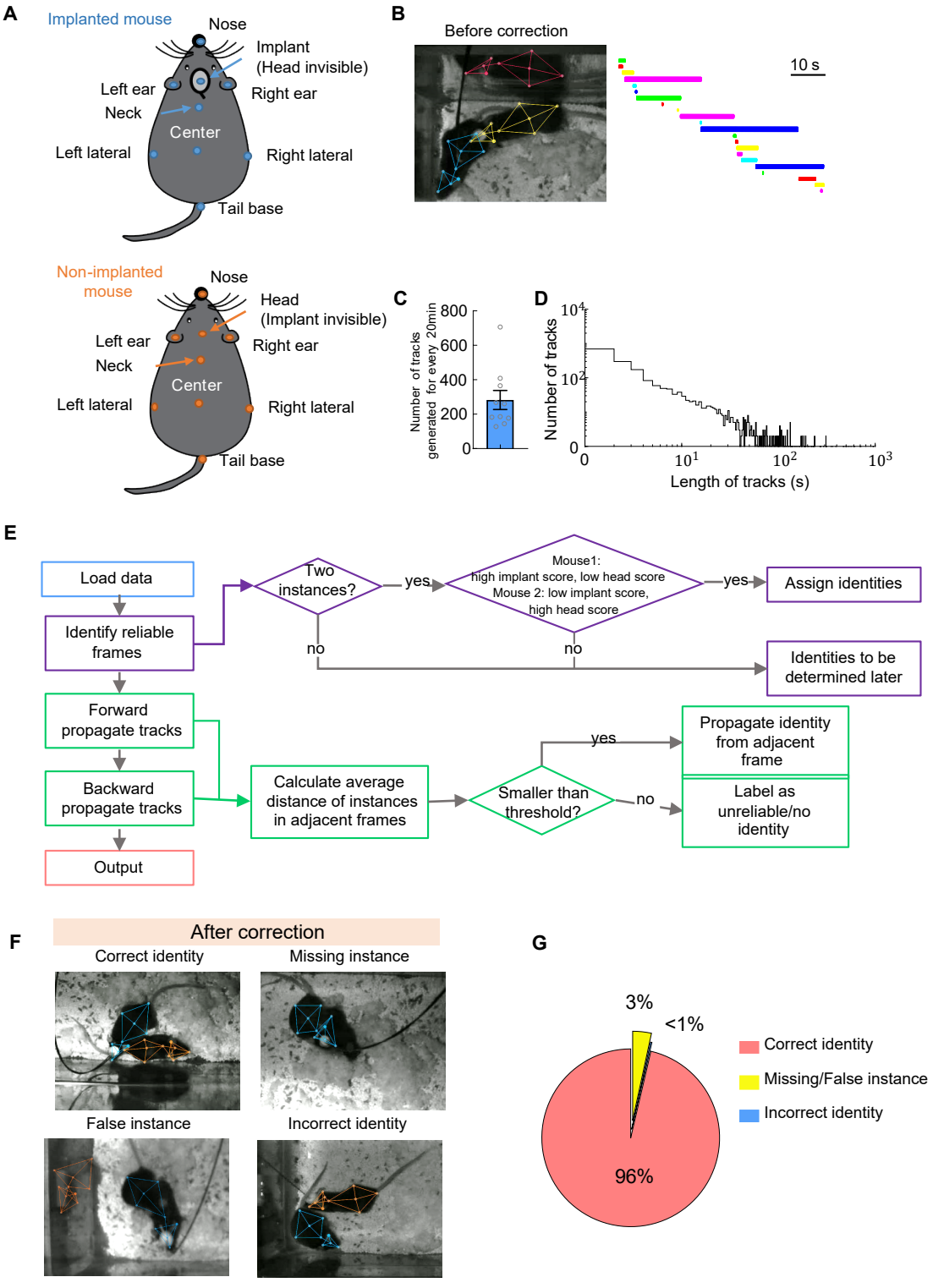
A
Implanted mouse
Nose
Implant
(Head invisible)
Left ear
Right ear
Neck
Center
Left lateral
Right lateral
Tail base
Non-implanted mouse
Nose
Head
(Implant invisible)
Left ear
Right ear
Neck
Center
Left lateral
Right lateral
Tail base
B
Before correction
10 s
C
Number of tracks generated for every 20min
800
600
400
200
0
D
Number of tracks
$10^4$
$10^2$
0
0
$10^1$
$10^2$
$10^3$
Length of tracks (s)
E
Load data
Identify reliable frames
Forward propagate tracks
Backward propagate tracks
Output
Two instances?
yes
no
Mouse1: high implant score, low head score
Mouse 2: low implant score, high head score
yes
no
Assign identities
Identities to be determined later
Calculate average distance of instances in adjacent frames
Smaller than threshold?
yes
no
Propagate identity from adjacent frame
Label as unreliable/no identity
F
After correction
Correct identity
Missing instance
False instance
Incorrect identity
G
3%
<1%
96%
Correct identity
Missing/False instance
Incorrect identity

**Figure S4 Procedures for tracking two same-color animals that differ in specific features. Realated to Figure 1.**
(A) Custom defined skeleton model in SLEAP. Nine body parts are labeled for each animal. “Head” will be labeled as invisible for implanted animals while “implant” will be labeled as invisible for non-implanted animals.
(B) A representative image overlaid with SLEAP model-identified animal skeletons (left) and representative tracks of candidate skeletons in 60 seconds (right). Each track represents one candidate skeleton over frames.
(C) The number of tracks generated for a 20-minute video with two freely interacting black mice using SLEAP. Error bar: ± SEM. n=10 videos.
(D) The distribution of track length from 10 videos with two freely interacting black mice of the opposite sexes.
(E) Flow chart showing the procedures to assign identities to the two mice based on the SLEAP results. The identity assignment starts with identifying frames that contains two skeletons, one with high “head” confidence score and low “implant” confidence score and the other with high “implant” confidence score and low “head” confidence scores. These frames are considered as reliable. The identities of animals in adjacent frames are then determined based on continuity in movement from the reliable frames. The movement based identity propagation occurs in both forward and backward directions. Lastly, candidate skeletons without identity assignments are eliminated.
(F) Representative images showing different outcomes of identity assignment.
(G) The percentage of frames with correct, missing/false and incorrect identity assignment based on a total of 2000 random images extracted from 10 videos.

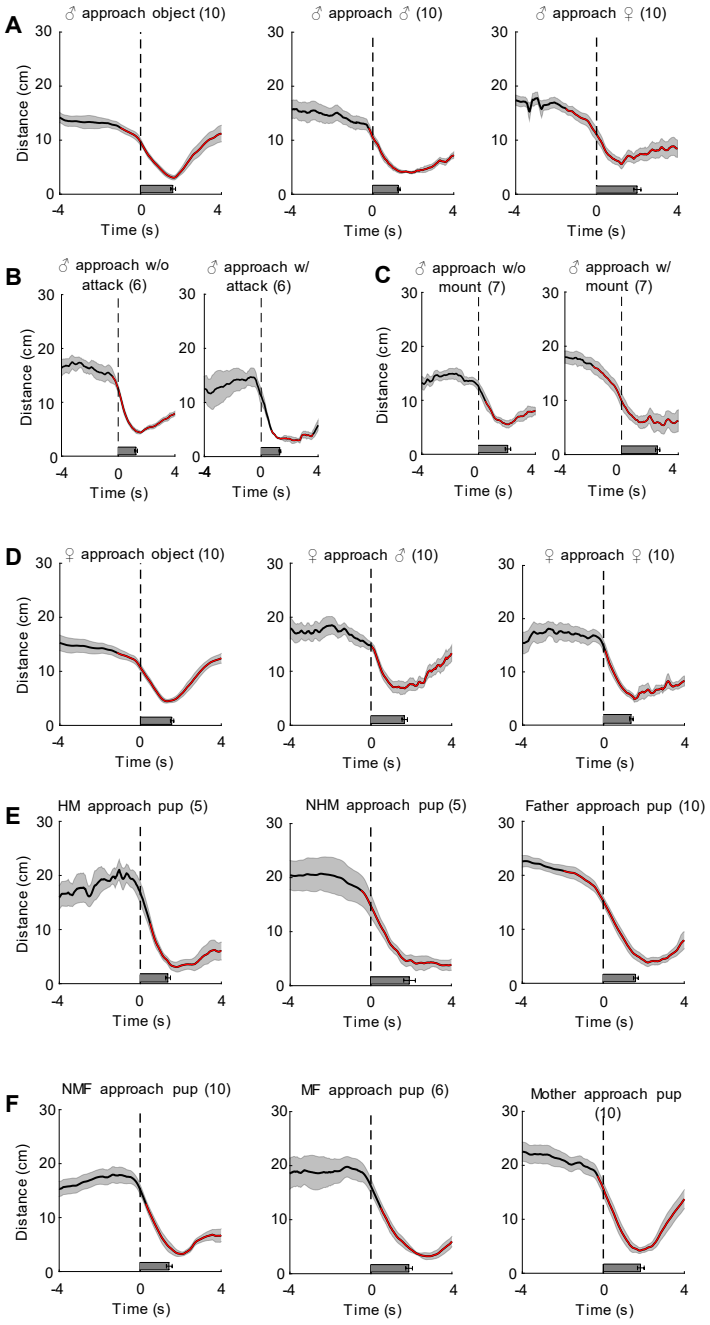


**Figure S5. Distance changes during approaching novel objects, male conspecific, female conspecific and pups. Related to Figures 1 and 7.**

(A-F) Averages PETHs aligned to onset of approach towards various targets in male (A-C, E) and female mice (D, F). Red indicates periods with significantly decreased distance from the baseline (-5 s to -3 s). Shaded area: ± S.E.M. Horizontal bars indicate the average duration of approach. Error bar: ± S.E.M. n = 10 (A), 6 (B), 7 (C), 10 (D), 5 (NHM, E), 5 (NNM, E) , 10 (father, E), 10 (NNF, F), 6 (NMF, F), 10 (Mother, F) mice.

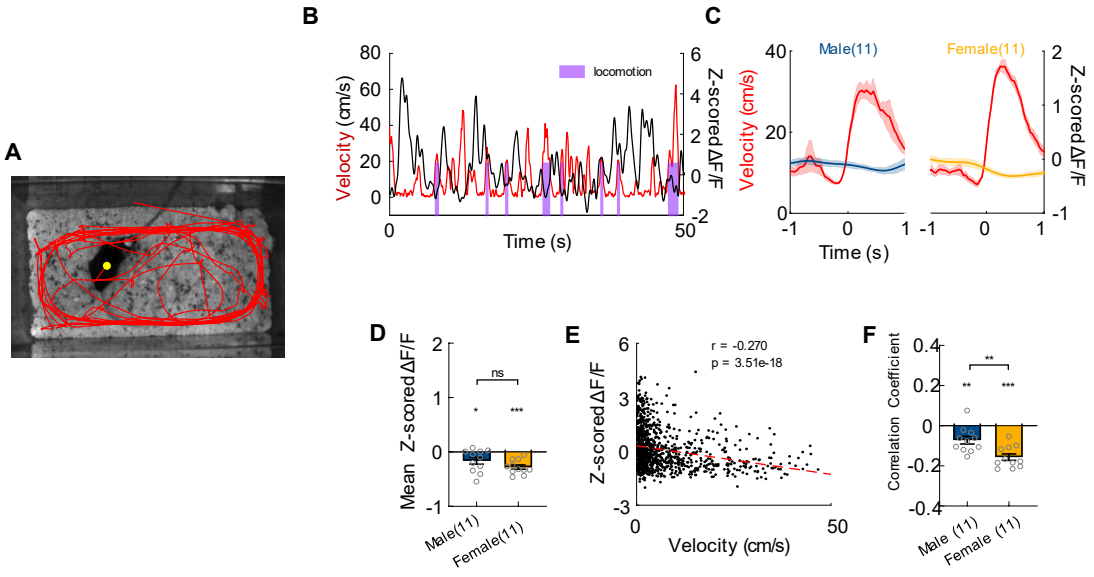


**Figure S6. No increase in DA activity at the onset of locomotion. Related to Figures 1 and 7.**
(A) Representative trajectory tracked by DeepLabCut.
(B) Representative traces of velocity (red) and Z-scored ΔF/F of GRAB$_{DA2m}$ (black) from an example male mouse. Color shades indicate periods of locomotion.
(C) Average PETHs of velocity (red) and GRAB$_{DA2m}$ signal aligned to the locomotion onset of males (left) and females (right). (n=11 male and 11 female mice.)
(D) Mean Z scored GRAB$_{DA2m}$ signal during 0-1s of all locomotion episodes. (n=11 male and 11 female mice.)
(E) A scatter plot showing the correlation between velocity and Z scored ΔF/F from one example female mouse. 1000 data points were randomly selected for visualization.
(F) Summary of correlation coefficient between velocity and Z scored ΔF/F. (n=11 male and 11 female mice.)

Error bars and shaded areas in (C-D, F) represent ± SEM. Numbers in bar graphs represent number of test animals. (D and F) Unparied t-test and one sample t test for individual groups; (E) Pearson correlation. *p<0.05, **p<0.01, and ***p<0.001.

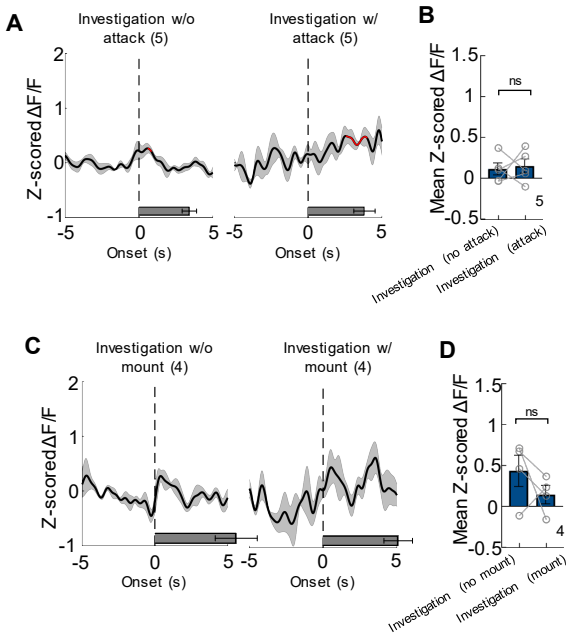


**Figure S7. No difference in DA response between investigation trials with and without immediately followed attack or mount. Related to Figure 2.**

(A) Average PETHs aligned to the onset of investigating male intruders without and with immediately followed attack in male mice.

(B) Mean Z scored GRAB$_{DA2m}$ responses during investigating male intruders without and with immediately followed attack in male mice. (n=5 mice.)

(C) Average PETHs aligned to the onset of investigating female intruders without and with immediately followed mount in male mice.

(D) Mean Z scored GRAB$_{DA2m}$ responses during investigating female intruders without and with immediately followed mount in male mice. (n=4 mice.)

Error bars and shaded areas represent ± SEM. Numbers in bar graphs represent number of test animals. (B and D) Paired t-test.

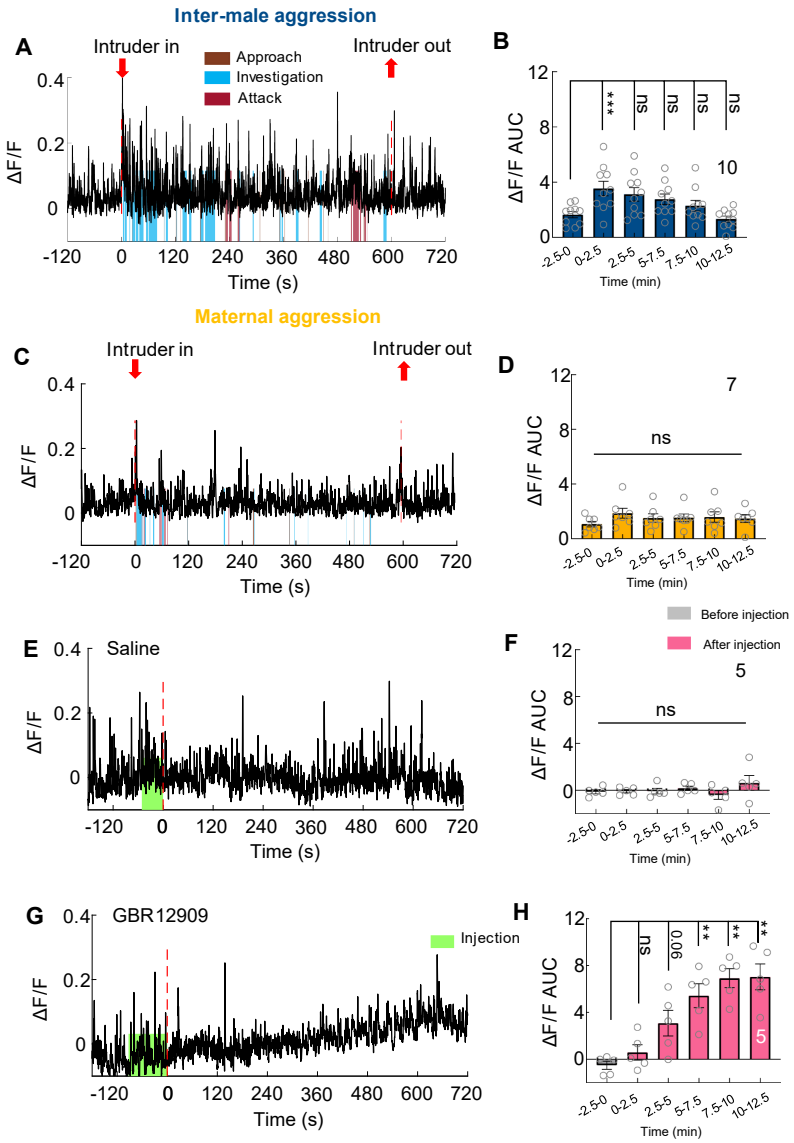


**Figure S8. Administration of DAT inhibitor induces a sustained increase in GRAB$_{DA2m}$ signal, but aggression does not. Related to Figure 5.**

(A) Representative trace of Z scored ΔF/F of GRAB$_{DA2m}$ before, during and after 10-min inter-male aggression.
(B) Accumulated DA signals before, during, and after inter-male aggression (n=10 male mice.)
(C) Representative trace of Z scored ΔF/F of GRAB$_{DA2m}$ before, during and after 10-min maternal aggression.
(D) Accumulated DA signals before, during, and after maternal aggression (n=7 female mice.)
(E) Representative traces of Z scored ΔF/F of GRAB$_{DA2m}$ before and after i.p. injection of saline.
(F) Accumulated DA signals before and after saline injection. (n = 5 mice.)
(G) Representative traces of Z scored ΔF/F of GRAB$_{DA2m}$ before and after i.p. injection of 20 mg/kg GBR 12909.
(H) Accumulated DA signals before and after GBR 12909 injection. (n = 5 mice.)

Error bars in (B, D, F, H) represent ± SEM. Numbers in bar graphs represent number of test animals. (B and D) Friedman test with Dunn's multiple comparison test; (F) Friedman test; (H) Tukey's multiple comparisons following one-way ANOVA. **p<0.01, and ***p<0.001.

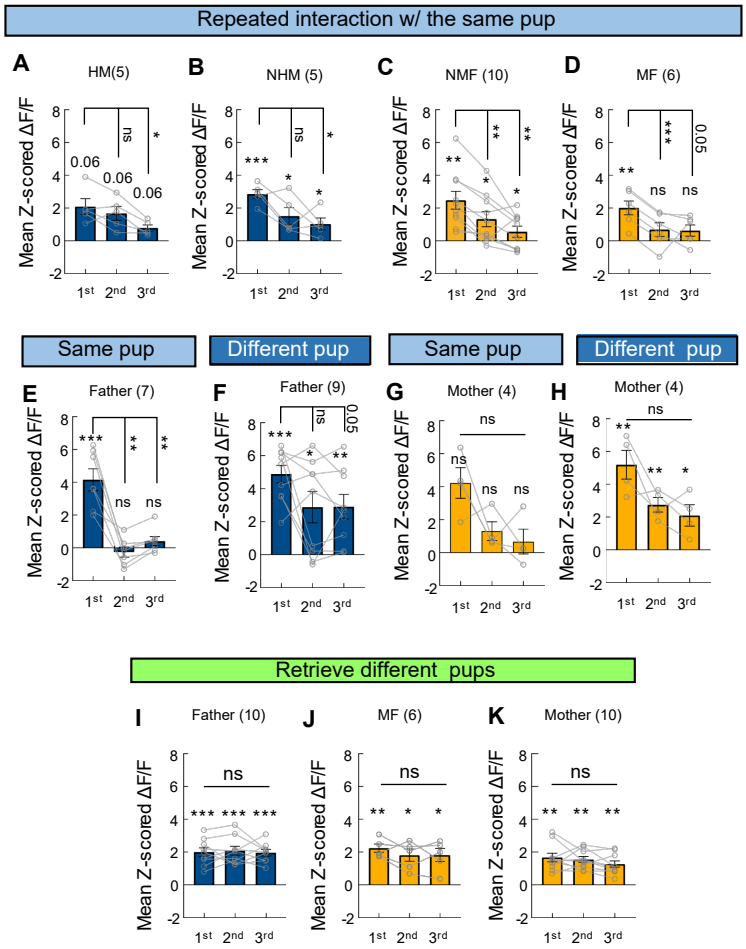


**Figure S9. Adaption of DA responses during pup-directed behaviors. Related to Figure 7.**
(A-D) Mean Z scored GRAB$_{DA2m}$ responses during the first three investigation towards the same pups in HMs (A, n=5), NHMs (B, n=5), NMFs (C, n=10) and MFs (D, n=6).
(E, F) Mean Z scored GRAB$_{DA2m}$ responses during the first three investigation towards the same pups (E, n =7) and the first investigation towards three different pups (F, n=9) in fathers.
(G, H) Mean Z scored GRAB$_{DA2m}$ responses during the first three investigation towards the same pups (E, n =4) and the first investigation towards three different pups (F, n=4) in mothers.
(I-K) Mean Z scored GRAB$_{DA2m}$ responses of the first retrieval towards three different pups in fathers (I, n=10), naïve maternal female (J, n=6) and mothers (K, n=10).

Error bars represent ± SEM. Numbers in bar graphs represent number of tested animals. (A) Friedman test with Dunn's multiple comparison test; (B-F) One-way ANOVA with Tukey's multiple comparisons test; (G, J) Friedman test; (H, I, K) One-way ANOVA; (A, G, J) One sample Wilcoxon test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR; (B-F, H, I, K) One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR. *p<0.05, **p<0.01, and ***p<0.001.