1 **Dopamine release in nucleus accumbens core during social behaviors in mice**

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Abstract

Social behaviors are among the most important and rewarding motivational behaviors. How dopamine, a "reward" signal, releases in the nucleus accumbens (NAc) during social behaviors has become a topic of interest for decades. However, limitations in early recording methods, such as microdialysis, prevented a complete understanding of moment-to-moment dopamine responses during social behaviors. Here, we employ a genetically encoded dopamine sensor, $GRAB_{DA2h}$, to record dopamine activity in the NAc core in mice and find acute changes in extracellular dopamine levels during all three phases of social behaviors: approach, investigation and consummation. Dopamine release during approach phase correlates with animal's motivation towards the conspecific whereas its release during consummatory phase signals the valence of the experience. Furthermore, dopamine release during sexual and aggressive behaviors shows sex differences that correlate with the potential value of those experiences. Overall, our results reveal rich and temporally precise motivation and value information encoded by NAc dopamine during social behaviors and beyond.

Introduction

The dopaminergic input from ventral tegmental area (VTA) to nucleus accumbens (NAc) is well known for its relevance to reward, but how so? One function of NAc dopamine release is to signal reward expectation which could be used as a motivating signal. In support of this hypothesis, altering the dopamine release artificially increases the willingness to work (Hamid et al., 2016; Phillips et al., 2003). The other function of NAc dopamine is to signal errors in reward prediction, providing a learning signal to guide future behavior (Bayer and Glimcher, 2005; Eshel et al., 2016). In cases when a reward arrives unexpectedly or in other words, the predicted value is zero, the reward prediction error is equivalent to the actual value of a stimulus. Thus, dopamine could signal the motivation to achieve a goal before the goal is obtained and the value of the goal upon obtaining it.

Social behaviors, such as sexual and parental behaviors, are among the most important and rewarding motivated behaviors (Trezza et al., 2011). The end goals of these behaviors, e.g., reproduction and fostering youngsters, are essential for the survival of a species. Thus, animals are innately motivated to engage in social behaviors and in some cases, willing to work hard for such opportunities. Social behaviors are intrinsically rewarding and can serve as unconditioned stimulus (US) for associative learning. For example, male and female rodents will

48   establish a preference for the context associated with copulation and postpartum female rats will

49   learn to lever press to gain access to a pup (Hauser and Gandelman, 1985; Tzschentke, 2007;

50   Wilsoncroft, 1968).

51   Many studies have been carried out since the early 90s to ask whether and how

52   dopamine level changes in NAc during social behaviors. Early microdialysis studies with a

53   temporal resolution of minutes revealed a gradual increase of dopamine in NAc in male rats that

54   copulated to ejaculation with receptive females (Damsma et al., 1992; Fumero et al., 1994;

55   Pfaus et al., 1990; Pleim et al., 1990; Wang et al., 1995; Wenkstern et al., 1993). Later

56   voltammetry recording with a higher temporal resolution (subsecond to second) found that

57   dopamine transients increase mainly during initial female encounter but not during

58   consummatory phase of sexual behaviors, such as deep thrust (Robinson et al., 2002). This is

59   surprising given that consummatory sexual actions are required for establishing conditioned

60   place preference – typically a dopamine-dependent learning process (Kippin and Pfaus, 2001;

61   Tenk et al., 2009). Recently, we used a genetically encoded dopamine sensor, namely $GRAB_{DA}$,

62   to optically record the dopamine signal in NAc in male mice with millisecond resolution and

63   found time-locked dopamine increase during each episode of thrust and ejaculation, supporting

64   a role of dopamine in encoding the hedonic value of sexual behaviors (Sun et al., 2018; Sun et

65   al., 2020). This result suggests that the early recording methods may lack the temporal

66   resolution and sensitivity to reveal the full details of dopamine responses during social

67   behaviors.

68   Similar to male sexual behaviors, dopamine increase in NAc during female sexual

69   behaviors and pup interactions have been reported using microdialysis and to a lesser extent

70   voltammetry (Afonso et al., 2008; Afonso et al., 2009; Afonso et al., 2013; Becker et al., 2001;

71   Champagne et al., 2004; Hansen et al., 1993; Jenkins and Becker, 2003; Kohlert and Meisel,

72   1999; Lavi-Avnon et al., 2008; Meisel et al., 1993; Mermelstein and Becker, 1995; Pfaus et al.,

73   1995; Shnitko et al., 2017). These studies unequivocally suggested that the dopamine release

74   in NAc is correlated with the animal's sexual or maternal motivation (Afonso et al., 2009;

75   Champagne et al., 2004; Kohlert and Meisel, 1999; Mermelstein and Becker, 1995). However,

76   considering the poor temporal resolution of microdialysis, dopamine responses during individual

77   behavioral events, especially those lasting for just a second or two, remain unclear. It also

78   remains to be determined whether the slow changes in dopamine levels truly reflects slow

79   dynamics of dopamine or whether they simply reflect methodological limitations. Furthermore,

80   nearly all studies on parental behaviors have focused on mothers probably due to the fact that

81 mother is the main care giver. One study showed that dopamine release to pups in NAc in naïve
82 and pair-bonded male prairie voles are quantitatively similar although pair-bonded males show
83 enhanced paternal behavior (Lei et al., 2017). These results raise the question of whether pup-
84 triggered dopamine release in males varies with the paternal state as is the case in females
85 (Afonso et al., 2008; Afonso et al., 2009; Champagne et al., 2004).

86 Aggression is another important type of motivational behaviors towards a social target.
87 Animals are willing to work for the opportunity to attack a conspecific, especially when the
88 outcome of attack is likely winning (Falkner et al., 2016; Fish et al., 2005; Fish et al., 2002; Fish
89 et al., 2008; Golden et al., 2017; Golden et al., 2019c; May and Kennedy, 2009). Winning also
90 supports associative learning: animals demonstrate a preference for the context where winning
91 occurs (Aleyasin et al., 2018; Golden et al., 2016; Golden et al., 2019c; Martinez et al., 1995;
92 Stagkourakis et al., 2018). Several studies investigated changes in dopamine levels related to
93 aggressive behaviors and found a slow and sustained increase in NAc (Beiderbeck et al., 2012;
94 van Erp and Miczek, 2000). However, unlike sexual and parental behaviors, dopamine rises
95 slowly and can remain elevated for over an hour (Beiderbeck et al., 2012; van Erp and Miczek,
96 2000). Whether the particularly slow dynamics reflect a qualitative difference in dopamine
97 release associated with aggressive vs. other social behaviors or whether they are merely
98 caused by low sampling rates remain to be investigated. Further complicating the findings is the
99 observation that NAc dopamine also increases after defeat (Tidey and Miczek, 1996). Since
100 defeat is clearly a negative experience, it suggests that dopamine increase during social
101 behaviors may signal salience instead of valence. Indeed, dopamine in NAc may signal both
102 salience and valence depending on the subregion. While dopamine increases to stimuli of both
103 positive and negative valence in the ventral NAc shell, dopamine only signals positive valence in
104 other parts of NAc (de Jong et al., 2019; Yuan et al., 2019).

105 Taken together, while dopamine release in NAc during social behaviors has been a topic
106 of interest for the last three decades, many questions remain unaddressed largely due to
107 technical limitations, heterogeneity of release pattern in NAc and differences in methodological
108 details across studies. Thus, the goal of our current study is to comprehensively investigate the
109 dopamine release in NAc during social behaviors using an optical recording method with fast
110 temporal resolution and cell type specificity. By recording from both males and females, our
111 study also examined potential sexual dimorphism in dopamine release during social behaviors.
112 Here, we specifically focused on NAc core given that this region is known to signal both

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113   motivation and reward, two important variables relevant for social behaviors (Hamid et al.,

114   2016).

115

116   **Results**

117   **Dopamine release in NAc core during approach and investigation**

118   To engage in social behaviors, animals need to first reach a social target. In laboratory

119   settings, this "approach" phase mainly consists of one animal walking towards another animal in

120   the same arena although more complicated movements, e.g., lever pressing or grid cross, can

121   also be involved (Golden et al., 2019c; Trezza et al., 2011; Wei et al., 2021). The seeking

122   behavior, most common approaching behavior, reflects an animal's motivation to a social target

123   and the relevant consummatory social actions. Upon gaining access to the social target, the

124   animal investigates it closely. After variable time of investigation, the consummatory action is

125   initiated. Although interaction with a social target alone can be rewarding, the consummatory

126   social actions are of higher hedonic value as social behavior-dependent associative learning

127   typically requires the successful completion of consummatory social actions (Trezza et al.,

128   2011).

129   To understand how dopamine levels change during different stages of social behaviors,

130   we performed optical recording of the dopamine signal by virally expressing Cre-dependent

131   (Cre-on) GRAB$_{DA2h}$, a genetically encoded fluorescent DA sensor, unilaterally or bilaterally in the

132   NAc core of Drd1-Cre mice, and implanting 400-μm multimode optic fiber(s) immediately above

133   the virus injection site(s) for light delivery and collection (Sun et al., 2018; Sun et al., 2020). In a

134   subset of animals, we also injected Cre-on or Cre-off GRAB$_{DA2h}$ virus into the contralateral side

135   of the NAc core to compare dopamine release onto D1R and non-D1R cells (**Figure 1a-1c**).

136   Control animals were injected with Cre-on GFP virus on both sides. Only animals with correct

137   fiber targeting are included in the analysis (**Figure 1 – figure supplement 1**).

138   In animals with bilateral GRAB$_{DA}$ expression in D1R cells of NAc core, we observed

139   highly correlated activity regardless of the presence of an intruder animal, suggesting

140   synchronized dopamine release in the two hemispheres (**Figure 1d, 1g and 1j**). Furthermore, in

141   animals with GRAB$_{DA}$ expression in D1R and non-D1R cells in the contralateral sides of NAc

142   core, we observed similarly highly correlated dopamine signals, suggesting that D1R and non-

143   D1R cells are likely sense similar level of dopamine fluctuation (**Figure 1e, 1h and 1j**). Of note,
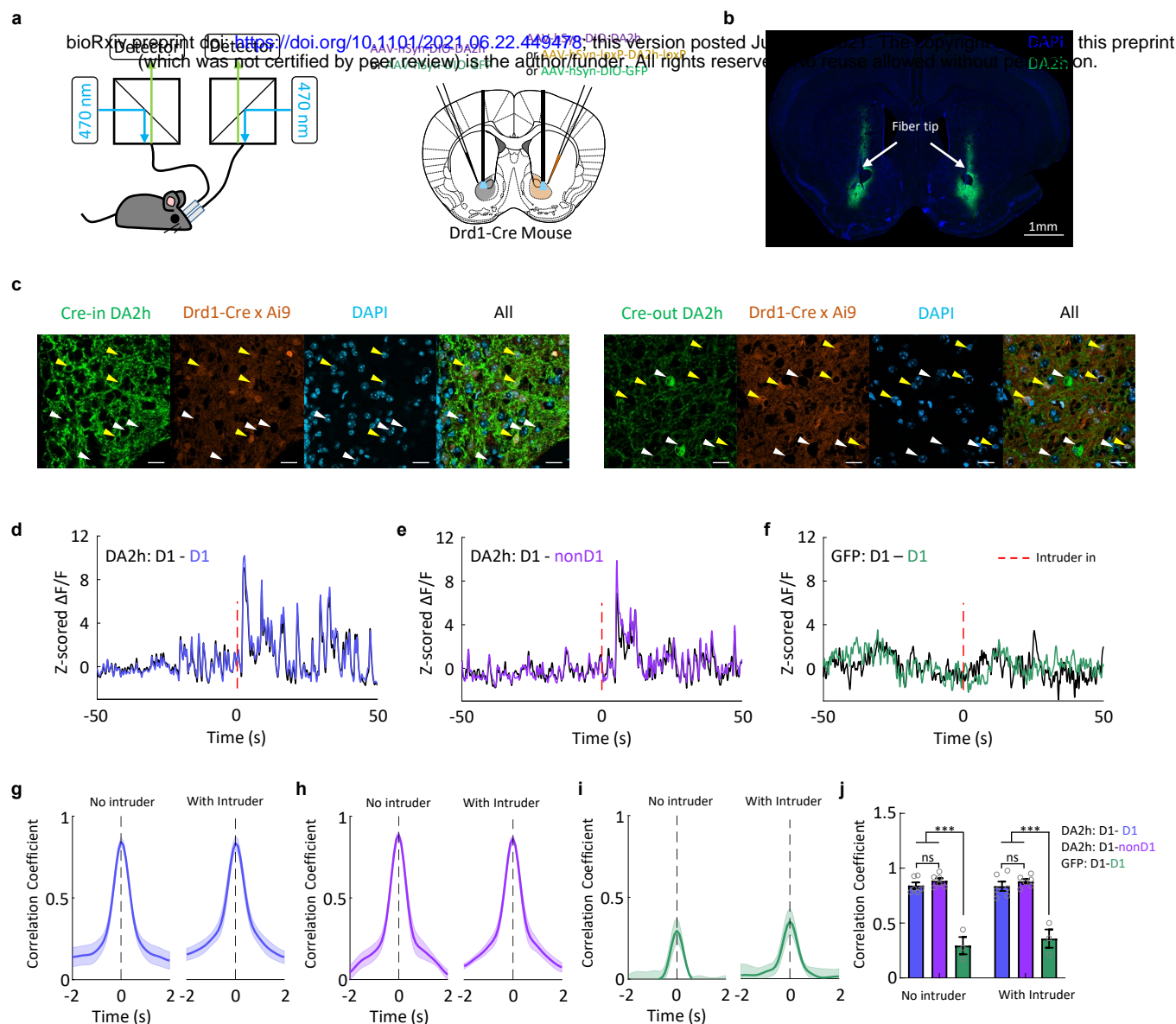
Figure 1

Figure 1. DA release sensed by D1 cells and non-D1 cells are highly correlated.

a, Schematic illustration of the recording setup and virus injection. Atlas image is adopted from (Franklin and Paxinos, 2013)

b, Representative image showing the expression of $GRAB_{DA2h}$ in both hemispheres and optic fiber tracks. (Scale bar, 1mm.)

c, Representative images showing the expression of $GRAB_{DA2h}$ 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 $GRAB_{DA2h}$ from D1R cells in both hemispheres. Time 0 indicates when a conspecific intruder is introduced. Similar results were observed from six mice.

e, Representative traces of $GRAB_{DA2h}$ from D1R cells in one hemisphere and that of non-D1R cells in the other hemisphere. Time 0 indicates when a conspecific intruder is introduced. Similar results were observed from six mice.

f, Representative traces of GFP from D1R cells in both hemispheres. Similar results were observed from three mice.

g, The time shifted correlation coefficient between the $GRAB_{DA2h}$ signals from two hemispheres before and after the introduction of an intruder. Shaded area: s.e.m. (n=6 mice.)

h, The time shifted correlation coefficient between $GRAB_{DA2h}$ in D1R and non-D1R cells in two different hemispheres before and after an intruder introduction. Shaded area: s.e.m. (n=6 mice.)

i, The time shifted correlation coefficient between GFP in D1R cells from different hemispheres before and after intruder introduction. Shaded area: s.e.m. (n=3 mice.)

j, Group summary of peak correlation coefficients of $GRAB_{DA2h}$ or GFP signals between hemispheres. Mean ± s.e.m overlaid for each group. (n= 6 mice for each $GRAB_{DA2h}$ group and n=3 for the GFP group. Bonferroni's multiple comparisons following two-way ANOVA. ***$p<0.001$.)

144   this does not rule out the possibility that D1R and non-D1R cells sense different dopamine

145   inputs at the microscopic level since it is beyond the spatial resolution allowed by our recording

146   method (Liu et al., 2018). The highly synchronized activity was not due to signal fluctuation

147   related to locomotion. In animals with bilateral GFP expression, we observed significantly lower

148   correlation in fluorescence signals between the two hemispheres (**Figure 1f, 1i and 1j**). Given

149   the highly correlated dopamine signal sensed by D1R and non-D1R cells, our subsequent

150   recordings were only obtained from D1R cells in NAc core (**Figures 2a-2c**).

151   During each recording session, we sequentially introduced a conspecific male, a female,

152   and a novel object into the tested mouse's home cage, each for 10 minutes or until ejaculation

153   was achieved in the case of opposite sex interaction. Upon introduction of a stimulus, the home-

154   cage animal approached the target quickly. For both male and female, the latency to approach a

155   social target was shorter than that towards an object (**Figures 2d-2e**). At the approach onset --

156   defined as the first step towards the target, the dopamine level was not elevated, suggesting

157   that dopamine increase was unlikely to play a role in initiating approach (**Figure 2f-2l**). During

158   approach, dopamine levels gradually increased (**Figure 2g-2h**). For males, the maximum

159   dopamine increase during approaching a female was significantly higher than that during

160   approaching an object (Female vs Object: $p = 0.04$, one-way ANOVA followed by Tukey's

161   multiple comparisons test) whereas females showed comparable dopamine increase during

162   approach towards all targets (**Figure 2j-2l**). At the offset of approach, which is often followed by

163   other social behaviors, e.g., investigation, the dopamine remained significantly elevated (**Figure**

164   **2j-2l**). We next asked whether the dopamine increase during approach adapted over trials and

165   found no consistent decrease especially towards a social target of the opposite sex (**Figure 2m-**

166   **o**).

167   To address whether the response during approach was due to movement per se, we

168   tracked the position of the test animal in the absence of an intruder and identified time points

169   when the animal initiated locomotion (**Figure 2 – figure supplement 1a-1b**). No increase in

170   dopamine activity was observed at the onset of locomotion (**Figure 2 – figure supplement 1c-**

171   **1d)** In fact, dopamine slightly but significantly decreased when the animal initiated locomotion

172   and the movement velocity was significantly negatively correlated with the dopamine signal,

173   suggesting that the dopamine increase during approach was not due to locomotion itself

174   (**Figure 2 – figure supplement 1c-1f**).

175   Upon reaching the target, test animals closely investigate the stimulus. For both females

176   and males, the average duration of investigation bout was longer towards social targets than
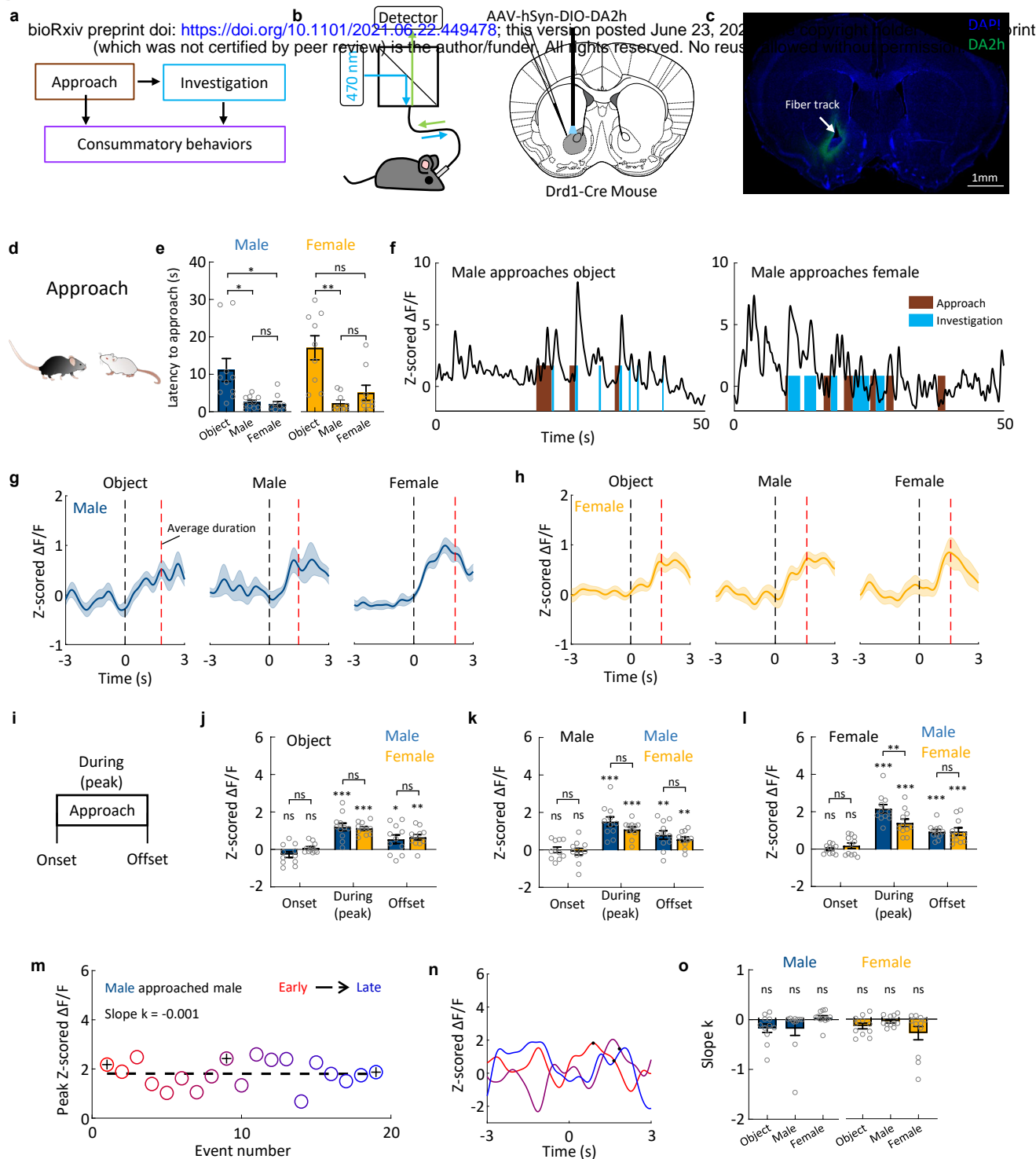
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Figure 2

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Figure 2. DA responses during approaching social and non-social targets.

a, Schematics illustrating different stages of social behaviors.

b, Schematics illustrating the experimental design.

c, Representative image showing the expression of GRAB$_{DA2h}$ and optic fiber track. (Scale bar, 1mm.)

d, A cartoon illustration of approaching behavior.

e, Group summary of the latency to approach towards different targets in male (blue) and female mice (yellow). Mean ± s.e.m overlaid with individual data points for each group. (n=10 male mice, Friedman test, p=0.007; n=10 female mice, Friedman test, p=0.006. Dunn's multiple comparisons post-tests revealed the difference within male and female groups, *p<0.05, **p<0.01)

f, Representative traces of Z scored ΔF/F of GRAB$_{DA2h}$ during social interaction from an example male mouse. Color shades indicate annotated behaviors. Similar results were observed from 11 male mice and 11 female mice.

g, Average post-event histograms aligned to the onset of approach for male mice. Shaded area: s.e.m. (n=11 mice.)

h, Average post-event histograms aligned to the onset of approach for female mice. Shaded area: s.e.m. (n=11 mice.)

i, Schematics showing the time periods used for characterizing DA responses related to approach.

j-l, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, and during approach a novel object (j), a male conspecific (k) and a female conspecific (l). (n=11 male mice and n=11 female mice. One sample t test followed by FDR correction (FDR = 0.05) to reveal significant responses. *p<0.05, **p<0.01, ***p<0.001. Bonferroni's multiple comparisons following two way ANOVA revealed no difference in responses between males and females. **p<0.01)

m, A scatter plot showing peak response during repeated approach trials from one example male-male encounter session.

n, Representative GRAB$_{DA2h}$ traces from early and late approach bouts. Black dots indicate the offset of behavior episodes.

o, Summary of slope k of GRAB$_{DA2h}$ responses over repeated approach episodes. (n=11 male mice and n=11 female mice. One sample Wilcoxon test followed by FDR correction (FDR = 0.05).

177   that towards novel objects (**Figure 3b**). Dopamine levels at the onset of investigation were

178   already significantly elevated above the baseline, likely due to the dopamine increase during

179   approach (**Figures 3c-3m**). During investigation, the dopamine further rose transiently before it

180   dropped (**Figures 3d-3m**). At the offset of social investigation, the dopamine level largely

181   returned to the baseline level in males and is slightly elevated in females (**Figure 3k-3m**). The

182   peak dopamine increases during investigation of social targets were significantly higher than

183   that during object investigation (Male: male vs object: p<0.001; female vs object: p = 0.009.

184   Female: male vs object: p = 0.01; female vs object: p = 0.005. One-way ANOVA followed by

185   Tukey's multiple comparisons test). Dopamine response during investigation adapted quickly

186   regardless of the targets (**Figures 3n-3p**). On average, dopamine increase during the tenth

187   investigation bout was around 20-40% of the first bout (**Figure 3q**).

188

189   **Dopamine release in NAc core during sexual behaviors**

190   During male and female encounters, after a period of investigation, male mice initiate

191   mounting towards the females. We used sexually experienced males and they often attempt to

192   mount females regardless of the female's receptivity level. In these males, the dopamine

193   dynamic during mounting towards receptive and non-receptive females was similar: it was

194   elevated at the mounting onset, continued to rise transiently during mounting before dropping

195   below the baseline at the offset of mounting if the mounting did not transition into intromission

196   (**Figures 4a-4e and 4j-4l**). Furthermore, the dopamine suppression remained for at least one

197   second after mounting termination (**Figures 4k-4l**). A different pattern of dopamine release was

198   observed in females. Both non-receptive and receptive females showed no change in dopamine

199   level at the onset of being mounted (**Figures 4d-4e and 4k-4l**) When being mounted, non-

200   receptive female showed a decrease in dopamine which was maintained throughout the

201   behavior and for at least one second after male stopped mounting (**Figures 4c-4d and 4k**). In

202   contrast, when being mounted, receptive females showed a transient increase in dopamine

203   which returned to baseline at the offset (**Figures 4c, 4e and 4l**)

204   If the females are receptive, males advance mounting to intromission, a rhythmic pelvic

205   movement presumably resulting in penile insertion. At the onset of intromission (the same

206   moment as mounting offset), dopamine level was significantly elevated and continued to rise for

207   approximately one second before it gradually decreased to the baseline level at the offset of

208   intromission (**Figures 4b, 4f-4g and 4m**). If the intromission did not transition to ejaculation,
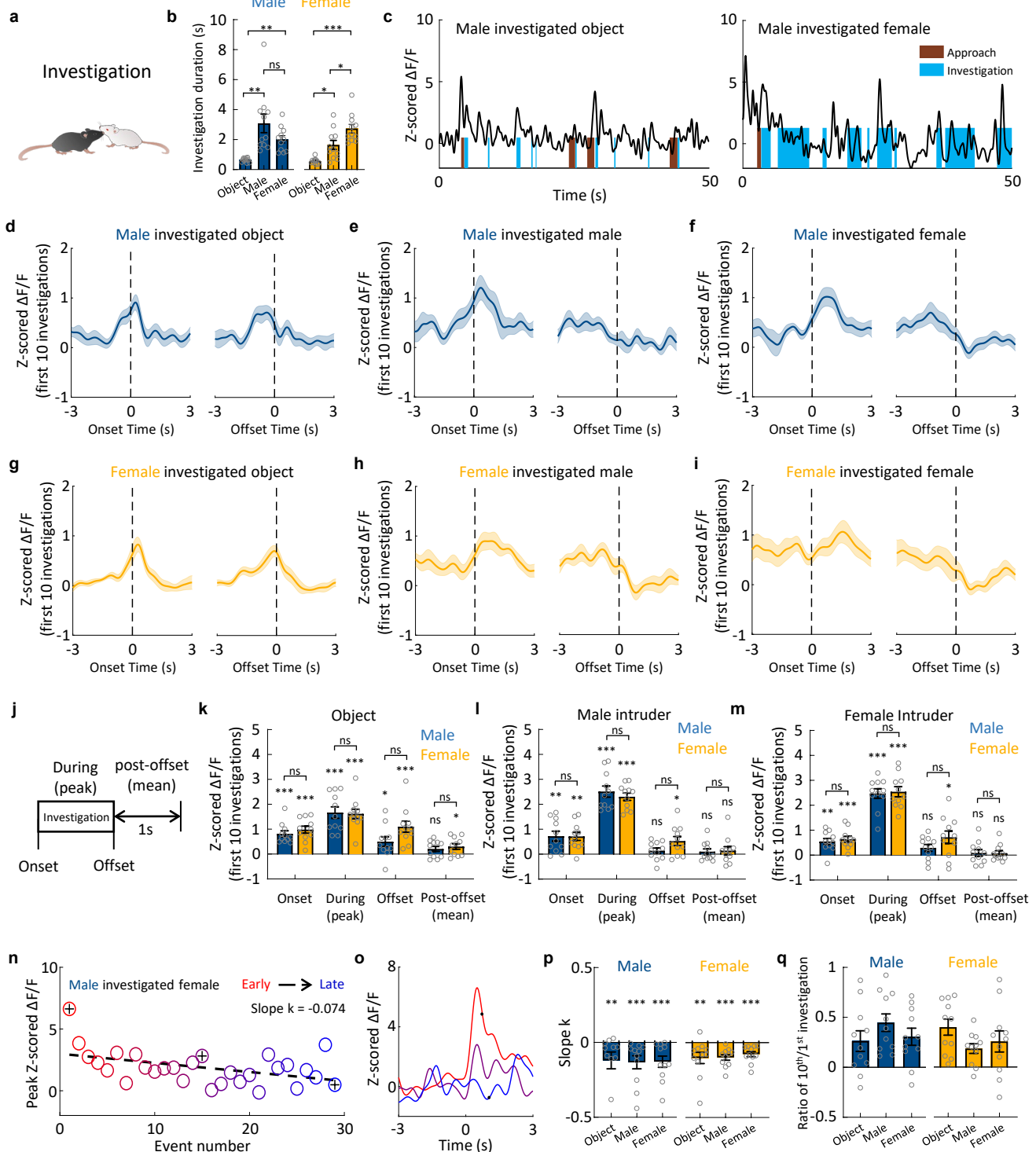
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Figure 3

Figure 3. DA responses during male and female investigatory behaviors.

a, A cartoon illustration of social investigation.

b, Summary of average investigation durations towards various targets in male and female mice. Mean $\pm$ s.e.m overlaid with individual data points for each group. (n=11 male mice, one-way ANOVA, $F_{(1.203, 12.03)}=9.151$, $p=0.008$; n=11 female mice, one-way ANOVA, $F_{(1.938,19.38)}=24.43$, $p<0.001$. Tukey's multiple comparisons post-tests revealed the differences within male and female groups, $*p<0.05$, $**p<0.01$, $***p<0.001$.)

c, Representative traces of Z scored ΔF/F of GRAB$_{DA2h}$ during object and female interaction from an example male mouse. Similar results were observed from 11 male mice and 11 female mice.

d-i, Average post-event histograms aligned to the onset (left) and offset (right) of investigation towards object (**d**), a male intruder (**e**) and a female intruder (**f**). Shaded area: s.e.m. (n=11 male mice and 11 female mice.)

j, Schematics showing the time periods used for characterizing DA responses related to investigation.

k-m, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, during and after investigating a novel object (**k**), a male mouse (**k**) and a female mouse (**m**). Signal changes across bouts. Mean $\pm$ s.e.m overlaid with individual data points for each group. (n=11 male mice and n=11 female mice. One sample t test followed by FDR correction revealed significant responses, $*p<0.05$, $**p<0.01$, $***p<0.001$. Bonferroni's multiple comparisons following two-way ANOVA revealed no difference between male's and female's responses.)

n, A scatter plot showing peak responses during repeated investigation trials from one example session of male-female encounter.

o, Representative GRAB$_{DA2h}$ traces from early and late investigation bouts. Black dots mark the end of investigation.

p. Summary of slope k of GRAB$_{DA2h}$ responses over repeated investigation events. Mean $\pm$ s.e.m overlaid with individual data points for each group. (n=11 male mice and n=11 female mice. One sample Wilcoxon rank test followed by FDR correction revealed significant adaptations. $**p<0.01$, $***p<0.001$.)

q, The ratio of peak DA signals during the tenth investigation bout to that of the first bout. Mean $\pm$ s.e.m overlaid individual data points for each group. (n=11 male mice and n=11 female mice.)

12

209   dopamine decreased below the baseline for at least one second after intromission terminated

210   (**Figures 4g and 4m**). In females, while the dopamine level was also elevated at the onset of

211   being intromitted, it gradually and monotonically decreased and returned to the baseline at the

212   offset of being intromitted without further post-behavior change (**Figures 4c, 4g and 4m**). After

213   repeated intromission, males achieve ejaculation which is characterized as a sudden cessation

214   of all movements. Ejaculation resulted in the highest dopamine release in both males and

215   females, but there was a noticeable temporal difference (**Figures 4b-4c, 4h-4i and 4n**). While

216   dopamine increased sharply in males at the onset of ejaculation, the dopamine increase in

217   females occurred approximately 2 s after the male ejaculation (**Figure 4i**). Within 5 seconds

218   after the onset of male ejaculation, dopamine reached its peak level and then gradually

219   decreased (**Figure 4h**). At the ejaculation offset, defined as the moment when male resumes

220   movement, dopamine levels have returned to baseline and no further post-ejaculation dopamine

221   changes were observed (**Figure 4n**). Lastly, in contrast to the gradual decrease of dopamine

222   release during investigation, the peak dopamine release during sexual actions was either

223   unchanged or slightly increased over repeated trials (**Figure 4o**).

224

Dopamine release in NAc core during aggressive behaviors

226   When the males encounter male intruders, they initiate attack after a period of

227   investigation. At the onset of each attack, dopamine levels were already elevated, likely due to

228   increase during approach or investigation (**Figures 5b-5c, 5f**). During attack, dopamine rose

229   transiently, reached peak and then dropped (**Figures 5b-5c and 5f**). At the offset of attack,

230   dopamine levels remained above the baseline but quickly returned to pre-attack levels within

231   one second (**Figure 5b-5c and 5f**). The maximum dopamine increase during male aggression

232   was quantitatively comparable to the dopamine increase during sexual intercourse and

233   consuming palatable food (i.e. peanut butter), supporting the notion that aggression could be

234   rewarding (**Figure 5- figure supplement 1**).

235   Non-lactating female mice typically show little aggression towards conspecific intruders.

236   During lactation, however, females show a marked increase in aggression towards all intruders

237   except pups, a phenomenon known as maternal aggression (St John and Corning, 1973).

238   Maternal aggression contains both offensive and defensive attacks and its main purpose is to

239   protect the young (Ferrari et al., 2000; Flannelly and Flannelly, 1985). In comparison to male

240   aggression, peak dopamine increase during female attack was significantly lower (**Figures 5f**).

13

Figure 4

14

Figure 4. DA responses during male and female sexual behaviors.

a, A cartoon illustration of mouse mating.

b-c, Representative traces of Z scored ΔF/F of GRAB$_{DA2h}$ during various stages of mating in male and female mice. Similar results were observed from 11 male mice (**b**), 11 unreceptive female mice(**c, left**), and 9 receptive female mice (**c, right**).

d, Average post-event histograms aligned to the onset (left) and offset (right) of the male mounting events, when the female mice were unreceptive. Shaded area: s.e.m. (n=11 male mice and n=11 female mice.)

e-h, Average post-event histograms aligned to the onset (left) and offset (right) of the indicated mating events, when the female mice were receptive. Shaded area: s.e.m. (n=11 male mice and n=9 female mice.)

i, Bar graphs showing the latency from the onset of ejaculation to the moment when DA signals start to increase. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice and n=9 female mice, Mann-Whitney test, ***p<0.001)

j, Schematics showing the time periods used for characterizing DA responses associated with mating.

k. Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, during and after male mounting when the females were non-receptive. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice and n=11 female mice. One sample t test followed by FDR correction to reveal significant responses, *p<0.05, **p<0.01, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed differences in responses between males and females, ***p<0.001.)

l, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, during and after male mounting that was not followed by intromission. The female mice were receptive. Mean ± s.e.m overlaid with individual data points for each group. (n=8 male mice and n=7 female mice. One sample t test followed by FDR correction to reveal significant responses, *p<0.05, **p<0.01, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed differences in responses between males and females, *p<0.05, **p<0.01, ***p<0.001.)

m, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, during and after male intromission that was not followed by ejaculation. The female mice were receptive. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice and n=9 female mice. One sample t test followed by FDR correction to reveal significant responses, **p<0.01, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed differences in responses between males and females, *p<0.05, ***p<0.001.)

n, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, during and after male ejaculation. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice and n=9 female mice. One sample t test followed by FDR correction to reveal significant responses, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed no difference in responses between males and females.)

o, Summary of slope k of GRAB$_{DA2h}$ responses over repeated mating events. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice, n=11 unreceptive female mice, and n=9 receptive female mice. One sample t test followed by FDR correction revealed no significant adaptation in response magnitude over trials.)

15

241 At the offset of female attack, dopamine levels returned to baseline (**Figures 5b, 5d and 5f**).

242 The sex difference in dopamine release during attack was not due to difference in attack

243 duration: in both males and females, each attack bout lasted approximately 2 seconds (**Figure**

244 **5g**).

245   Previous microdialysis measurements suggest a sustained elevation of dopamine levels

246 after male-male confrontation (Beiderbeck et al., 2012; van Erp and Miczek, 2000). We thus

247 analyzed the accumulated dopamine signal before, during and after the inter-male aggression

248 and maternal aggression (**Figure 5h-5i**). While dopamine levels were significantly elevated

249 during initial encounter with the intruder in males, we found no significant difference in

250 dopamine levels between the pre-intruder and post-intruder periods (**Figure 5h**). To ensure that

251 our recording method can detect a sustained increase in dopamine, we i. p. injected dopamine

252 transporter (DAT) inhibitor (GBR12909 20 mg/kg) in a subset of animals. DAT mediates

253 dopamine reuptake and its blockage is known to cause an elevation of extracellular dopamine

254 concentration (Westerink et al., 1987). Ten minutes after injecting DAT inhibitor but not saline,

255 the GRAB$_{DA2h}$ signal showed a consistent and sustained upward shift, supporting that our

256 method was capable of detecting a general increase in dopamine level (**Figure 5 – figure**

257 **supplement 2**).

258

259 **Dopamine responses in NAc during pup-directed behaviors**

260   We also examined the dopamine release during pup-directed behaviors. Pup-directed

261 behaviors are unique in that they show qualitative differences based on the motivational state of

262 the animals. Naïve male mice either ignore or attack pups while fathers readily care for the

263 young (e.g., quickly retrieve a wandering pup back to the nest; (Perrigo et al., 1990)). Virgin

264 female mice from laboratory stocks typically do not attack pups but many show avoidance

265 (Mann et al., 1983). With repeated pup exposure, however, virgin females can stop avoiding

266 pups and care for them, a process known as pup sensitization (Rosenblatt, 1967). Mothers are

267 strongly motivated for pups. They not only care for pups for most of the day but are willing to

268 trade other high value rewards for pups (Trezza et al., 2011). Thus, the varying motivation to

269 pups across different reproductive stage makes pups a particular useful social stimulus for

270 evaluating the neural representation of motivation.

271   During recording, we introduced 5 - 7 pups, one at time, into the home cage of the

272 recorded mouse for a total of 10 minutes. Among the 10 recorded naïve males, 5 ignored the
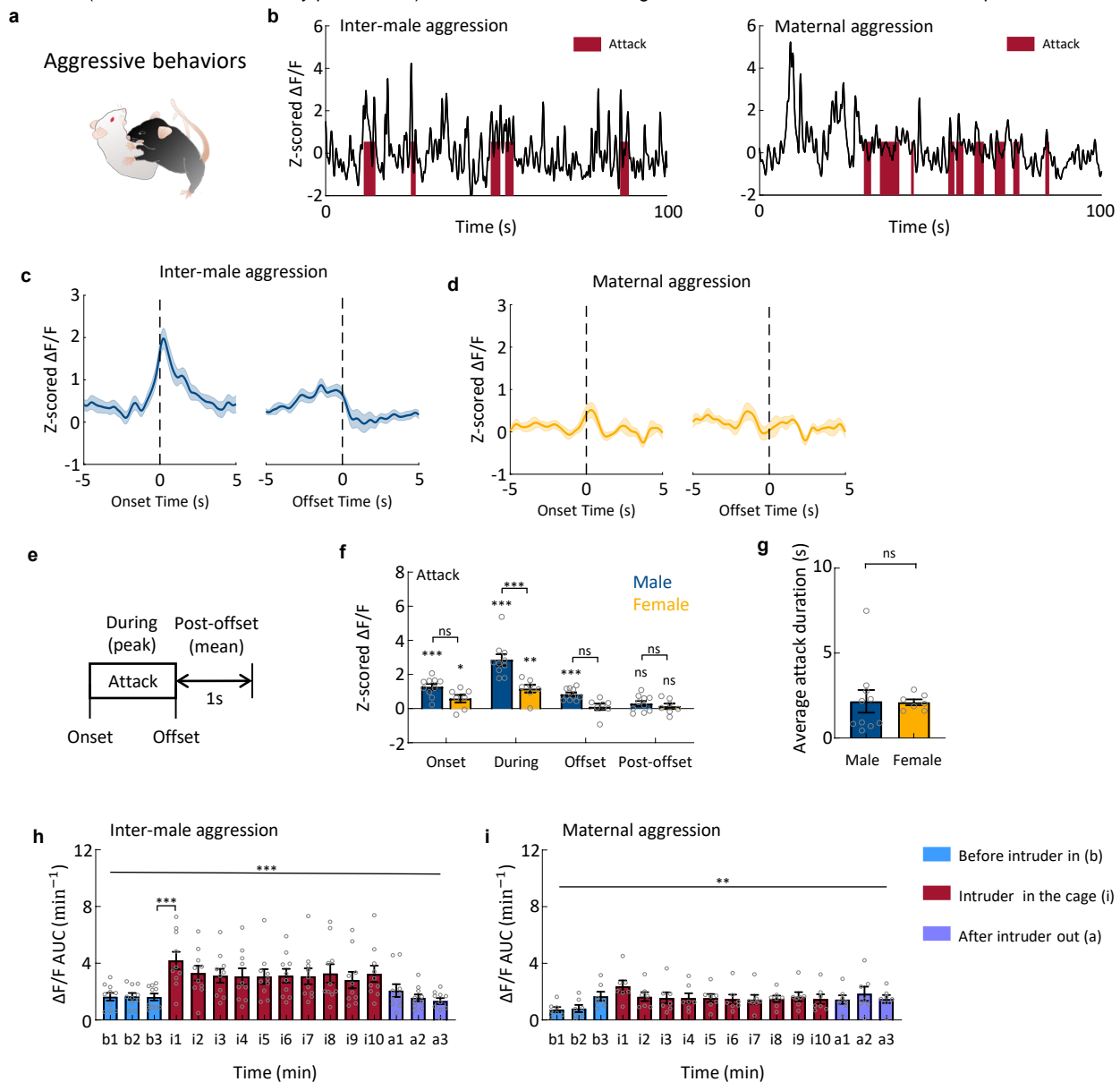
Figure 5

Figure 5. DA responses during aggressive behaviors.

a, A cartoon illustration of mouse attack.

b, Representative traces of Z scored ΔF/F of GRAB$_{DA2h}$ during inter-male aggression and maternal aggression. Similar results were observed from 10 male mice and 7 female mice.

c-d, Average post-event histograms aligned to the onset (left) and offset (right) of attack. Shaded area: s.e.m. (n=10 male mice and n=7 female mice.)

e, Schematics showing the time periods used for characterizing DA responses related to attack.

f, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, during and after attack. Mean ± s.e.m overlaid with individual data points for each group. (n=10 male mice and n=7 female mice. One sample t test followed by FDR revealed significant responses, *p<0.05, **p<0.01, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed differences in responses between males and females, ***p<0.001.)

g, Bar graph showing average attack durations for male and female mice. Mean ± s.e.m overlaid with individual data points for each group. (n=10 male mice and n=7 female mice, Mann-Whitney test, p=0.94.)

h. Accumulated DA signals before, during, and after the male-male interaction. Mean ± s.e.m overlaid with individual data points for each group. (n = 11 mice, Friedman test, p<0.001. Dunn's multiple comparisons revealed the significant difference between b3 and i1, ***p<0.001.)

i, Accumulated DA signals before, during, and after the interaction between the lactating female with a female intruder. Mean ± s.e.m overlaid with individual data points for each group. (n = 9 mice, Friedman test, p=0.002. Dunn's multiple comparisons revealed no difference between b3 and any the other time points.)

17

273   pups (non-hostile males, NHM) and 5 attacked and killed pups (hostile males, HM). 10 (5 non-

274   hostile and 5 hostile males) of those males were then paired with a female and became a father

275   (father males, FM). We then repeated the recording of the test males between postpartum day 2

276   and 5 of their cohoused females. During recording, all fathers showed paternal behaviors

277   including pup retrieval.

278   The temporal dynamics of dopamine release during pup approach were similar among

279   naïve hostile males, naïve non-hostile males, and fathers: dopamine was not elevated at the

280   onset of approach and increased during approach and reached the maximum level towards the

281   end of approach (**Figures 6a-6c, 6f and 6h**). However, there was a significant difference in

282   peak dopamine release during pup approach among the three groups of males. Specifically,

283   dopamine increase was the highest in fathers and the lowest in hostile males (**Figure 6k**). Upon

284   reaching the pup, the males closely interacted with the pups, including investigating, licking, and

285   grooming the pups. As pups were often occluded by the body of the male, we did not attempt to

286   distinguish these behaviors. During close interaction, dopamine level transiently increased and

287   then returned to the baseline in all recorded animals and the peak level did not differ among

288   groups (**Figure 6d, 6g, 6i and 6l**). In hostile males, dopamine level also transiently increased

289   during infanticide although the peak increase was slightly lower than that during retrieval in

290   fathers ($p = 0.05$, unpaired t test) (**Figures 6e, 6j and 6m-6n**). Interestingly, we noticed a

291   transient dopamine suppression below the baseline after fathers completed retrieval and

292   disengaged with the pups (**Figures 6n**).

293   Similarly, we examined dopamine release in NAc core in both naïve and lactating

294   females (**Figure 7a**). 6/16 virgin females showed pup retrieval (naïve maternal females, NMF,

295   latency to retrieve: 254.2 ± 108.5 s), while the rest of females only investigated and groomed

296   the pups (naïve non-maternal females, NNF). All lactating females (10/10, mother females, MF)

297   showed pup retrieval quickly after pup introduction (Latency to retrieve (mean ± STD): 23.51 ±

298   20.9 s). Dopamine release patterns during pup-interaction in females were qualitatively similar

299   to that in males. Specifically, dopamine increased during pup approach, investigation, and

300   retrieval **(Figure 7)**. The level of increase during pup approach was higher in females that

301   showed maternal behaviors than those not (**Figure 7k**).

302   Overall, these results suggest that dopamine increased acutely in NAc core during all

303   phases of social behaviors. Dopamine increase during approach may signal the motivation

304   towards a social target while dopamine increase during the consummatory social actions may

305   signal the hedonic value of the behavior.
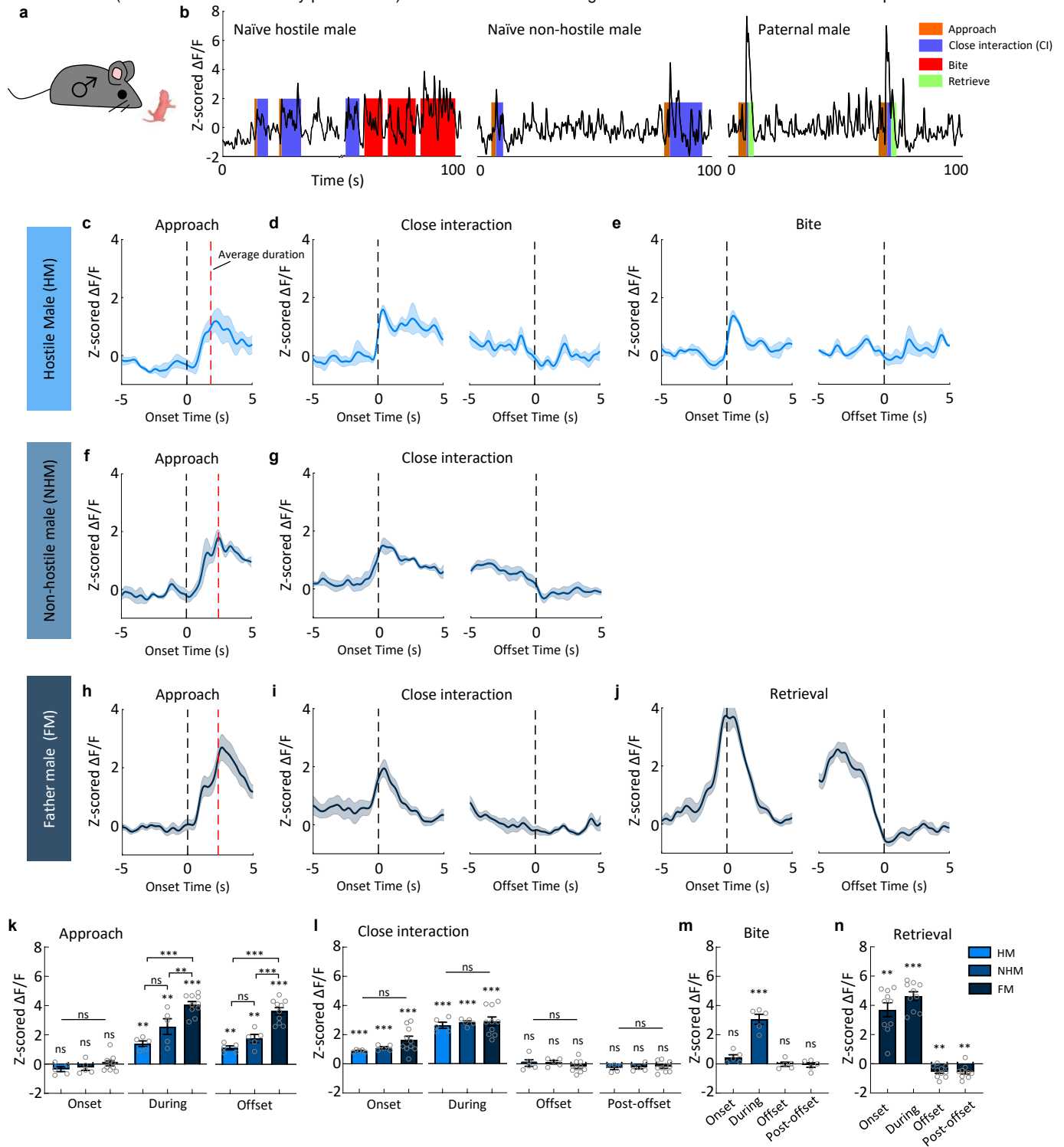
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Figure 6

Figure 6. DA responses during pup-directed behaviors in naïve males and fathers.

a, A cartoon illustration showing an adult male approaches a pup.

b, Representative traces of Z scored ΔF/F of GRAB$_{DA2h}$ during pup-directed behaviors in naive hostile males, naive non-hostile males, and fathers. Similar results were observed for 5 naive hostile male mice, 5 naive non-hostile male mice, and 10 father male mice.

c-e, Average post-event histograms aligned to the onset (left) and offset (right) of the indicated pup-directed behaviors for the naive hostile male mice. Shaded area: s.e.m. (n=5 mice.)

f-g, Average post-event histograms aligned to the onset (left) and offset (right) of the indicated pup-directed behaviors for the naive non-hostile male mice. Shaded area: s.e.m. (n=5 mice.)

h-j, Average post-event histograms aligned to the onset (left) and offset (right) of the indicated pup-directed behaviors for fathers. Shaded area: s.e.m. (n=10 mice.)

k, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, and during approach a pup for the naive hostile males, naive non-hostile males, and fathers. Mean $\pm$ s.e.m overlaid with individual data points for each group. (n=5 for naive hostile male mice, n=5 for naive non-hostile male mice, and n=10 for fathers. One sample t test followed by FDR correction to reveal responses, **p<0.01, ***p<0.001. Tukey's multiple comparisons following one way ANOVA revealed the differences in responses between groups at the indicated moments, *p<0.05, **p<0.01, ***p<0.001.)

l, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, during and after close interaction with a pup for the naive hostile male mice, naive non-hostile male mice, and fathers. Mean $\pm$ s.e.m overlaid with individual data points for each group. (n=5 for naive hostile male mice, n=5 for naive non-hostile male mice, and n=10 for father male mice. One sample t test followed by FDR correction to reveal significant responses, *p<0.05, **p<0.01, ***p<0.001. Tukey's multiple comparisons following one-way ANOVA revealed no differences between groups at any moment. *p<0.05, **p<0.01, ***p<0.001.)

m, Summary of Z scored GRAB$_{DA2h}$ responses at the indicated moments of biting for the naive hostile male. Mean $\pm$ s.e.m overlaid with individual data points for each group. (n=5 male mice, one sample t test followed by FDR correction to reveal significant responses, ***p<0.001.)

n, Summary of Z scored GRAB$_{DA2h}$ responses at the indicated moments of retrieval for the fathers. Mean $\pm$ s.e.m overlaid with individual data points for each group. (n=10 male mice, one sample t test followed by FDR correction to reveal significant responses, *p<0.05, **p<0.01, ***p<0.001.)
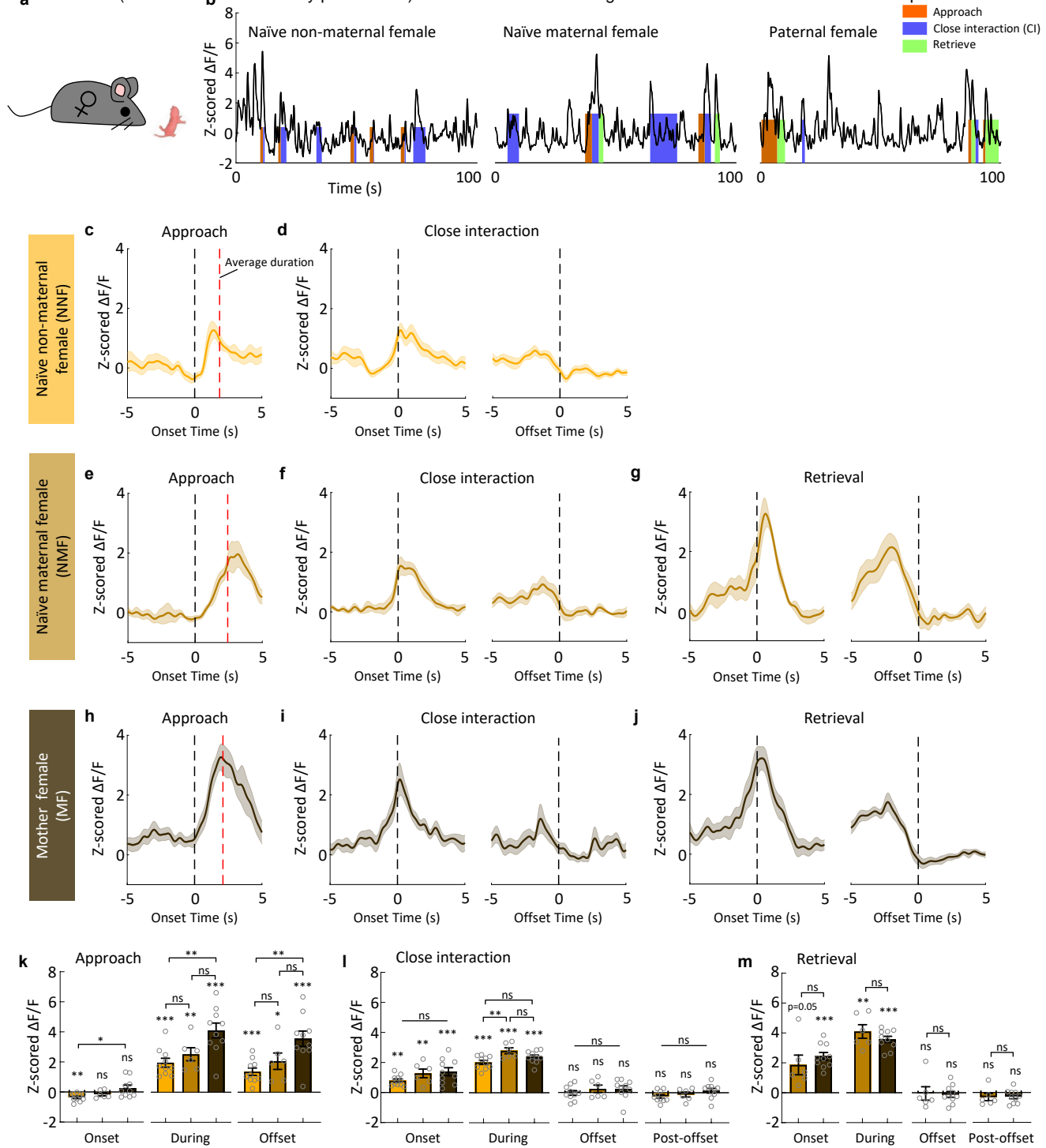
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Figure 7

Figure 7: DA responses during pup-directed behaviors in naive females and mothers.

a, A cartoon illustration showing an adult female approaches a pup.

b, Representative traces of Z scored ΔF/F of GRAB$_{DA2h}$ during pup-directed behaviors in naive non-maternal female, naive maternal female, and paternal female mice. Similar results were observed from 10 naive non-maternal female mice, 6 naive maternal female mice, and 10 mother mice.

c-d, Average post-event histograms aligned to the onset (left) and offset (right) of the indicated pup-directed events for the naive non-maternal female mice. Shaded area: s.e.m. (n=10 mice.)

e-g, Average post-event histograms aligned to the onset (left) and offset (right) of the indicated pup-directed events for the naive maternal female mice. Shaded area: s.e.m. (n=6 mice.)

h-j, Average post-event histograms aligned to the onset (left) and offset (right) of the indicated pup-directed events for mother mice. Shaded area: s.e.m. (n=10 mice.)

k, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, and during approach a pup of the naive non-maternal female mice, naive maternal female mice, and paternal female mice. Mean ± s.e.m overlaid with individual data points for each group. (n=10 naive non-maternal female mice, n=6 naive maternal female mice, and n=10 mother female mice. One sample t test followed by FDR correction to reveal significant responses, *p<0.05, **p<0.01, ***p<0.001. Tukey's multiple comparisons following one-way ANOVA revealed the differences between groups at the indicated moments, *p<0.05, **p<0.01, ***p<0.001.)

l, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, during and after close interaction with a pup for the naive non-maternal female mice, naive maternal female mice, and paternal female mice. Mean ± s.e.m overlaid with individual data points for each group. (n=10 naive non-maternal female mice, n=6 naive maternal female mice, and n=10 mother female mice. One sample t test with FDR correction revealed significant responses, *p<0.05, **p<0.01, ***p<0.001. Tukey's multiple comparisons following one-way ANOVA revealed the differences between groups at the indicated moments, **p<0.01.)

m, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, during and after retrieving a pup for the naive maternal females and paternal females. Mean ± s.e.m overlaid with individual data points for each group. (n=6 naive maternal female mice, and n=10 mother female mice. One sample t test with FDR correction revealed significant responses,*p<0.05, **p<0.01, ***p<0.001. Unpaired t tests revealed no differences between groups of mice.)

306

Dopamine responses towards aversive experience

307

308   Do dopamine levels increase during all social behaviors or only the behaviors with

309   positive valence? To address this question, we recorded GRAB$_{DA2h}$ signal during defeat, a

310   robust negative social experience. During recording, an aggressive Swiss Webster (SW) male

311   mouse was introduced into the home cage of the recorded male for 10 minutes. Upon being

312   attacked, the recording male mouse attempted to escape from the aggressor by flight and

313   pushing and did not attempt to attack back. After a couple bouts of fighting, the recording male

314   was clearly defeated: the SW intruder initiated all the attacks and the recorded mice stayed in

315   corners and showed submissive postures. In contrast to the dopamine increase during attack,

316   dopamine consistently and transiently decreased during defeat (**Figure 8b-d**). Interestingly, we

317   noticed a rebound increase of dopamine after the termination of each defeat episode (**Figure**

318   **8b-d**).

319   To induce defeat in females, we introduced each recording female mouse to the home

320   cage of a lactating SW female. Similar to that in males, dopamine signal decreased during

321   defeat and rebounded after each defeat episode (**Figure 8b-d**).

322   We next examined dopamine response during aversive experience that is not socially

323   relevant. We presented 0.05% TMT, a pungent chemical found in fox feces, on a Q-tip to the

324   recorded mouse for 10 minutes when the test animal was head-fixed and positioned on a

325   running wheel (**Figure 8e**); we suspended the test mouse by lifting its tail for 10 seconds to

326   simulate the situation when it was caught by a predator (**Figure 8i**); we also delivered a series

327   of 5-second 0.4 mA foot shocks to elicit pain (**Figure 8m**). In all cases, we observed a

328   consistent decrease in dopamine signal during the aversive experience (**Figures 8e-8p**). For

329   defeat, tail suspension and TMT exposure, the dopamine typically decreased to its lowest level

330   within 2s after the onset and then gradually increases despite the ongoing aversive experience

331   (**Figures 8a-8l**). For foot shock, the dopamine decreased sharply immediately after shock onset

332   and remained maximally suppressed throughout the shocking period (**Figures 8n-8p**). At the

333   offset of all aversive experience, dopamine showed a rebound increase (**Figures 8a-p**). While

334   the decrease in dopamine response during aversive experience was comparable between

335   males and females, the male showed a higher rebound after all aversive experiences except

336   foot shock (**Figures 8d, 8h, 8l and 8p**). Neither dopamine decrease during aversive experience
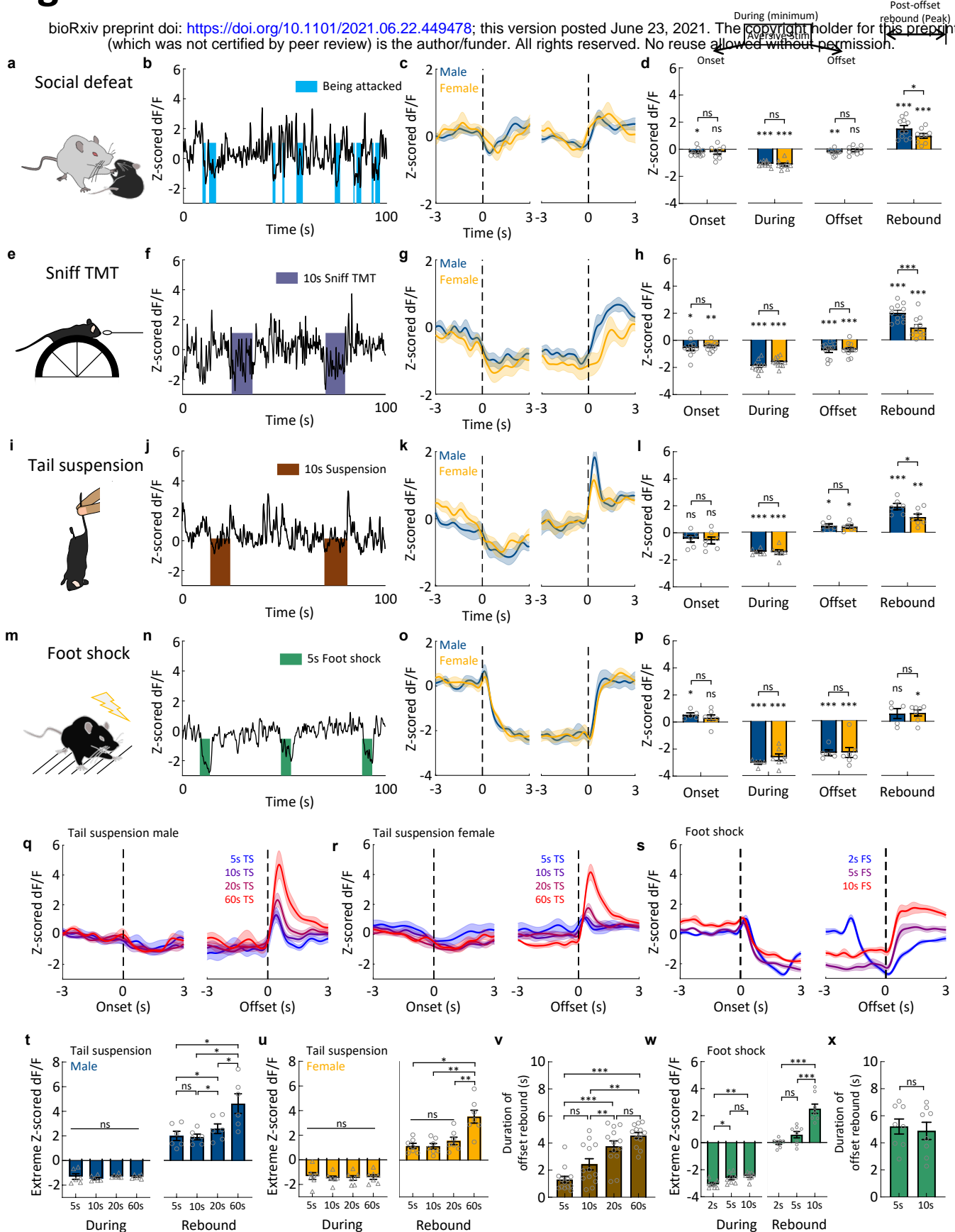
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Figure 8

Figure 8. DA responses during aversive social and non-social experiences.

a, A cartoon illustration of social defeat.

b, Representative traces of Z-scored ΔF/F of GRAB$_{DA2h}$ during defeat. Similar results were observed for 11 male mice and 9 female mice.

c, Average post-event histograms aligned to the onset (left) and offset (right) of being attacked. Shaded area: s.e.m. (n=11 male mice and n=9 female mice.)

d, Top: Schematics showing the time periods used for characterizing DA responses related to aversive experiences. Bottom: Summary of Z scored GRAB$_{DA2h}$ responses at the indicated moments of being attacked. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice and n=9 female mice. One sample t test with FDR correction revealed significant responses, *p<0.05, **p<0.01, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed the difference in rebound responses between males and females, *p<0.05.)

e, A cartoon illustration of TMT exposure.

f, Representative traces of Z scored ΔF/F of GRAB$_{DA2h}$ during sniffing TMT. Similar results were observed for 11 male mice and 11 female mice.

g, Average post-event histograms aligned to the onset (left) and offset (right) of TMT exposure. Shaded area: s.e.m. (n=11 male mice and n=11 female mice.)

h, Summary of Z scored GRAB$_{DA2h}$ responses at the indicated moments of sniffing TMT. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice and n=11 female mice. One sample t test with FDR correction revealed significant responses, *p<0.05, **p<0.01, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed the difference in rebound response between males and females, ***p<0.001.)

i, A cartoon illustration of tail suspension.

j, Representative traces of z-scored ΔF/F of GRAB$_{DA2h}$ during the tail suspension. Similar results were observed from 6 male mice and 7 female mice.

k, Average post-event histograms aligned to the onset (left) and offset (right) of tail suspension for the tested male and female mice. Shaded area: s.e.m. (n=6 male mice and n=7 female mice.)

l Summary of Z scored GRAB$_{DA2h}$ responses at the indicated moments of tail suspension. Mean ± s.e.m overlaid with individual data points for each group. (n=6 male mice and n=7 female mice. One sample t test with FDR correction revealed significant responses,*p<0.05, **p<0.01, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed the difference in rebound response between males and females, *p<0.05)

m, A cartoon illustration of foot shock.

n, Representative traces of Z scored ΔF/F of GRAB$_{DA2h}$ during 5s foot shock. Similar results were observed from 5 male mice and 7 female mice.

o, Average post-event histograms aligned to the onset (left) and offset (right) of the 5s foot shock. Shaded area: s.e.m. (n=5 male mice and n=7 female mice.)

p, Summary of Z scored GRAB$_{DA2h}$ responses at the indicated moments of foot shock. Mean ± s.e.m overlaid for each group. (n=5 male mice and n=7 female mice. One sample t test with FDR correction revealed significant responses, *p<0.05, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed no difference in responses between males and females.)

q-r, Average post-event histograms aligned to the onset (left) and offset (right) of the tail suspension with different suspension duration in males (**q**) and females (**r**). Shaded area: s.e.m. (n=6 male mice and n=7 female mice.)

s, Average post-event histograms aligned to the onset (left) and offset (right) of the foot shock with different shock durations. Shaded area: s.e.m. (n=5 males and 3 females.)

t-u, Maximum GRAB$_{DA2h}$ decrease during tail suspension and maximum post-suspension rebound responses at different suspension durations in males **(t)** and females **(u)**. Mean ± s.e.m overlaid with individual data points for each group. (n=6 male mice: Friedman test for the during period, p=0.43; Tukey's multiple comparisons following one-way ANOVA revealed the differences in rebound responses between groups, *p<0.05. n=7 female mice: Tukey's multiple comparisons following one-way ANOVA revealed the differences in rebound responses between groups, *p<0.05, **p<0.01.)

v, Post-suspension rebound durations vary with the length of tail suspension. Mean ± s.e.m overlaid with individual data points for each group. (n=6 males and 7 females, Tukey's multiple comparisons following one-way ANOVA revealed the differences in offset rebound duration between groups, **p<0.01, **p<0.001.)

w, Maximum GRAB$_{DA2h}$ decrease during foot-shock and maximum post-shock rebound responses at different shock durations. Mean ± s.e.m overlaid with individual data points for each group. (n=5 male mice and 3 female mice, Tukey's multiple comparisons following one-way ANOVA revealed the differences in responses between groups, *p<0.05, **p<0.01, ***p<0.001).

x, The post-shock rebound duration is similar between 5s and 10s shocks. Mean ± s.e.m overlaid with individual data points for each group. (n=5 male mice and 3 female mice, paired t-test, p=0.69.)

25

337   nor the rebound response after the experience showed any adaptation over repeated trials

338   (**Figure 8– figure supplement 1**).

339   The dopamine rebound may represent a rewarding signal related to relief from aversive

340   experience. We wondered whether the extent of relief (i.e., dopamine rebound), correlates with

341   the negativity of the experience. We thus varied the duration of tail suspension and foot shock

342   and asked whether dopamine responses varied with the length of the aversive experience.

343   While the magnitude of dopamine decrease during tail suspension was similar across all

344   durations, the rebound increase was significantly higher and lasted longer after 60-s suspension

345   than that of shorter durations in both males and females (**Figure 8q-8r and 8t-8v**). Similarly, 10-

346   s foot shock induced significantly higher dopamine rebound than 5-s shock while 2-s shock

347   induced no appreciable rebound, supporting the idea that rebound increase was correlated with

348   the extent of negativity of the experience (**Figure 8s and 8w-8x**).

349   Lastly, changes in our fluorescence signal could not be accounted by the movement

350   artifacts. In our control animals expressing GFP, we observed no significant change in

351   fluorescence during any behaviors (**Figure 8 – figure supplement 2**).

352

353   **Discussion**

354   Using a recently developed genetically encoded dopamine sensor, our study revealed

355   details of dopamine responses during social behaviors and identified sexual dimorphism in the

356   response pattern. We found that dopamine increases in all three phases of social behaviors:

357   approach, investigation and consummation.

358   The dopamine increase during approach likely signals the motivational state of the

359   animal towards the target. For example, males, not females, show a higher dopamine increase

360   during approaching opposite-sex than same-sex conspecifics, consistent with the fact that

361   males prefer females over males in three-chamber social preference test while females show no

362   preference to either social target (Yao et al., 2017). For another example, mothers and fathers

363   are known to be highly motivated for pups as shown by their willingness to work hard to gain

364   access to pups or giving up other high-value rewards for pups (Hauser and Gandelman, 1985;

365   Lee et al., 2000; Mattson et al., 2001; Wang et al., 2012; Wilsoncroft, 1968). In parents,

366   dopamine increases in NAc core during pup approach is consistently higher than that in non-

367   parental animals. This result is in contrast to the previous finding showing that dopamine

26

368   responses to pups are similar in males with low and high parental motivation (Lei et al., 2017).

369   This discrepancy is likely due to the low temporal resolution of microdialysis. Although we found

370   that the response during approach differs in fathers and naïve males, dopamine increase during

371   investigation is similar. During infanticide, dopamine level also increased. Thus, the difference in

372   dopamine release during pup approach may be averaged out using microdialysis that has a

373   temporal resolution of 10-20 minutes.

374   The motivation-dependent release of dopamine during approach is in line with previous

375   functional studies that demonstrated an indispensable role of dopamine in social approach, or

376   more broadly speaking, acquiring access to a social target. For example, blocking the dopamine

377   signaling in the NAc reduces males' willingness to lever press to gain access to a weaker

378   intruder and attack (Golden et al., 2019a). Increase dopamine release in NAc by activating

379   inputs from the medial preoptic nucleus to the ventral tegmental area promotes pup and male

380   approach in female mice (Fang et al., 2018; McHenry et al., 2017) . Importantly, we found that

381   dopamine rises after the onset of approach in all cases, suggesting that it is not required for

382   approach initiation but might play a role in invigorating the process.

383   Dopamine also increases during social investigation. This increase is likely driven by the

384   sensory cues from the conspecific and adapts quickly. Similar increase and fast adaption occur

385   during investigation of a non-social target. This response pattern may explain why mice are less

386   interested in familiar conspecific or object than novel ones (Leger et al., 2013; Moy et al., 2004).

387   A previous study suggested that dopamine neurons in VTA show an increase in Ca^{2+} activity as

388   animals withdraw from a novel object (Gunaydin et al., 2014). Here, we found dopamine

389   increases occur mainly during object approach and reaches maximum during object

390   investigation. Although dopamine levels remain elevated above the pre-investigation level at the

391   offset of object investigation, no acute upward change was observed when the animal retreated

392   from the object. This difference in response pattern may be due to discrepancies of dopamine

393   neuron activity and dopamine release at the terminals (Gunaydin et al., 2014).

394   Dopamine consistently and transiently increases during consummatory phase of social

395   behaviors with little adaption over repeated trials. In general, dopamine rises, reaches peak

396   levels soon after behavior onset, and gradually drops afterwards. At behavior offset, dopamine

397   levels have often returned to baseline, suggesting that dopamine signals may be most important

398   for marking transitions in behaviors instead of their maintenance. The sex dimorphism of

399   dopamine release is most noticeable during the consummatory phase. During attack, males

400   show higher dopamine increase than postpartum female mice. While dopamine increases

27

401   during all phases of sexual behaviors in males, only receptive, not unreceptive, females show
402   dopamine increase and only when she is initially mounted and soon after male ejaculation. The
403   sex difference in dopamine release during aggression and sexual behaviors likely stem from the
404   sex differences in behaviors themselves. Indeed, aggressive behaviors in male and female mice
405   are triggered by different sensory cues while sexual behaviors differ between sexes in both
406   sensory triggers and motor execution. Regardless of the cause of sex differences in dopamine
407   release, this difference in release pattern likely signals different valence of the experience and
408   reinforces the behavior in a sex specific way. Indeed, while in male mice repeated attacks lead
409   to an increase in aggression, a phenomenon known as winner effect, female mice have not
410   been found to show a clear winner effect (Hashikawa et al., 2018).

411   What is the function of dopamine release during the consummatory social behaviors?
412   One likely role of dopamine is for reward associative learning. Inputs to NAc cells that carry
413   information regarding the environmental contexts and mating partners could be strengthened by
414   the dopamine release during consummatory social actions and in turn facilitate approach to the
415   cues and enhance the chance to be engaged in similar social behaviors in the future (Aragona
416   et al., 2003; Aragona et al., 2006; Balfour et al., 2004; Gingrich et al., 2000; Meisel et al., 1996).
417   Beyond reward learning, does dopamine release also play a role in the expression of
418   consummatory social behaviors? Answer to this question could be behavior-specific. Pup
419   retrieval, for example, has been shown to be critically dependent on dopamine receptor
420   activation in the NAc shell (Keer and Stern, 1999; Numan et al., 2005). Blocking D1 receptor
421   signaling in NAc disrupts pup retrieval whereas D1 receptor agonist in the NAc facilitates the
422   onset of maternal behavior (Numan et al., 2005; Stolzenberg et al., 2007). Inhibiting D1R
423   expressing cells in NAc reduced attack duration in male mice (Golden et al., 2019b). In contrast,
424   male sexual behaviors are not affected by dopamine depletion in the NAc although manipulated
425   males showed a decrease in noncontact erection, suggesting a decrease in sexual motivation
426   (Liu et al., 1998; Moses et al., 1995). The differential importance of dopamine signaling in the
427   expression of various social behaviors suggests distinct roles for the NAc in driving these
428   behaviors. Indeed, NAc has been suggested as a key part of the maternal circuit but largely left
429   out of consummatory sexual behavior circuits (Jennings and de Lecea, 2020; Kohl et al., 2017;
430   Numan, 2007). Of note, deficits in social reward conditioning are mainly caused by D2 receptor
431   antagonists in the NAc while deficits in social behavior expression mainly resulted from D1
432   receptor antagonism (Aragona et al., 2006; Gingrich et al., 2000; Golden et al., 2019b; Meisel et
433   al., 1996; Numan et al., 2005). Thus, it is possible that dopamine release in the NAc serves dual

434   roles: it promotes the execution of certain social behaviors through D1R and facilitates reward
435   conditioning through D2R.

436   Previous microdialysis study showed an overall increase in dopamine during defeat
437   (Tidey and Miczek, 1996). Here, using a method with finer temporal resolution, we observed a
438   decrease in extracellular dopamine during defeat followed by rebound increase after the defeat
439   terminates. Indeed, dopamine rebound is commonly observed at the termination of an aversive
440   experience regardless of its exact nature and the magnitude of rebound is positively correlated
441   with the duration of the experience. This suppression-rebound response pattern is in line with
442   previous electrophysiological recordings in the VTA and FSCV recording in the NAc (Brischoux
443   et al., 2009; Budygin et al., 2012). Behavioral experiments also demonstrated that relief from the
444   pain can be used as a "reward" to induce preference to the associated context or sensory cues
445   (Navratilova et al., 2015). We speculate the dopamine rebound at the end of defeat could
446   contribute to the rapid emergence of avoidant behavior towards the aggressor after a short
447   period of defeat as the rebound often occurs when the defeated animal moves away from the
448   aggressor. Interestingly, we did not observe a rebound increase at the termination of forced
449   mounting in non-receptive females and females do not develop avoidance towards the male
450   afterwards. In addition to the rebound increase after aversive experience, we also noticed a
451   transient suppression in dopamine activity after the termination of positive social behavior, such
452   as mounting and intromission in males and pup retrieval in fathers. The biphasic activity change
453   at the onset and offset of an experience may together facilitate learning of cues predictive of the
454   transition of behaviors.

455   In short, our study demonstrated fast and dynamic dopamine responses in the NAc
456   during various phases of social behaviors. The functional role of dopamine is likely to be
457   complex, multifaceted and across both fast and slow time scales. Future studies with temporally
458   precise manipulation tools that can block and enhance the dopamine release in a behavior-
459   locked manner will facilitate our understanding of the mesolimbic dopamine function in social
460   behaviors.

461

462   **Material and Methods**

463   **Animals**
464   All procedures were approved by the IACUC of NYULH in compliance with the NIH guidelines
465   for the care and use of laboratory animals. Mice were housed at 18–23 °C with 40–60%

29

466   humidity under a 12 h light–dark cycle (dark cycle, 10 p.m. to 10 a.m.), with food and water

467   available ad libitum. Test animals were adult Drd1-cre (> 8 weeks, MMRRC_030989-UCD). Ai9

468   mice (Jackson stock no.007909) were crossed with Drd1-cre mice for revealing D1R

469   expression. Stimulus animals were adult C57BL/6N male and female mice, adult BALB/c male

470   and female mice, or sexually experienced C57BL/6N and Swiss Webster males (aggressor)

471   purchased from Charles River, and 3-7 days old pups were bred from test mice or wildtype

472   C57BL/6N breeders. After surgery, all the animals are single housed. All experiments were

473   performed during the dark cycle of the animals.

474

475   **Immunofluorescence**

476   For histological analysis, animals were deeply anesthetized and transcardially perfused with 20

477   mL of PBS, followed by 20 mL of 4% paraformaldehyde (Electron Microscopy Sciences, cat. no.

478   15714) in PBS. After perfusion, brains were harvested, post-fixed in 4% paraformaldehyde for

479   4h at 4 °C and cryoprotected in 20% (w/v) sucrose for 24h. The brains were then embedded in

480   an O.C.T compound (Fisher Healthcare, cat. no. 23730571) and sectioned into 60-μm-thick

481   slices using a CM1900 cryostat (Leica). GRAB$_{DA2h}$ was immunostained using a chicken anti-

482   GFP antibody (1:1,000, Abcam, cat. no. ab13970) followed by an Alexa 488-conjugated donkey

483   anti-chicken secondary antibody (1:1,000, Jackson ImmunoResearch, cat. no. 116967). DAPI

484   (1:20,000; Thermo Fisher, cat. no. D1306) was used with the secondary antibody to visualize

485   the nucleus. The GRAB$_{DA2h}$ fluorescence images were acquired with a virtual slide microscope

486   (Olympus, VS120) in 10x mode or a confocal microscope (Zeiss LSM 510 or 700 microscope)

487   for the high-resolution image in 40x mode.

488

489   **Fiber photometry**

490   The male mice were screened for aggression before the surgery. For the test mouse

491   with a single fiber implanted, 80 nL AAV9.hSyn.DIO. GRAB$_{DA2h}$ (Vigene Biosciences, titer:

492   4.97e+13 gc/ml) was injected into one side of NAc core (anterior-posterior (AP): +0.98 mm

493   relative to Bregma; medial-lateral (ML): ±1.2mm relative to Bregma; dorsal-ventral (DV): 4.2 mm

494   from brain surface) using a nanoinjector (World Precision Instruments, Nanoliter 2000). For

495   bilateral recording, AAV9.hSyn.DIO. GRAB$_{DA2h}$ (Vigene Biosciences) was injected into one side

496   and AAV9.hSyn.DIO. GRAB$_{DA2h}$ or AAV9.hSyn.loxP. GRAB$_{DA2h}$.loxP (Vigene Biosciences, titer:

497   1.43e+13 gc/ml) was injected into the contralateral side of NAc core for the D1-D1 mice or D1-

498   nonD1 mice respectively. Control mice were injected with AAV2.CAG.Flex.GFP (UNC, titer:

499   4.00e+12 gc/ml) bilaterally. After virus injection, a custom-made optic fiber assembly (Thorlabs,

500   FT400EMT and SFLC440-10) was implanted approximately 300 μm above each injection site.

501   Fiber photometry recording was performed two weeks after AAV injection. The setup used for

502   recording was constructed as described previously (Falkner et al., 2016). In brief, a 400-Hz 472-

503   nm bandpass (passing band: 472 ± 15 nm, FF02-472/30-25, Semrock) filtered light-emitting

504   diode (Thorlabs, LED light: M470F1; LED driver: LEDD1B) was used to excite $GRAB_{DA2h}$ or

505   GFP. The emission light collected from the recording site was bandpass filtered (passing bands:

506   535 ± 25 nm, FF01-535/505, Semrock), detected by a Femtowatt Silicon Photoreceiver

507   (Newport, 2151), and recorded using a real-time processor (RZ5, TDT). The 400-Hz signals

508   carrying fluorescence intensity of $GRAB_{DA2h}$ or GFP were extracted in real time using a custom

509   TDT program. To analyze the recording data, the MATLAB function ''msbackadj'' with a moving

510   window of 25% of the total recording duration was first applied to obtain the instantaneous

511   baseline signal. The instantaneous ΔF/F was calculated as $(F_{raw} - F_{baseline})/F_{baseline}$. The Z scored

512   ΔF/F of the entire recording session was calculated as $(\Delta F/F - mean(\Delta F/F))/std(\Delta F/F)$. The

513   peri-event histogram (PETH) of a given behavior was plotted by aligning the Z scored ΔF/F

514   signal to the onset or offset of the behavior. The response during each behavior episode is

515   defined as the maximum Z during the behavior if the average Z increases during the behavior

516   and the minimum Z if average Z decreases during the behavior. The onset and offset response

517   are determined as the Z values at the moment of behavior onset and offset. The post-offset

518   response is defined as the average Z between 0-1 second after the end of the behavior. The

519   post-offset rebound response is the maximum Z 0-2 seconds after the termination of aversive

520   experience. The velocity of the animal was calculated as the displacement of the body location

521   of the animal between every other frame, and the acceleration was calculated as the velocity

522   difference between two adjacent frames. The onset of the movement is defined as the

523   movement following at least 2s immobility (velocity < 2 cm/s) and reaches a velocity of minimally

524   15 cm/s for at least 1.5 s. The correlation coefficient was calculated using MATLAB function

525   'corrcoef'.

526

527   **Behavioral paradigm and analysis**

528   Animal behaviors in all experiments were video recorded from both the side and top of

529   the cage using two synchronized cameras (Basler, acA640-100 gm) and a commercial video

530   acquisition software (StreamPix 8, Norpix) in a semi-dark room with infrared illumination at a

531   frame rate of 25 frames/s. Manual behavioral annotation was performed on a frame-by-frame

31

532 basis using custom software written in MATLAB (https://pdollar.github.io/toolbox/). DeepLabCut

533 was used for animal tracking (Mathis et al., 2018)

534   *Social interaction between animals of the same sex:* An adult BALB/c male was

535 introduced to the home cage of the test male mouse, or an adult BALB/c female was introduced

536 to the home cage of the test female mouse. If the test female is lactating, pups were removed

537 10 minutes prior to the intruder introduction. During social encounters, we identified three

538 behaviors of the test mice -- approach, investigation and attack. "Approach" was defined as

539 continuous movement toward a stationary intruder mouse until the center mass of the two

540 animals are below 100 pixels. "Investigation" was defined as close contact to any part of the

541 intruder's body. "Attack" was defined as a suite of intense actions aiming at biting the intruders,

542 including pushes, lunges, bites, tumbling, and fast locomotion episodes between such

543 movements.

544   *Social interaction between animals of different sexes:* An adult receptive or unreceptive

545 female intruder mouse was introduced into the home cage of the test male mouse. For the test

546 female mouse, an adult sexually experienced male mouse was introduced into the female's

547 home cage. During social encounters, we annotated "approach" and "investigation" of the test

548 mouse, and "mount", "intromit" and "ejaculate" of the male mouse. "Mount" is when the male

549 grasped and mounted the female's flanks. "Intromit" includes both rapid thrust against the

550 female's rear and deep rhythmic thrust. "Ejaculate" starts when the male suddenly ceases all

551 thrusting movements but still holding onto the female's flank and then after a few seconds

552 slumps to the side of the female. "Ejaculate" ends when the male resumes movements.

553   *Food Intake* A cup of peanut butter (around 2 grams, Jif Creamy Peanut Butter) was

554 introduced into the home cage of the test mouse. The test mouse freely interacted with the

555 peanut butter for 10 minutes. During the interaction, we identified "approach" and "eat".

556 "Approach" is defined as continuous movement toward the peanut butter cup until the mouse

557 head is above the cup. "Eat" is defined as active licking and chewing peanut butter.

558   *Pup-related behaviors:* 5 -7 pups from test mice or C57BL/6N breeders were

559 introduced, one at a time, into the home cage of the test mouse for a total of 10 minutes.

560 Approach, close interaction, biting, and retrieval are annotated. "Approach" is defined as

561 continuous movement toward a pup until the head of the test mouse is above the pup. "Close

562 interaction" is defined as close contact with pups including sniffing, licking, and grooming.

563 "Biting" is when the test mouse holds and bites the pup and causes harm. "Retrieval" starts

564 when the mouse picks up a pup with its mouth and ends when it drops the pup into or around

565 the nest.

566   *Social defeat:* To induce defeat, the male test mouse was introduced into a Swiss

567   Webster male's (aggressor) cage for 10-15 minutes. The female test mouse was introduced into

568   a lactating Swiss Webster female's cage. During interaction with the aggressor, we annotated

569   "being attacked" of test mice, which is defined as when the aggressor attacks the test mouse.

570   No other behavior tests were performed after social defeat for at least 24 hours.

571   *Footshock*: The test mouse was placed in a fear conditioning chamber (Med

572   Associates). After 5 minutes habituation, a series of electric shocks were delivered through the

573   floor grids (2-s shock: 0.4 mA for 2 s, 40 s interval, 6 times; 5-s shock: 0.4 mA for 5 s, 60 s

574   interval, 4 times; 10-s shock: 0.4 mA for 10 s, 90 s interval, 4 times). The shock was controlled

575   by TTL pulses generated using a real-time processor (RZ5, TDT). No other behavior tests were

576   performed after footshock for at least 24 hours.

577   *TMT exposure:* The test mouse was habituated on a 3D printed head-fixed apparatus 10

578   minutes a day for 3 days. On the test day, 0.05% TMT (diluted in mineral oil) was delivered to

579   the head-fixed mouse on a Q-tip moving along a linear track for 6 times. Each TMT

580   representation lasted for 10 s with a 120-s between trials. The onset of TMT exposure was

581   defined as when the Q-tip reached the closest point (< 1cm) to the mouse nostrils. The offset

582   was defined as when the Q-tip started to retract. No other behavior tests were performed after

583   TMT exposure for at least 24 hours.

584   *Tail suspension:* During the test, the experimenter grabbed the tail of the mouse and

585   lifted it approximately 25 cm above the floor of the mouse cage. 5-s tail suspension was first

586   performed for 6 times with 120-s between trials. Then, 10-s, 20-s, 60-s tail suspension trials

587   were performed with 180 s between trials. No other behavior tests were performed after tail

588   suspension for at least 24 hours.

589

590   **Drug Injection**

591   The test mouse was habituated for head fixation for 3 continuous days. On the test day,

592   the mouse was head-fixed while DA signals is continuously monitored. After 10 minutes

593   baseline, we intraperitoneally injected 0.9% NaCl (10ml/kg, Hanna Pharmaceutical Supply, cat.

594   no. NC9054335) and 40 minutes later, 20 mg/kg GBR 12909 (Tocris, cat. no. 0421) was

595   injected and the signal was recorded for at least 30 minutes after injection.

596

597   **Statistics**

598   All the data were tested for normality first by using Kolmogorov-Smirnov test. If all the

599   data was normally distributed, paired t tests were performed for comparisons between two

600   groups within the animal, unpaired t tests between animals, one-way ANOVA with Turkey's post

601   hoc test, or ordinary (or mixed effect) two-way ANOVA with Bonferroni's multiple-comparison

602   post hoc test were performed for comparisons between groups. In addition, one sample t-test

603   was performed to determine whether the Z score is significantly different from 0, followed by

604   false discovery rate correction with a false discovery rate of 0.05. If one or more groups were

605   not normally distributed, Mann–Whitney test, Wilcoxon matched-pairs signed-rank test, the

606   Friedman test with Dunn's multiple-comparison post hoc test, or repeated-measures two-way

607   ANOVA with Bonferroni's multiple-comparison test were performed. Wilcoxon rank test was

608   used to compare the means of two groups or the mean of a single group with 0, followed by

609   false discovery rate correction with a false discovery rate of 0.05. Details of each statistical test

610   can be found in the **supplementary table 1**.All error bars or error shades represent ± SEM. *, p

611   < 0.05; **, p < 0.01; ***, p < 0.001.

612

616   **Competing Interests:** The authors declare no competing interests.

References

Afonso, V.M., Grella, S.L., Chatterjee, D., and Fleming, A.S. (2008). Previous maternal experience affects accumbal dopaminergic responses to pup-stimuli. Brain Res *1198*, 115-123.

Afonso, V.M., King, S., Chatterjee, D., and Fleming, A.S. (2009). Hormones that increase maternal responsiveness affect accumbal dopaminergic responses to pup-and food-stimuli in the female rat. Hormones and Behavior *56*, 11-23.

Afonso, V.M., Shams, W.M., Jin, D., and Fleming, A.S. (2013). Distal pup cues evoke dopamine responses in hormonally primed rats in the absence of pup experience or ongoing maternal behavior. J Neurosci *33*, 2305-2312.

Aleyasin, H., Flanigan, M.E., Golden, S.A., Takahashi, A., Menard, C., Pfau, M.L., Multer, J., Pina, J., McCabe, K.A., Bhatti, N.*, et al.* (2018). Cell-Type-Specific Role of DeltaFosB in Nucleus Accumbens In Modulating Intermale Aggression. J Neurosci *38*, 5913-5924.

Aragona, B.J., Liu, Y., Curtis, J.T., Stephan, F.K., and Wang, Z. (2003). A critical role for nucleus accumbens dopamine in partner-preference formation in male prairie voles. J Neurosci *23*, 3483-3490.

Aragona, B.J., Liu, Y., Yu, Y.J., Curtis, J.T., Detwiler, J.M., Insel, T.R., and Wang, Z. (2006). Nucleus accumbens dopamine differentially mediates the formation and maintenance of monogamous pair bonds. Nat Neurosci *9*, 133-139.

Balfour, M.E., Yu, L., and Coolen, L.M. (2004). Sexual behavior and sex-associated environmental cues activate the mesolimbic system in male rats. Neuropsychopharmacology *29*, 718-730.

Bayer, H.M., and Glimcher, P.W. (2005). Midbrain dopamine neurons encode a quantitative reward prediction error signal. Neuron *47*, 129-141.

Becker, J.B., Rudick, C.N., and Jenkins, W.J. (2001). The role of dopamine in the nucleus accumbens and striatum during sexual behavior in the female rat. J Neurosci *21*, 3236-3241.

Beiderbeck, D.I., Reber, S.O., Havasi, A., Bredewold, R., Veenema, A.H., and Neumann, I.D. (2012). High and abnormal forms of aggression in rats with extremes in trait anxiety--involvement of the dopamine system in the nucleus accumbens. Psychoneuroendocrinology *37*, 1969-1980.

Brischoux, F., Chakraborty, S., Brierley, D.I., and Ungless, M.A. (2009). Phasic excitation of dopamine neurons in ventral VTA by noxious stimuli. Proc Natl Acad Sci U S A *106*, 4894-4899.

Budygin, E.A., Park, J., Bass, C.E., Grinevich, V.P., Bonin, K.D., and Wightman, R.M. (2012). Aversive stimulus differentially triggers subsecond dopamine release in reward regions. Neuroscience *201*, 331-337.

Champagne, F.A., Chretien, P., Stevenson, C.W., Zhang, T.Y., Gratton, A., and Meaney, M.J. (2004). Variations in nucleus accumbens dopamine associated with individual differences in maternal behavior in the rat. J Neurosci *24*, 4113-4123.

Damsma, G., Pfaus, J.G., Wenkstern, D., Phillips, A.G., and Fibiger, H.C. (1992). Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion. Behav Neurosci *106*, 181-191.

de Jong, J.W., Afjei, S.A., Pollak Dorocic, I., Peck, J.R., Liu, C., Kim, C.K., Tian, L., Deisseroth, K., and Lammel, S. (2019). A Neural Circuit Mechanism for Encoding Aversive Stimuli in the Mesolimbic Dopamine System. Neuron *101*, 133-151 e137.

Eshel, N., Tian, J., Bukwich, M., and Uchida, N. (2016). Dopamine neurons share common response function for reward prediction error. Nat Neurosci *19*, 479-486.

Falkner, A.L., Grosenick, L., Davidson, T.J., Deisseroth, K., and Lin, D. (2016). Hypothalamic control of male aggression-seeking behavior. Nature neuroscience *19*, 596.

Fang, Y.Y., Yamaguchi, T., Song, S.C., Tritsch, N.X., and Lin, D. (2018). A Hypothalamic Midbrain Pathway Essential for Driving Maternal Behaviors. Neuron *98*, 192-207 e110.

663 Ferrari, P., Palanza, P., and Parmigiani, S. (2000). Does fear modulate defensive and offensive types of
664 maternal attack in mice? Aggressive Behavior *26*, 193-203.
665 Fish, E.W., DeBold, J.F., and Miczek, K.A. (2005). Escalated aggression as a reward: corticosterone and
666 GABA A receptor positive modulators in mice. Psychopharmacology *182*, 116-127.
667 Fish, E.W., Joseph, F., and Miczek, K.A. (2002). Aggressive behavior as a reinforcer in mice: activation by
668 allopregnanolone. Psychopharmacology *163*, 459-466.
669 Fish, E.W., McKenzie-Quirk, S.D., Bannai, M., and Miczek, K.A. (2008). 5-HT(1B) receptor inhibition of
670 alcohol-heightened aggression in mice: comparison to drinking and running. Psychopharmacology (Berl)
671 *197*, 145-156.
672 Flannelly, K.J., and Flannelly, L. (1985). Opponents' size influences maternal aggression. Psychological
673 reports *57*, 883-886.
674 Fumero, B., Fernandez-Vera, J.R., Gonzalez-Mora, J.L., and Mas, M. (1994). Changes in monoamine
675 turnover in forebrain areas associated with masculine sexual behavior: a microdialysis study. Brain Res
676 *662*, 233-239.
677 Gingrich, B., Liu, Y., Cascio, C., Wang, Z., and Insel, T.R. (2000). Dopamine D2 receptors in the nucleus
678 accumbens are important for social attachment in female prairie voles (Microtus ochrogaster). Behav
679 Neurosci *114*, 173-183.
680 Golden, S.A., Heins, C., Venniro, M., Caprioli, D., Zhang, M., Epstein, D.H., and Shaham, Y. (2017).
681 Compulsive Addiction-like Aggressive Behavior in Mice. Biol Psychiatry *82*, 239-248.
682 Golden, S.A., Heshmati, M., Flanigan, M., Christoffel, D.J., Guise, K., Pfau, M.L., Aleyasin, H., Menard, C.,
683 Zhang, H., and Hodes, G.E. (2016). Basal forebrain projections to the lateral habenula modulate
684 aggression reward. Nature *534*, 688.
685 Golden, S.A., Jin, M., Heins, C., Venniro, M., Michaelides, M., and Shaham, Y. (2019a). Nucleus
686 Accumbens Drd1-Expressing Neurons Control Aggression Self-Administration and Aggression Seeking in
687 Mice. The Journal of Neuroscience *39*, 2482-2496.
688 Golden, S.A., Jin, M., Heins, C., Venniro, M., Michaelides, M., and Shaham, Y. (2019b). Nucleus
689 Accumbens Drd1-Expressing Neurons Control Aggression Self-Administration and Aggression Seeking in
690 Mice. J Neurosci *39*, 2482-2496.
691 Golden, S.A., Jin, M., and Shaham, Y. (2019c). Animal Models of (or for) Aggression Reward, Addiction,
692 and Relapse: Behavior and Circuits. J Neurosci *39*, 3996-4008.
693 Gunaydin, L.A., Grosenick, L., Finkelstein, J.C., Kauvar, I.V., Fenno, L.E., Adhikari, A., Lammel, S.,
694 Mirzabekov, J.J., Airan, R.D., Zalocusky, K.A.*, et al.* (2014). Natural neural projection dynamics underlying
695 social behavior. Cell *157*, 1535-1551.
696 Hamid, A.A., Pettibone, J.R., Mabrouk, O.S., Hetrick, V.L., Schmidt, R., Vander Weele, C.M., Kennedy,
697 R.T., Aragona, B.J., and Berke, J.D. (2016). Mesolimbic dopamine signals the value of work. Nat Neurosci
698 *19*, 117-126.
699 Hansen, S., Bergvall, A.H., and Nyiredi, S. (1993). Interaction with pups enhances dopamine release in
700 the ventral striatum of maternal rats: a microdialysis study. Pharmacol Biochem Behav *45*, 673-676.
701 Hashikawa, K., Hashikawa, Y., Lischinsky, J., and Lin, D. (2018). The Neural Mechanisms of Sexually
702 Dimorphic Aggressive Behaviors. Trends Genet *34*, 755-776.
703 Hauser, H., and Gandelman, R. (1985). Lever pressing for pups: evidence for hormonal influence upon
704 maternal behavior of mice. Horm Behav *19*, 454-468.
705 Jenkins, W.J., and Becker, J.B. (2003). Dynamic increases in dopamine during paced copulation in the
706 female rat. Eur J Neurosci *18*, 1997-2001.
707 Jennings, K.J., and de Lecea, L. (2020). Neural and Hormonal Control of Sexual Behavior. Endocrinology
708 *161*.

709 Keer, S.E., and Stern, J.M. (1999). Dopamine receptor blockade in the nucleus accumbens inhibits
710 maternal retrieval and licking, but enhances nursing behavior in lactating rats. Physiol Behav *67*, 659-
711 669.
712 Kippin, T.E., and Pfaus, J.G. (2001). The development of olfactory conditioned ejaculatory preferences in
713 the male rat. I. Nature of the unconditioned stimulus. Physiol Behav *73*, 457-469.
714 Kohl, J., Autry, A.E., and Dulac, C. (2017). The neurobiology of parenting: A neural circuit perspective.
715 Bioessays *39*, 1-11.
716 Kohlert, J.G., and Meisel, R.L. (1999). Sexual experience sensitizes mating-related nucleus accumbens
717 dopamine responses of female Syrian hamsters. Behav Brain Res *99*, 45-52.
718 Lavi-Avnon, Y., Weller, A., Finberg, J.P., Gispan-Herman, I., Kinor, N., Stern, Y., Schroeder, M., Gelber, V.,
719 Bergman, S.Y., Overstreet, D.H.*, et al.* (2008). The reward system and maternal behavior in an animal
720 model of depression: a microdialysis study. Psychopharmacology (Berl) *196*, 281-291.
721 Lee, A., Clancy, S., and Fleming, A.S. (2000). Mother rats bar-press for pups: effects of lesions of the
722 mpoa and limbic sites on maternal behavior and operant responding for pup-reinforcement. Behav Brain
723 Res *108*, 215-231.
724 Leger, M., Quiedeville, A., Bouet, V., Haelewyn, B., Boulouard, M., Schumann-Bard, P., and Freret, T.
725 (2013). Object recognition test in mice. Nat Protoc *8*, 2531-2537.
726 Lei, K., Liu, Y., Smith, A.S., Lonstein, J.S., and Wang, Z. (2017). Effects of pair bonding on parental
727 behavior and dopamine activity in the nucleus accumbens in male prairie voles. Eur J Neurosci *46*, 2276-
728 2284.
729 Liu, C., Kershberg, L., Wang, J., Schneeberger, S., and Kaeser, P.S. (2018). Dopamine secretion is
730 mediated by sparse active zone-like release sites. Cell *172*, 706-718. e715.
731 Liu, Y.C., Sachs, B.D., and Salamone, J.D. (1998). Sexual behavior in male rats after radiofrequency or
732 dopamine-depleting lesions in nucleus accumbens. Pharmacol Biochem Behav *60*, 585-592.
733 Mann, M.A., Kinsley, C., Broida, J., and Svare, B. (1983). Infanticide exhibited by female mice: genetic,
734 developmental and hormonal influences. J Physiology behavior Research Methods *30*, 697-702.
735 Martinez, M., Guillen-Salazar, F., Salvador, A., and Simon, V.M. (1995). Successful intermale aggression
736 and conditioned place preference in mice. Physiol Behav *58*, 323-328.
737 Mathis, A., Mamidanna, P., Cury, K.M., Abe, T., Murthy, V.N., Mathis, M.W., and Bethge, M. (2018).
738 DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. Nat Neurosci
739 *21*, 1281-1289.
740 Mattson, B.J., Williams, S., Rosenblatt, J.S., and Morrell, J.I. (2001). Comparison of two positive
741 reinforcing stimuli: pups and cocaine throughout the postpartum period. Behav Neurosci *115*, 683-694.
742 May, M.E., and Kennedy, C.H. (2009). Aggression as positive reinforcement in mice under various ratio-
743 and time-based reinforcement schedules. Journal of the experimental analysis of behavior *91*, 185-196.
744 McHenry, J.A., Otis, J.M., Rossi, M.A., Robinson, J.E., Kosyk, O., Miller, N.W., McElligott, Z.A., Budygin,
745 E.A., Rubinow, D.R., and Stuber, G.D. (2017). Hormonal gain control of a medial preoptic area social
746 reward circuit. Nature neuroscience *20*, 449.
747 Meisel, R.L., Camp, D.M., and Robinson, T.E. (1993). A microdialysis study of ventral striatal dopamine
748 during sexual behavior in female Syrian hamsters. Behav Brain Res *55*, 151-157.
749 Meisel, R.L., Joppa, M.A., and Rowe, R.K. (1996). Dopamine receptor antagonists attenuate conditioned
750 place preference following sexual behavior in female Syrian hamsters. Eur J Pharmacol *309*, 21-24.
751 Mermelstein, P.G., and Becker, J.B. (1995). Increased extracellular dopamine in the nucleus accumbens
752 and striatum of the female rat during paced copulatory behavior. Behav Neurosci *109*, 354-365.
753 Moses, J., Loucks, J.A., Watson, H.L., Matuszewich, L., and Hull, E.M. (1995). Dopaminergic drugs in the
754 medial preoptic area and nucleus accumbens: effects on motor activity, sexual motivation, and sexual
755 performance. Pharmacol Biochem Behav *51*, 681-686.

756 Moy, S.S., Nadler, J.J., Perez, A., Barbaro, R.P., Johns, J.M., Magnuson, T.R., Piven, J., and Crawley, J.N.
757 (2004). Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-
758 like behavior in mice. Genes Brain Behav *3*, 287-302.
759 Navratilova, E., Atcherley, C.W., and Porreca, F. (2015). Brain Circuits Encoding Reward from Pain Relief.
760 Trends Neurosci *38*, 741-750.
761 Numan, M. (2007). Motivational systems and the neural circuitry of maternal behavior in the rat. Dev
762 Psychobiol *49*, 12-21.
763 Numan, M., Numan, M.J., Pliakou, N., Stolzenberg, D.S., Mullins, O.J., Murphy, J.M., and Smith, C.D.
764 (2005). The effects of D1 or D2 dopamine receptor antagonism in the medial preoptic area, ventral
765 pallidum, or nucleus accumbens on the maternal retrieval response and other aspects of maternal
766 behavior in rats. Behav Neurosci *119*, 1588-1604.
767 Perrigo, G., Bryant, W.C., and vom Saal, F.S. (1990). A unique neural timing system prevents male mice
768 from harming their own offspring. J Animal Behaviour *39*, 535-539.
769 Pfaus, J.G., Damsma, G., Nomikos, G.G., Wenkstern, D.G., Blaha, C.D., Phillips, A.G., and Fibiger, H.C.
770 (1990). Sexual behavior enhances central dopamine transmission in the male rat. Brain Res *530*, 345-
771 348.
772 Pfaus, J.G., Damsma, G., Wenkstern, D., and Fibiger, H.C. (1995). Sexual activity increases dopamine
773 transmission in the nucleus accumbens and striatum of female rats. Brain Res *693*, 21-30.
774 Phillips, P.E., Stuber, G.D., Heien, M.L., Wightman, R.M., and Carelli, R.M. (2003). Subsecond dopamine
775 release promotes cocaine seeking. Nature *422*, 614-618.
776 Pleim, E.T., Matochik, J.A., Barfield, R.J., and Auerbach, S.B. (1990). Correlation of dopamine release in
777 the nucleus accumbens with masculine sexual behavior in rats. Brain Res *524*, 160-163.
778 Robinson, D.L., Heien, M.L., and Wightman, R.M. (2002). Frequency of dopamine concentration
779 transients increases in dorsal and ventral striatum of male rats during introduction of conspecifics. J
780 Neurosci *22*, 10477-10486.
781 Rosenblatt, J.S. (1967). Nonhormonal basis of maternal behavior in the rat. Science *156*, 1512-1514.
782 Shnitko, T.A., Mace, K.D., Sullivan, K.M., Martin, W.K., Andersen, E.H., Avram, S.K.W., Johns, J.M., and
783 Robinson, D.L. (2017). Use of fast scan cyclic voltammetry to assess phasic dopamine release in rat
784 models of early postpartum maternal behavior and neglect. Behavioural pharmacology *28*, 648.
785 St John, R.D., and Corning, P.A. (1973). Maternal aggression in mice. Behav Biol *9*, 635-639.
786 Stagkourakis, S., Spigolon, G., Williams, P., Protzmann, J., Fisone, G., and Broberger, C. (2018). A neural
787 network for intermale aggression to establish social hierarchy. Nat Neurosci *21*, 834-842.
788 Stolzenberg, D.S., McKenna, J.B., Keough, S., Hancock, R., Numan, M.J., and Numan, M. (2007).
789 Dopamine D$_1$ receptor stimulation of the nucleus accumbens or the medial preoptic area promotes the
790 onset of maternal behavior in pregnancy-terminated rats. Behavioral neuroscience *121*, 907.
791 Sun, F., Zeng, J., Jing, M., Zhou, J., Feng, J., Owen, S.F., Luo, Y., Li, F., Wang, H., Yamaguchi, T.*, et al.*
792 (2018). A Genetically Encoded Fluorescent Sensor Enables Rapid and Specific Detection of Dopamine in
793 Flies, Fish, and Mice. Cell *174*, 481-496 e419.
794 Sun, F., Zhou, J., Dai, B., Qian, T., Zeng, J., Li, X., Zhuo, Y., Zhang, Y., Wang, Y., Qian, C.*, et al.* (2020). Next-
795 generation GRAB sensors for monitoring dopaminergic activity in vivo. Nat Methods *17*, 1156-1166.
796 Tenk, C.M., Wilson, H., Zhang, Q., Pitchers, K.K., and Coolen, L.M. (2009). Sexual reward in male rats:
797 effects of sexual experience on conditioned place preferences associated with ejaculation and
798 intromissions. Horm Behav *55*, 93-97.
799 Tidey, J.W., and Miczek, K.A. (1996). Social defeat stress selectively alters mesocorticolimbic dopamine
800 release: an in vivo microdialysis study. Brain Res *721*, 140-149.
801 Trezza, V., Campolongo, P., and Vanderschuren, L.J. (2011). Evaluating the rewarding nature of social
802 interactions in laboratory animals. Dev Cogn Neurosci *1*, 444-458.

803   Tzschentke, T.M. (2007). Review on CPP: Measuring reward with the conditioned place preference (CPP)
804   paradigm: update of the last decade. J Addiction biology *12*, 227-462.
805   van Erp, A.M., and Miczek, K.A. (2000). Aggressive behavior, increased accumbal dopamine, and
806   decreased cortical serotonin in rats. J Neurosci *20*, 9320-9325.
807   Wang, C.T., Huang, R.L., Tai, M.Y., Tsai, Y.F., and Peng, M.T. (1995). Dopamine release in the nucleus
808   accumbens during sexual behavior in prenatally stressed adult male rats. Neurosci Lett *200*, 29-32.
809   Wang, J., Tai, F., Yu, P., and Wu, R. (2012). Reinforcing properties of pups versus cocaine for fathers and
810   associated central expression of Fos and tyrosine hydroxylase in mandarin voles (Microtus mandarinus).
811   Behav Brain Res *230*, 149-157.
812   Wei, D., Talwar, V., and Lin, D. (2021). Neural circuits of social behaviors: innate yet flexible. Neuron.
813   Wenkstern, D., Pfaus, J.G., and Fibiger, H.C. (1993). Dopamine transmission increases in the nucleus
814   accumbens of male rats during their first exposure to sexually receptive female rats. Brain Res *618*, 41-
815   46.
816   Westerink, B.H., Damsma, G., De Vries, J.B., and Koning, H. (1987). Dopamine re-uptake inhibitors show
817   inconsistent effects on the in vivo release of dopamine as measured by intracerebral dialysis in the rat. J
818   European journal of pharmacology *135*, 123-128.
819   Wilsoncroft, W. (1968). Babies by bar-press: maternal behavior in the rat. Behavior Research Methods

820   Instrumentation *1*, 229-230.
821   Yao, S., Bergan, J., Lanjuin, A., and Dulac, C. (2017). Oxytocin signaling in the medial amygdala is required
822   for sex discrimination of social cues. Elife *6*.
823   Yuan, L., Dou, Y.N., and Sun, Y.G. (2019). Topography of Reward and Aversion Encoding in the
824   Mesolimbic Dopaminergic System. J Neurosci *39*, 6472-6481.

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Figure 1 – figure supplement 1
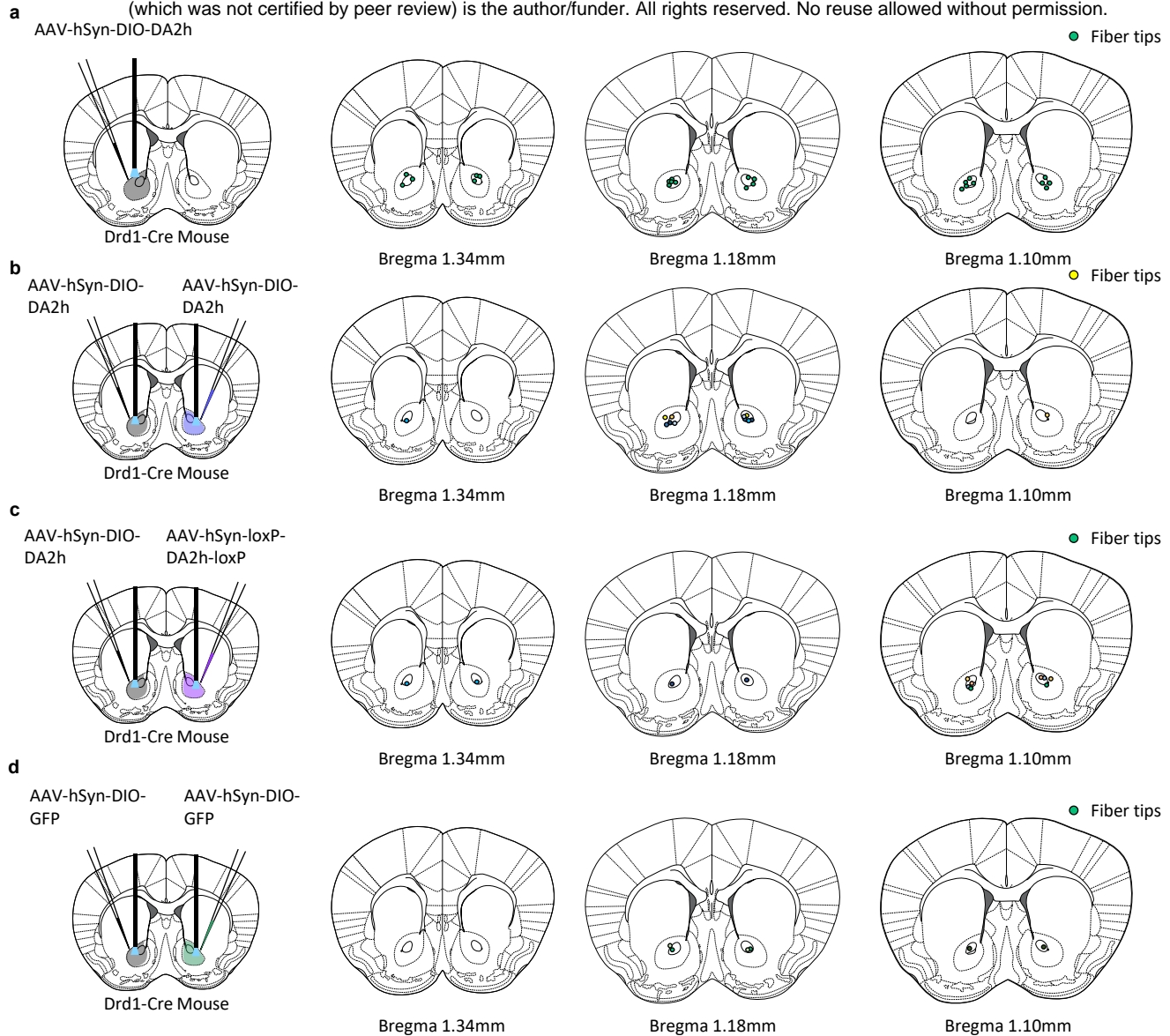
Figure 1 - figure supplement 1. Optic fiber placements for recordings.

a-d, Experimental design (left) and coronal brain sections (right) at the bregma level of NAc showing the optic fiber ends. Each dot represents one animal in **(a)**, and dots with same color in **(b-d)** represent same animal. Brain atlas images are modified from (Franklin and Paxinos, 2013).

Figure 2—figure supplement 1
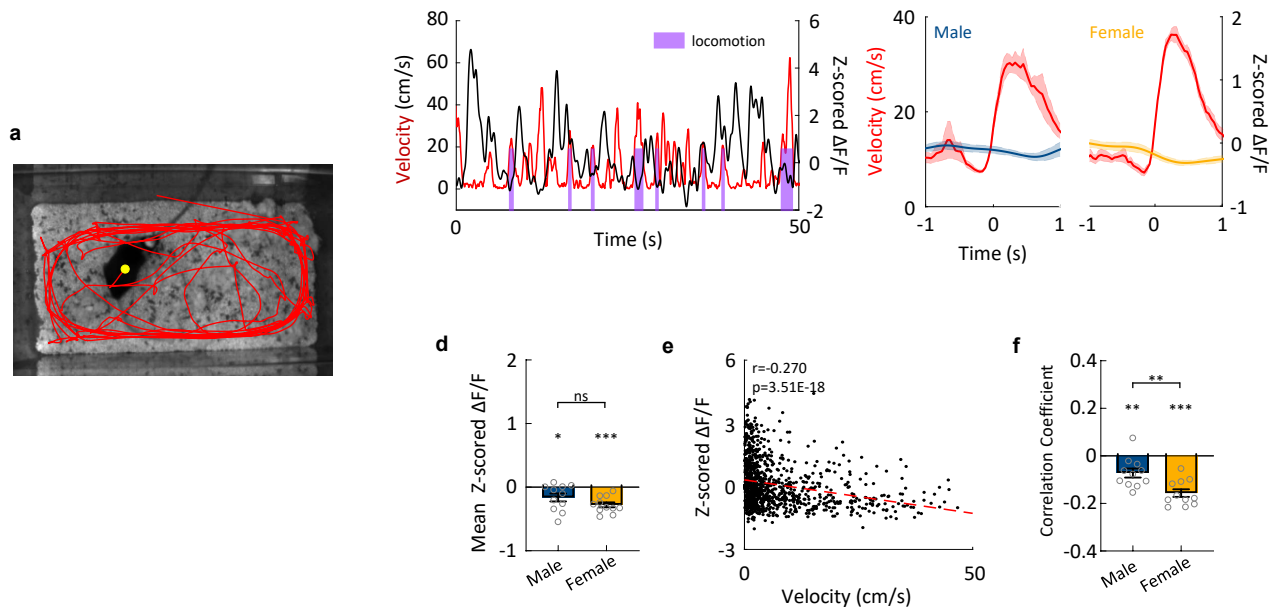
Figure 2 - figure supplement 1. No increase in DA activity at the onset of locomotion or acceleration.
a, Representative trajectory tracked by DeepLabCut (Mathis et al., 2018).
b, Representative traces of velocity (red) and z-scored ΔF/F of GRAB$_{DA2h}$ (black) from an example male mouse. Color shades indicate periods of locomotion. Similar results were observed from 11 male mice and 11 female mice.
c, Average post-event histograms of velocity (red) and GRAB$_{DA2h}$ signal aligned to the locomotion onset of males (left) and females (right). Shaded area: s.e.m. (n=11 male mice and n=11 female mice.)
d, Averaged Z scored GRAB$_{DA2h}$ signal during 0-1s of all locomotion episodes. (n=11 male mice and n=11 female mice. One sample t test revealed significant activity changes, *p<0.05, ***p<0.001. Unpaired t-test found no difference between two groups, p=0.12.)
e, A scatter plot showing the correlation between velocity and Z scored ΔF/F from one example female mouse. Similar results were observed from 11 male mice and 11 female mice. (A thousand data points were randomly selected for visualization, Pearson correlation, r = -0.27, p=3.15E-18.)
f, Summary of correlation coefficient between velocity and Z scored ΔF/F. (n=11 male mice and n=11 female mice. One sample t test revealed the correlation coefficients are significantly negative across animals, **p<0.01, ***p<0.001. Unpaired t-test found a difference between males and females, p = 0.003.)

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Figure 5—figure supplement 1
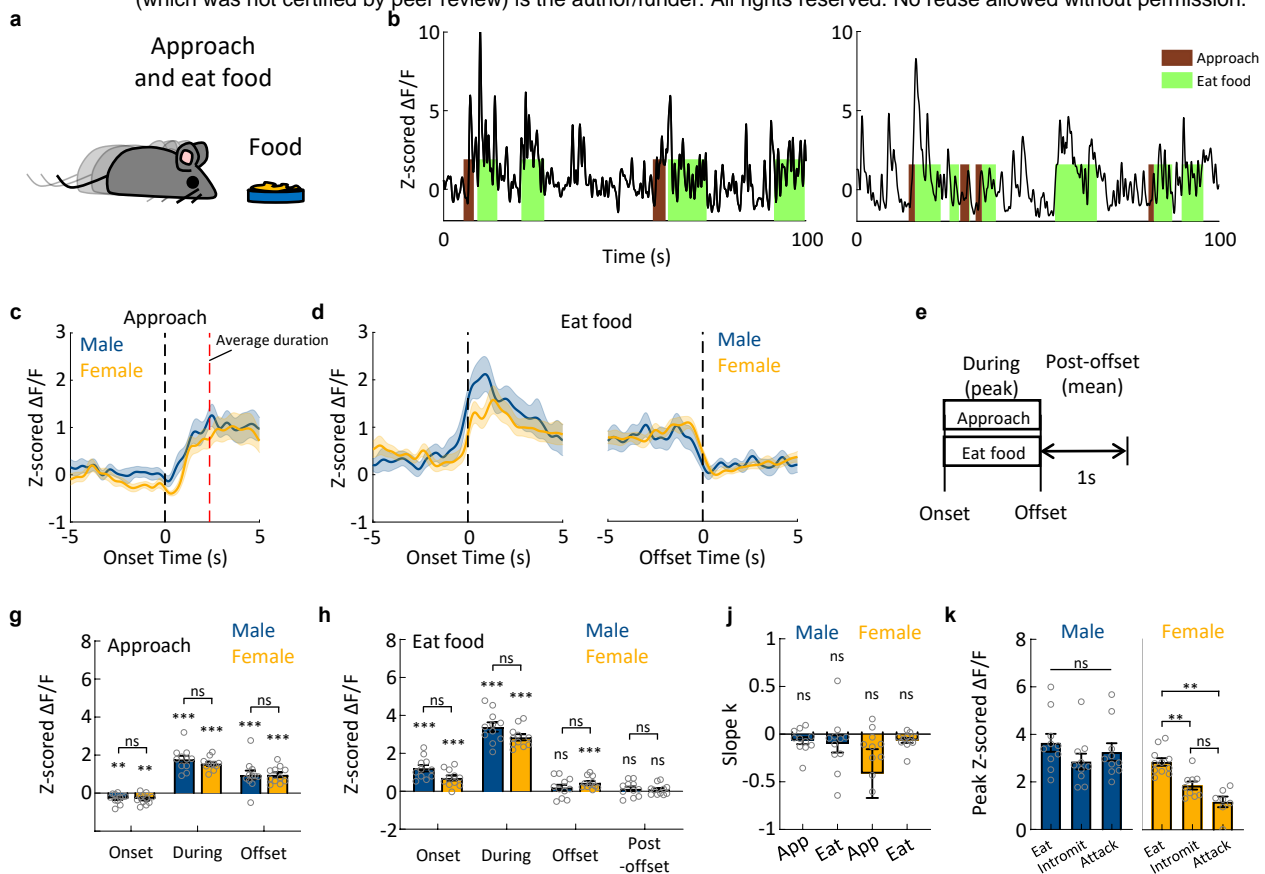
Figure 5 - figure supplement 1. DA responses during food intake.

a, A cartoon illustration of mouse approaching food.

b, Representative traces of z-scored ΔF/F of GRAB$_{DA2h}$ during approach and eat peanut butter. Similar results were observed from 11 male and 11 female mice.

c, Average post-event histograms aligned to the onset of approaching food. Shaded area: s.e.m. (n=11 male mice and n=11 female mice.)

d, Average post-event histograms aligned to the onset (left) and offset (right) of eating. Shaded area: s.e.m. (n=11 male mice and n=11 female mice.)

e, Schematics showing the periods used for characterizing DA responses related to food intake.

g, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, and during approach food. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice and n=11 female mice. One sample t test followed by FDR correction to reveal significant responses, **p<0.01, ***p<0.001. Two-way ANOVA group x time interaction, F(2,40)=0.6226, p=0.54.)

h, Summary of Z scored GRAB$_{DA2h}$ responses at the onset and offset of, and during and after eating food. (n=11 male mice and n=11 female mice. One sample t test followed by FDR correction to reveal significant responses, ***p<0.001. Bonferroni's multiple comparisons following two-way ANOVA revealed no significant difference in responses between males and females.)

j, Summary of slope k of GRAB$_{DA2h}$ responses over repeated eating episodes. (n=11 male mice, n=11 female mice. One sample t test followed by FDR correction to reveal significant adaptations.)

k, Summary of Z scored GRAB$_{DA2h}$ responses during food intake, intromission, and attack. (n=10 male mice, one-way ANOVA, F(1.340,12.06)=1.166, p=0.32. n=11 female mice for food intake, n=9 female mice for intromission, n=7 female mice for attack, Tukey's multiple comparisons following one-way ANOVA revealed differences in responses between groups in females, **p<0.01.)
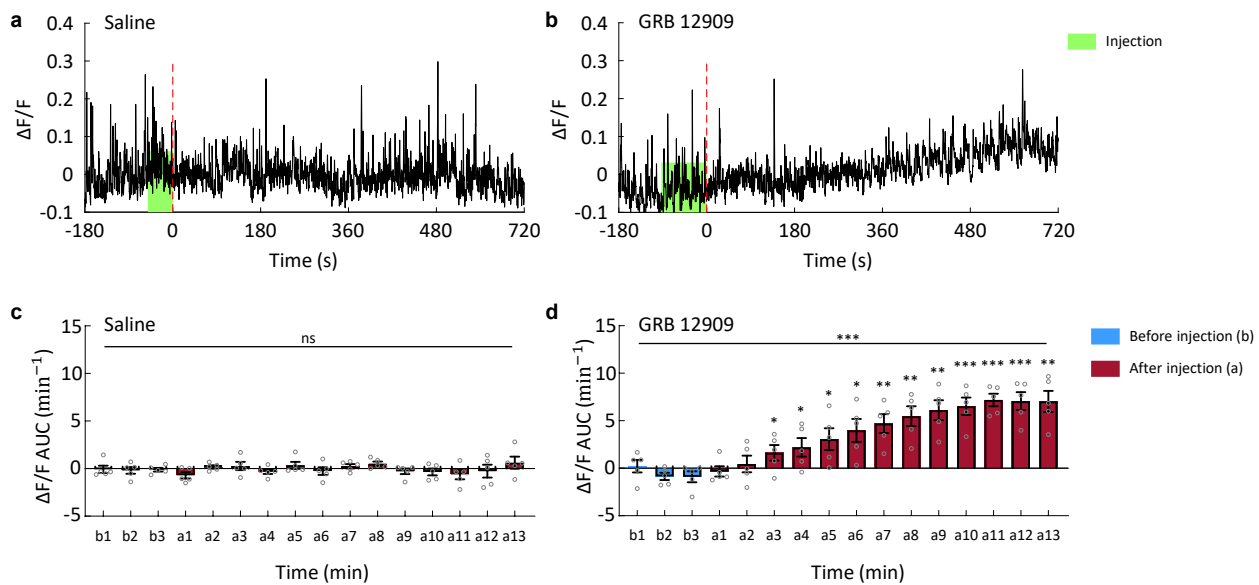
Figure 5—figure supplement 2

Figure 5 - figure supplement 2. Administration of DAT inhibitor induces a sustained increase in GRAB$_{DA2h}$ signal.

a-b, Representative traces of z-scored ΔF/F of GRAB$_{DA2h}$ after i.p. injection of saline (left) and 20 mg/kg GBR 12909 (right). Similar results were observed from 5 male mice.

c, Accumulated DA signals before and after saline treatment. Mean ± s.e.m overlaid with data points from individual animals for each group. (n = 5 mice, Friedman test, p=0.58.)

d, Accumulated DA signals before and after GBR 12909 treatment. Mean ± s.e.m overlaid for each group. (n = 5 mice, Tukey's multiple comparisons following one-way ANOVA revealed the difference between b3 and other time periods, *p<0.05, **p<0.01, ***p<0.001.)

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Figure 8– figure supplement 1
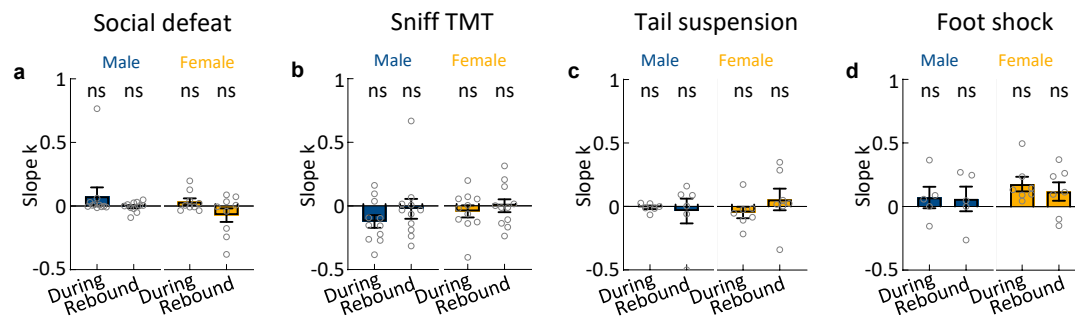
Figure 8 - figure supplement 1. Dopamine responses during and immediately after aversive experiences do not adapt over repeated trials.

a, Summary of slope k of $GRAB_{DA2h}$ responses over repeated defeat events. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice and n=female mice. One sample t test followed by FDR correction revealed no significant adaption.)

b, Summary of slope k of $GRAB_{DA2h}$ responses over repeated TMT exposure. Mean ± s.e.m overlaid with individual data points for each group. (n=11 male mice and n=11 female mice. One sample t test followed by FDR correction revealed no significant adaption.)

c, Summary of slope k of $GRAB_{DA2h}$ responses over repeated tail suspension. Mean ± s.e.m overlaid with individual data points for each group. (n=6 male mice and n=7 female mice. One sample t test followed by FDR correction revealed no significant adaption.)

d, Summary of slope k of $GRAB_{DA2h}$ responses over repeated 5s foot-shock. Mean ± s.e.m overlaid with individual data points for each group. (n=5 male mice and n=7 female mice. One sample t test followed by FDR correction revealed no significant adaption.)
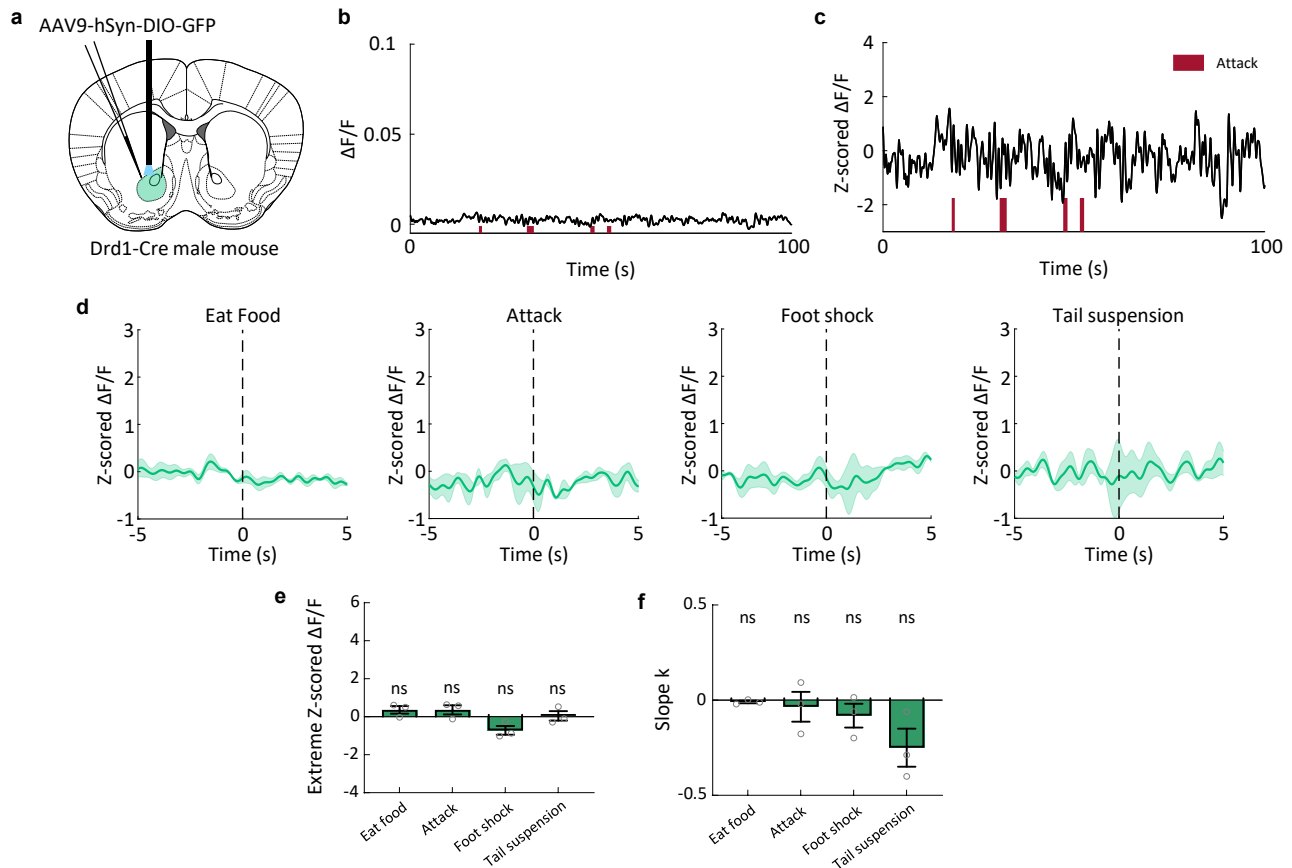
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Figure 8– figure supplement 2

Figure 8 - figure supplement 2. No change in fluorescence signal during any behaviors in GFP control animals.

a, Schematics illustrating the experimental design. Brain atlas images are modified from (Franklin and Paxinos, 2013).

b-c, Representative traces of ΔF/F **(b)** and Z scored ΔF/F **(c)** of GFP during inter-male aggression. Similar results were observed for 3 male mice.

d, Average post-event histograms aligned to the onset of eat, attack, foot-shock, and tail suspension. Shaded area: s.e.m. (n = 3 male mice.)

e, Maximum GFP increase during food intake and attack, and maximum GFP decrease during tail suspension and foot shock. Mean ± s.e.m overlaid with individual data points for each group. (n = 3 male mice. One sample t test followed by FDR correction revealed no significant response.)

f, Summary of slope k of GFP responses over repeated events. Mean ± s.e.m overlaid for each group. (n=3 male mice. One sample t test followed by FDR correction revealed no significant adaption.)

Supplementary Table 1

Figure	Part	Test	Exact P-value, F-value with degree of freedom for ANOVAs, t-value with degree of freedom for t-tests
1	j	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(1,12)=0.27, df=1, p=0.32. Group: F(2,12)=50.84, df= 2, p<0.001. Group x time interaction: F(2,12)=1.474, df=2, p=0.27. Bonferroni's multiple comparison: For no-intruder group, p >0.99 (DA2h: D1-D1 vs. DA2h: D1-nonD1), p<0.001(DA2h: D1-D1 vs. GFP: D1-D1), p<0.001(DA2h: D1-nonD1 vs. GFP: D1-D1); For with-intruder group, p >0.99 (DA2h: D1-D1 vs. DA2h: D1-nonD1), p<0.001(DA2h: D1-D1 vs. GFP: D1-D1), p<0.001(DA2h: D1-nonD1 vs. GFP: D1-D1); for the comparisions between no-intruder and with-intruder groups, p>0.99 (DA2h: D1-D1), p>0.99 (DA2h: D1-nonD1), p=0.□ (GFP: D1-D1).
2	e	Friedman test with Dunn's multiple comparison test	For the male group, p=0.007, Dunn's multiple comparisions, p=0.02 (Object vs. Male), p=0.02 (Object vs. Female), p>0.99 (Male vs. Female). For the female group, p=0.006, Dunn's multiple comparisions, p=0.007 (Object vs. Male), p=0.18 (Object vs. Female), p=0.72 (Male vs. Female).
2	j	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(2,40)=73.10, df=2, p<0.001. Group: F(1,20)=0.4296, df=1, p=0.52. Group x time interaction: F(2,40)=2.149, df=2, p=0.13. Bonferroni's multiple comparisons: p=0.34 (Onset), p=0.98 (Peak), p=0.95(Offset).
2		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10, p=0.11, q=0.069 (onset); df=10, p<0.001, q=0.001(peak);df=10, p=0.04, q=0.032(offset). For the female, df=10, p=0.36, q=0.18 (onset); df=10, p<0.001, q=0.001(peak); df=10, p=0.001, q=0.001(offset).
2	k	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(2,40)=69.85, df=2, p<0.001. Group: F(1,20)=1.243, df=1, p=0.28. Group x time interaction: F(2,40)=0.9988, df=2, p=0.38. Bonferroni's multiple comparisons: p=0.97 (Onset), p=0.30 (Peak), p=0.76(Offset).
2		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10, p=0.97, q=0.34 (onset); df=10, p<0.001, q=0.001(peak); df=10, p=0.005, q=0.003(offset). For the female, df=10, p=0.61, q=0.25 (onset), df=10, p<0.001, q=0.001(peak), df=10, p=0.001, q=0.001(offset).
2	l	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(2,40)=75.26, df=2, p<0.001. Group: F(1,20)=1.060, df=1, p=0.32. Group x time interaction: F(2,40)=6.557, df=2, p=0.003. Bonferroni's multiple comparisons: p=0.84 (Onset), p=0.008 (Peak), p>0.99(Offset).
2		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10, p=0.99, q=0.347 (onset); df=10, p<0.001, q=0.001(peak); df=10, p<0.001, q=0.001(offset). For the female, df=10, p=0.26, q=0.99 (onset); df=10, p<0.001, q=0.001(peak); df=10, p<0.001, q=0.001(offset).
2	o	One sample Wilcoxon test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, p=0.08, q=0.16(object); p=0.41, q=0.43(male); p=0.1, q=0.16(female). For the female, p=0.05, q=0.16(object); p=0.24, q=0.3(male); p=0.05, q=0.16(female).
2-supplement 1	d	One sample t test	df=10, p=0.03 (male), df=10, p<0.001(female).
2-supplement 1		Unpaired t test	t=1.611, df=20, p=0.12.
2-supplement 1	f	One sample t test	df=10, p=0.004 (male), df=10, p<0.001(female).
2-supplement 1		Unpaired t test	t=3.368, df=20, p=0.003.
3	b	One-way ANOVA with Tukey's multiple comparisons test	For the male, F(1.203,12.03)=9.151, df=2, p=0.008, Tukey's multiple comparisons, p=0.008 (object vs. male), p=0.001 (object vs. female), p=0.34 (male vs. female). For the female, F(1.938,19.38)=24.43, df=2, p<0.001, Tukey's multiple comparisons, p=0.02 (object vs. male), p<0.001 (object vs. female), p=0.01 (male vs. female).
3	k	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(2.294,45.86)=34.77, df=3, p<0.001. Group: F(1,20)=1.379, df=1, p=0.25. Group x time interaction: F(3,60)=2.100, df=3, p=0.11. Bonferroni's multiple comparisons: p>0.99 (Onset), p>0.99 (Peak), p=0.22(Offset), p>0.99(Post-offset).
3	k	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10, p<0.001, q=2.1e-4 (onset); df=10, p<0.001, q=2.1e-4 (peak); df=10, p=0.03, q=0.005(offset); df=10, p=0.07, q=0.009(post-offset). For the female, df=10, p<0.001, q=2.1e-4 (onset); df=10, p<0.001, q=2.1e-4 (peak); df=10, p<0.001, q=2.1e-4 (offset); df=10, p=0.02, q=0.004 (post-offset).
3	l	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(2.027,40.55)=119.0, df=3, p<0.001. Group: F(1,20)=0.1028, df=1, p=0.75. Group x time interaction: F(3,60)=1.707, df=3, p=0.18. Bonferroni's multiple comparisons: p>0.99 (Onset), p>0.99 (Peak), p=0.32(Offset), p>0.99(Post-offset).
3	l	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10, p=0.005, q=0.004 (onset); df=10, p<0.001, q=0.001 (peak); df=10, p=0.24, q=0.126(offset); df=10, p=0.04, q=0.158(post-offset). For the female, df=10, p<0.001, q=0.001 (onset); df=10, p<0.001, q=0.001 (peak); df=10, p=0.01, q=0.006 (offset); df=10, p=0.04, q=0.158 (post-offset).
3	m	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(2.473,45.45)=127.4, df=3, p<0.001. Group: F(1,20)=0.8114, df=1, p=0.38. Group x time interaction: F(3,60)=1.030, df=3, p=0.39. Bonferroni's multiple comparisons: p>0.99 (Onset), p>0.99 (Peak), p=0.61(Offset), p>0.99(Post-offset).
3	m	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10, p=0.001, q=0.001 (onset); df=10, p<0.001, q=0.001 (peak); df=10, p=0.06, q=0.032(offset); df=10, p=0.52, q=0.205(post-offset). For the female, df=10, p<0.001, q=0.001 (onset); df=10, p<0.001, q=0.001 (peak); df=10, p=0.02, q=0.013 (offset); df=10, p=0.45, q=0.203 (post-offset).
3	p	One sample Wilcoxon test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, p=0.01, q=0.011(object); p<0.001, q=0.002(male); p<0.001, q=0.002(female). For the female, p=0.01, q=0.11(object); p<0.001, q=0.002(male); p<0.001, q=0.002(female).

4	i	Mann-Whitney test	p<0.001
	k	Two-way ANOVA with Bonferroni's multiple comparison test	Time: $F_{(3,60)}$=36.9, df=3, p<0.001. Group: $F_{(1,20)}$=35.21, df=1, p<0.001. Group x time interaction: $F_{(3,60)}$=47.76, df=3, p<0.001. Bonferroni's multiple comparisons: p<0.001 (Onset), p<0.001 (Peak), p>0.99(Offset), p>0.99(Post-offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10, p=0.006, q=0.001 (onset); df=10, p<0.001, q=2.625e-004 (peak); df=10, p=0.003, q=0.001(offset); df=10, p=0.02, q=0.003(post-offset). For the female, df=10, p=0.05, q=0.007 (onset); df=10, p<0.001, q=2.625e-004 (peak); df=10, p<0.001, q=2.625e-004 (offset); df=10, p<0.001, q=2.625e-004 (post-offset).
	l	Two-way ANOVA with Bonferroni's multiple comparison test	Time: $F_{(3,39)}$=85.48, df=3, p<0.001. Group: $F_{(1,13)}$=1.546, df=1, p=0.24. Group x time interaction: $F_{(3,39)}$=17.70, df=3, p<0.001. Bonferroni's multiple comparisons: p=0.2(Onset), p>0.99(Peak), p<0.001 (Offset), p=0.005(Post-offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, p=0.007, q=0.004 (onset); p<0.001, q=0.001 (peak); p<0.001, q=0.001(offset); p<0.001, q=0.001(post-offset). For the female, p=0.58, q=0.261 (onset); p<0.001, q=0.001 (peak); p=0.16, q=0.084 (offset); p=0.85, q=0.335 (post-offset).
	m	Two-way ANOVA with Bonferroni's multiple comparison test	Time: $F_{(3,54)}$=135.6, df=3, p<0.001. Group: $F_{(1,18)}$=0.6076, df=1, p=0.45. Group x time interaction: $F_{(3,54)}$=19.51, df=3, p<0.001. Bonferroni's multiple comparisons: p=0.2(Onset), p<0.001(Peak), p=0.41 (Offset), p=0.02(Post-offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=7, p<0.001, q=0.001 (onset); df=7, p<0.001, q=0.001 (peak); df=7, p=0.05, q=0.001(offset); df=7, p<0.001, q=0.001(post-offset). For the female, df=6, p=0.003, q=0.002 (onset); df=6, p<0.001, q=0.001 (peak); df=6, p=0.25, q=0.113 (offset); df=6, p=0.81, q=0.319 (post-offset).
	n	Two-way ANOVA with Bonferroni's multiple comparison test	Time: $F_{(3,54)}$=160.8, df=3, p<0.001. Group: $F_{(1,18)}$=0.2174, df=1, p=0.65. Group x time interaction: $F_{(3,54)}$=1.949, df=3, p=0.13. Bonferroni's multiple comparisons: p>0.99(Onset), p>0.99(Peak), p=0.38 (Offset), p>0.99(Post-offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10, p=0.07, q=0.147 (onset); df=10, p<0.001, q=0.003 (peak); df=10, p=0.13 q=0.167(offset); df=10, p=0.10, q=0.158(post-offset). For the female, df=8, p=0.61, q=0.558 (onset); df=8, p<0.001, q=0.003 (peak); df=8, p=0.62, q=0.558 (offset); df=8, p=0.80, q=0.63 (post-offset).
	o	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, p=0.14, q=0.44(mount unreceptive); p=0.07, q=0.25(mount receptive); p=0.51, q=0.64(intromission). For the female, p=0.47, q=0.64(mount unreceptive); p=0.7, q=0.74(mount receptive); p=0.46, q=0.64(intromission).
5	f	Two-way ANOVA with Bonferroni's multiple comparison test	Time: $F_{(3,45)}$=31.16, df=3, p<0.001. Group: $F_{(1,15)}$=22.92, df=1, p<0.001. Group x time interaction: $F_{(3,45)}$=4.979, df=3, p=0.005. Bonferroni's multiple comparisons: p=0.10(Onset), p<0.001(Peak), p=0.07(Offset), p>0.99(Post-offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=9, p<0.001, q=0.001 (onset); df=9, p<0.001, q=0.001 (peak); df=9, p<0.001 q=0.001(offset); df=9, p=0.08, q=0.056(post-offset). For the female, df=6, p=0.04, q=0.034(onset); df=6, p=0.002, q=0.002 (peak); df=6, p=0.59, q=0.31(offset); df=6, p=0.43, q=0.258 (post-offset).
	g	Mann-Whitney test	p=0.60
	h	Friedman test with with Dunn's multiple comparison test	p<0.001. Dunn's multiple comparisions, p>0.99 (b3 vs. b1), p>0.99 (b3 vs. b2), p<0.001 (b3 vs. i1), p=0.10 (b3 vs. i2), p=0.52 (b3 vs. i3), p>0.99 (b3 vs. i4), p=0.25 (b3 vs. i5), p=0.46 (b3 vs. i6), p=0.36 (b3 vs. i7), p=0.19 (b3 vs. i8), p>0.99 (b3 vs. i9), p=0.25 (b3 vs.i10), p>0.99 (b3 vs.a1), p>0.99 (b3 vs.a2), p>0.99 (b3 vs.a3).
	i	Friedman test with with Dunn's multiple comparison test	p=0.004. Dunn's multiple comparisions, p=0.32 (b3 vs. b1), p=0.65 (b3 vs. b2), p=0.37 (b3 vs. i1), p>0.99 (b3 vs. i2), p>0.99 (b3 vs. i3), p>0.99 (b3 vs. i4), p>0.99 (b3 vs. i5), p>0.99 (b3 vs. i6), p>0.99 (b3 vs. i7), p>0.99 (b3 vs. i8), p>0.99 (b3 vs. i9), p>0.99 (b3 vs.i10), p>0.99 (b3 vs.a1), p>0.99 (b3 vs.a2), p>0.99 (b3 vs.a3).
5-supplement 1	g	Two-way ANOVA with Bonferroni's multiple comparison test	Time: $F_{(3,40)}$=141.6, df=3, p<0.001. Group: $F_{(1,20)}$=0.2653, df=1, p=0.61. Group x time interaction: $F_{(2,40)}$=0.6226, df=2, p=0.54. Bonferroni's multiple comparisons: p>0.99(Onset), p=0.80(Peak), p>0.99(Offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10, p=0.005, q=0.005 (onset); df=10, p<0.001, q=0.002(peak);df=10, p=0.002, q=0.003(offset). For the female, df=10,p=0.004, q=0.005 (onset); df=10,p<0.001, q=0.002(peak); df=10,p<0.001, q=0.002(offset).
	h	Two-way ANOVA with Bonferroni's multiple comparison test	Time: $F_{(3,60)}$=303.7, df=3, p<0.001. Group: $F_{(1,20)}$=1.537, df=1, p=0.23. Group x time interaction: $F_{(3,60)}$=5.945, df=3, p=0.001. Bonferroni's multiple comparisons: p=0.08(Onset), p=0.06(Peak), p=0.96(Offset), p>0.99(Post-offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10,p<0.001, q=0.001 (onset); df=10,p<0.001, q=0.001 (peak); df=10,p=0.25, q=0.131(offset); df=10,p=0.31, q=0.134 (post-offset). For the female, df=10,p<0.001, q=0.001 (onset); df=10, p<0.001, q=0.001 (peak); df=10, p<0.001, q=0.001 (offset); df=10, p=0.34, q=0.134 (post-offset).
	j	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=10,p=0.1, q=0.14(app); df=10,p=0.28, q=0.29(eat). For the female, df=10,p=0.02, q=0.06 (app), df=10,p=0.03, q=0.06(eat).
	k	One-way ANOVA with Tukey's multiple comparisons test	For the male, $F_{(1.34,12.06)}$=1.166, df=2, p=0.32. For the female, $F_{(1.546, 18.55)}$=21.78, p<0.001, Tukey's multiple comparisons, p=0.008 (eat vs. intromit), p=0.001 (eat vs. attack), p=0.30 (intromit vs. attack).
	c	Friedman test	p=0.58.

5-supplement 2	d	One-way ANOVA with Tukey's multiple comparisons test	F(1.976, 7.906)=37.67, df=15,p<0.001. Tukey's multiple comparisions, p=0.90 (b3 vs. b1), p>0.99 (b3 vs. b2), p=0.91 (b3 vs. a1), p=0.52 (b3 vs. a2), p=0.03 (b3 vs. a3), p=0.03 (b3 vs. a4), p=0.03 (b3 vs. a5), p=0.01 (b3 vs. a6), p=0.002 (b3 vs. a7), p=0.002 (b3 vs. a8), p=0.001 (b3 vs. a9), p<0.001 (b3 vs. a10), p<0.001 (b3 vs. a11), p<0.001 (b3 vs. a12), p=0.001 (b3 vs. a13).
6	k	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=4, p=0.11, q=0.05 (HM), df=4, p=0.31,q=0.122 (NHM), df=9, p=0.77,q=0.249(FM); for the during, df=4, p=0.002,q=0.002 (HM), df=4, p=0.009,q=0.005 (NHM), df=9, p<0.001,q=0.001 (FM); for the offset, df=4, p=0.001,q=0.001 (HM),df=4, p=0.003,q=0.002 (NHM), df=9, p<0.001, q=0.001(FM).
		One-way ANOVA with Tukey's multiple comparisons test	For the onset, F(2,17)=1.280,df=2,p=0.3. For the during, F(2,17)=19.85,df=2,p<0.001; Tukey's multiple conparisions, p=0.08(HM vs. NHM), p<0.001(HM vs. FM), p=0.008(NHM vs. FM). For the offset, F(2,17)=27.99,df=2,p<0.001; Tukey's multiple conparisions, p=0.3(HM vs. NHM), p<0.001(HM vs. FM), p<0.001(NHM vs. FM).
	l	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=4, p<0.001, q=0.001 (HM), df=4, p<0.001,q=0.001 (NHM), df=9,p<0.001,q=0.001(FM); for the during, df=4,p<0.001,q=0.001 (HM), df=4,p<0.001,q=0.001 (NHM), df=9,p<0.001,q=0.001 (FM); for the offset, df=4,p=0.82,q=0.431 (HM), df=4,p=0.39,q=0.223 (NHM),df=9, p=0.20, q=0.126(FM); for the post-offset, df=4, p=0.07,q=0.063 (HM), df=4, p=0.09,q=0.071 (NHM), df=9, p=0.12, q=0.084(FM).
		One-way ANOVA with Tukey's multiple comparisons test	For the onset, F(2,17)=2.737,df=2,p=0.09. For the during, F(2,17)=19.85,df=2,p<0.001; Tukey's multiple conparisions, p=0.08(HM vs. NHM), p<0.001(HM vs. FM), p=0.008(NHM vs. FM). For the offset, F(2,17)=27.99,df=2,p<0.001; Tukey's multiple conparisions, p=0.3(HM vs. NHM), p<0.001(HM vs. FM), p<0.001(NHM vs. FM).
	m	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=4,p=0.07, q=0.110; for the during, df=4,p<0.001,q=0.003; for the offset, df=4,p=0.90,q=0.709; for the post-offset, df=4,p=0.66,q=0.693.
	n	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=9, p<0.001, q=0.002; for the during, df=9, p<0.001,q=0.002; for the offset, df=9, p=0.002,q=0.003; for the post-offset, df=9, p=0.003,q=0.003.
7	k	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=9, p=0.002, q= 0.001(NNF), df=5, p=0.22,q=0.051 (NMF), df=9, p=0.21,q=0.051(MF); for the during, df=9, p<0.001,q=0.001 (NNF), df=5, p=0.002,q=0.001 (NMF), df=9, p<0.001,q=0.001 (MF); for the offset, df=9, p<0.001,q=0.001 (NNF), df=5, p=0.01,q=0.003 (NMF), df=9, p<0.001, q=0.001(MF).
		One-way ANOVA with Tukey's multiple comparisons test	For the onset, F(2,23)=5.216,df=2,p=0.01; Tukey's multiple conparisions, p=0.63(NNF vs. NMF), p=0.01(NNF vs. MF), p=0.18(NMF vs. MF). For the during, F(2,23)=7.914,df=2,p=0.002; Tukey's multiple conparisions,p=0.66(NNF vs. NMF), p=0.002(NNF vs. MF), p=0.05(NMF vs. MF). For the offset, F(2,23)=7.564,df=2,p=0.003; Tukey's multiple conparisions, p=0.55(NNF vs. NMF), p=0.002(NNF vs. MF), p=0.08(NMF vs. MF).
	l	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=9, p<0.001, q= 0.001(NNF), df=5, p=0.007,q=0.007 (NMF), df=9, p<0.001,q=0.001(MF); for the during, df=9, p<0.001,q=0.001 (NNF), df=5, p<0.001,q=0.001 (NMF), df=9, p<0.001,q=0.001 (MF); for the offset, df=9, p=0.87,q=0.457 (NNF), df=5, p=0.36,q=0.252 (NMF), df=9, p=0.27, q=0.213(MF);for the post-offset, df=9, p=0.07,q=0.063 (NNF), df=5, p=0.51,q=0.292(NMF), df=9, p=0.42, q=0.265(MF).
		One-way ANOVA with Tukey's multiple comparisons test	For the onset, F(2,23)=2.195,df=2,p=0.13. For the during, F(2,23)=5.842,df=2,p=0.009; Tukey's multiple conparisions,p=0.007(NNF vs. NMF), p=0.19(NNF vs. MF), p=0.18(NMF vs. MF). For the offset, F(2,23)=0.6654,df=2,p=0.52. For the post-offset, F(2,23)=1.946,df=2,p=0.17.
	m	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=5, p=0.04,q=0.053 (NMF), df=9, p<0.001,q=0.002(MF); for the during, df=5, p<0.001,q=0.002 (NMF), df=9, p<0.001,q=0.002 (MF); for the offset, df=5, p=0.92,q=0.604 (NMF), df=9, p=0.58, q=0.435(MF);for the post-offset, df=5, p=0.27,q=0.236(NMF), df=9, p=0.09, q=0.095(MF).
		Unpaired t test	For the onset, t=0.9661, df=14, p=0.35; for the during, t=1.208, df=14, p=0.25; for the offset, t=0.1535, df=14, p=0.88; for the post-offset, t=0.1388, df=14, p=0.89.
	d	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(3,54)=117.7, df=3, p<0.001. Group: F(1,18)=1.443, df=1, p=0.25. Group x time interaction: F(3,54)=2.594, df=3, p=0.06. Bonferroni's multiple comparisons: p>0.99(Onset), p>0.99(Peak), p>0.99(Offset), p=0.02(Post-offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=10, p=0.03, q= 0.011(male); df=8, p=0.22, q=0.066(female).For the during, p<0.001, df=10,q=0.001(male); df=8, p<0.001, q=0.001(female). For the offset, df=10,p=0.005, q=0.002 (male); df=8, p=0.31, q=0.081(female). For the post-offset, df=10, p<0.001, q=0.001(male); df=8, p<0.001, q=0.001(female).
	h	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(3,60)=183.0, df=3, p<0.001. Group: F(1,20)=1.143, df=1, p=0.30. Group x time interaction: F(3,60)=9.848, df=3, p<0.001. Bonferroni's multiple comparisons: p>0.99(Onset), p=0.86(Peak), p>0.99(Offset), p<0.001(Post-offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=10, p=0.01, q= 0.011(male); df=10, p=0.001, q=0.001(female).For the during,df=10, p<0.001, q=0.001(male); df=10, p<0.001, q=0.001(female). For the offset,df=10, p<0.001, q=0.001 (male); df=10, p<0.001, q=0.001(female). For the post-offset, df=10,p<0.001, q=0.001(male); df=10, p=0.002, q=0.002(female).
		Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(3,33)=92.07, df=3, p<0.001. Group: F(1,11)=2.695, df=1, p=0.13. Group x time interaction: F(3,33)=9.848, df=3, p=0.17. Bonferroni's multiple comparisons: p>0.99(Onset), p>0.99(Peak), p>0.99(Offset), p=0.03(Post-offset).

8	l	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=5, p=0.14, q= 0.037(male); df=6, p=0.07, q=0.021(female).For the during, p<0.001, df=5,q=0.001(male); df=6, p<0.001, q=0.001(female). For the offset, df=5,p=0.03, q=0.011(male); df=6, p=0.01, q=0.004(female). For the post-offset, df=5,p<0.001, q=0.001(male); df=6, p=0.003, q=0.002(female).
	p	Two-way ANOVA with Bonferroni's multiple comparison test	Time: F(3,30)=74.71, df=3, p<0.001. Group: F(1,10)=0.3665, df=1, p=0.56. Group x time interaction: F(3,30)=0.3650, df=3, p=0.78. Bonferroni's multiple comparisons: p>0.99(Onset), p>0.99(Peak), p>0.99(Offset), p>0.99(Post-offset).
		One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the onset, df=4, p=0.02, q= 0.008(male); df=6, p=0.18, q=0.053(female).For the during, df=4, p<0.001, q=0.001(male); df=6, p<0.001, q=0.001(female). For the offset, df=4, p<0.001, q=0.001(male); df=6, p<0.001, q=0.001(female). For the post-offset,df=4, p=0.02, q=0.053(male); df=6, p=0.03, q=0.011(female).
	t	During: Friedman test	p=0.43.
		Rebound: One-way ANOVA with Tukey's multiple comparisons test	F(1.124, 5.618)=19.17, df=3, p=0.005. Tukey's multiple conparisions, p=0.97 (5s vs. 10s), p=0.04 (5s vs. 20s), p=0.01 (5s vs. 60s), p=0.05 (10s vs. 20s), p=0.03 (10s vs. 60s), p=0.03 (20s vs. 60s).
	u	During: One-way ANOVA with Tukey's multiple comparisons test	F(1.567, 9.405)=0.5401, df=3, p=0.56. Tukey's multiple conparisions, p=0.59 (5s vs. 10s), p=0.73 (5s vs. 20s), p=0.98 (5s vs. 60s), p>0.99 (10s vs. 20s), p=0.95 (10s vs. 60s), p=0.85 (20s vs. 60s).
		Rebound: One-way ANOVA with Tukey's multiple comparisons test	F(1.255, 7.527)=19.25, df=3, p=0.002. Tukey's multiple conparisions, p>0.99 (5s vs. 10s), p=0.53 (5s vs. 20s), p=0.02 (5s vs. 60s), p=0.1 (10s vs. 20s), p=0.009 (10s vs. 60s), p=0.006 (20s vs. 60s).
	v	One-way ANOVA with Tukey's multiple comparisons test	F(2.286, 27.43)=27.27, df=3, p<0.001. Tukey's multiple comparisons, p=0.06 (5s vs. 10s), p<0.001 (5s vs. 20s), p<0.001 (5s vs. 60s), p=0.005 (10s vs. 20s), p=0.004 (10s vs. 60s), p=0.31 (20s vs. 60s).
	w	During: One-way ANOVA with Tukey's multiple comparisons test	F(1.751, 12.26)=9.89, df=2, p=0.003. Tukey's multiple conparisions, p=0.03 (2s vs. 5s), p=0.004 (2s vs. 10s), p=0.64 (5s vs. 10s).
		Rebound: One-way ANOVA with Tukey's multiple comparisons test	F(1.359, 9.512)=41.62, df=2, p<0.001. Tukey's multiple conparisions, p=0.09 (2s vs. 5s), p<0.001 (2s vs. 10s), p<0.001 (5s vs. 10s).
	x	Paired t test	t=0.4245, df=7, p=0.68.
8-supplement 1	a	One sample Wilcoxon test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, p=0.28, q=0.7(During); p=0.97, q=1(Rebound). For the female, p=0.50, q=0.7(During); p=0.50, q=0.7(Rebound).
	b	One sample Wilcoxon test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, p=0.05, q=0.21(During); p=0.28, q=0.59(Rebound). For the female, p=0.46, q=0.64(During); p=0.97, q=1(Rebound).
	c	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=5, p=0.61, q=0.77(During); df=5, p=0.73, q=0.77(Rebound). For the female,df=6, p=0.34, q=0.77(During); df=6, p=0.53, q=0.77(Rebound).
	d	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	For the male, df=4, p=0.45, q=0.6(During); df=4, p=0.57, q=0.6(Rebound). For the female, df=6, p=0.02, q=0.08 (During); df=6, p=0.15, q=0.32(Rebound).
8-supplement 2	e	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	df=2, p=0.21, q= 0.38(Eat food); df=2, p=0.27, q=0.38 (Attack); df=2, p=0.09, q=0.38(Foot shock); df=2, p=0.88, q=0.92(Tail suspension).
	f	One sample t test followed by two-stage step-up method of Benjamini, Krieger and Yekutieli with 0.05 FDR	df=2, p=0.3, q= 0.45(Eat food); df=2, p=0.7, q=0.74 (Attack); df=2, p=0.32, q=0.45(Foot shock); df=2, p=0.13, q=0.45(Tail suspension).