Activation of GLP-1 Receptors Suppresses Pathological Defensive Aggression via DMPAG in Mice

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Figure S1. Temporal effects of a single central liraglutide administration on defensive aggression in male and female mice. (A) Experimental timeline. Group-housed male and female mice were implanted with ICV cannulas and allowed to recover for 7 days. Liraglutide or saline was administered 20 min before DAT on day 14, and DAT was subsequently assessed once daily from day 15 to day 18. **(B)** Quantification of latency of biting, number of biting, and time for biting in male mice. **(C)** Quantification of latency of biting, number of biting, and time for biting in female mice. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001. GLP-1RA, glucagon-like peptide-1 receptor agonist; ICV, intracerebroventricular; DAT, defensive attack test.

Figure S2. Acute peripheral liraglutide administration does not alter OFT or DAT performance in male and female mice. (A) Experimental timeline. Group-housed male and female mice received I.P. administration of liraglutide or saline 20 min before testing. The OFT was performed 1 day before the DAT. **(B, D)** Representative traces and quantification of total distance traveled and time spent in the center in the OFT in male (B)

and female (D) mice. **(C, E)** Quantification of latency of biting, number of biting, and time for biting in the DAT in male (C) and female (E) mice. GLP-1RA, glucagon-like peptide-1 receptor agonist; I.P., intraperitoneal; OFT, open field test; DAT, defensive attack test.

Figure S3. Local co-administration of carnosine and MK-801 in the DMPAG does not clearly reverse the suppressive effect of carnosine on defensive aggression. (A) Experimental timeline. Group-housed male mice were implanted with cannulas and allowed to recover for 7 days. Carnosine, MK-801, or carnosine + MK-801 was administered into the DMPAG 10 min before DAT. **(B)** Quantification of latency of biting, number of biting, and time for biting in the DAT. *$p < 0.05$; **$p < 0.01$. DAT, defensive attack test; DMPAG, dorsomedial periaqueductal gray.

Figure S4. Representative immunofluorescence image showing GLP-1R immunoreactivity and c-Fos-positive cells in the DMPAG. (A) Representative image of GLP-1R immunoreactivity (green), c-Fos-positive cells (red), and DAPI (blue) staining in the DMPAG, with an enlarged view of the highlighted region. DMPAG, dorsomedial periaqueductal gray; GLP-1R, glucagon-like peptide-1 receptor.

Figure.S1

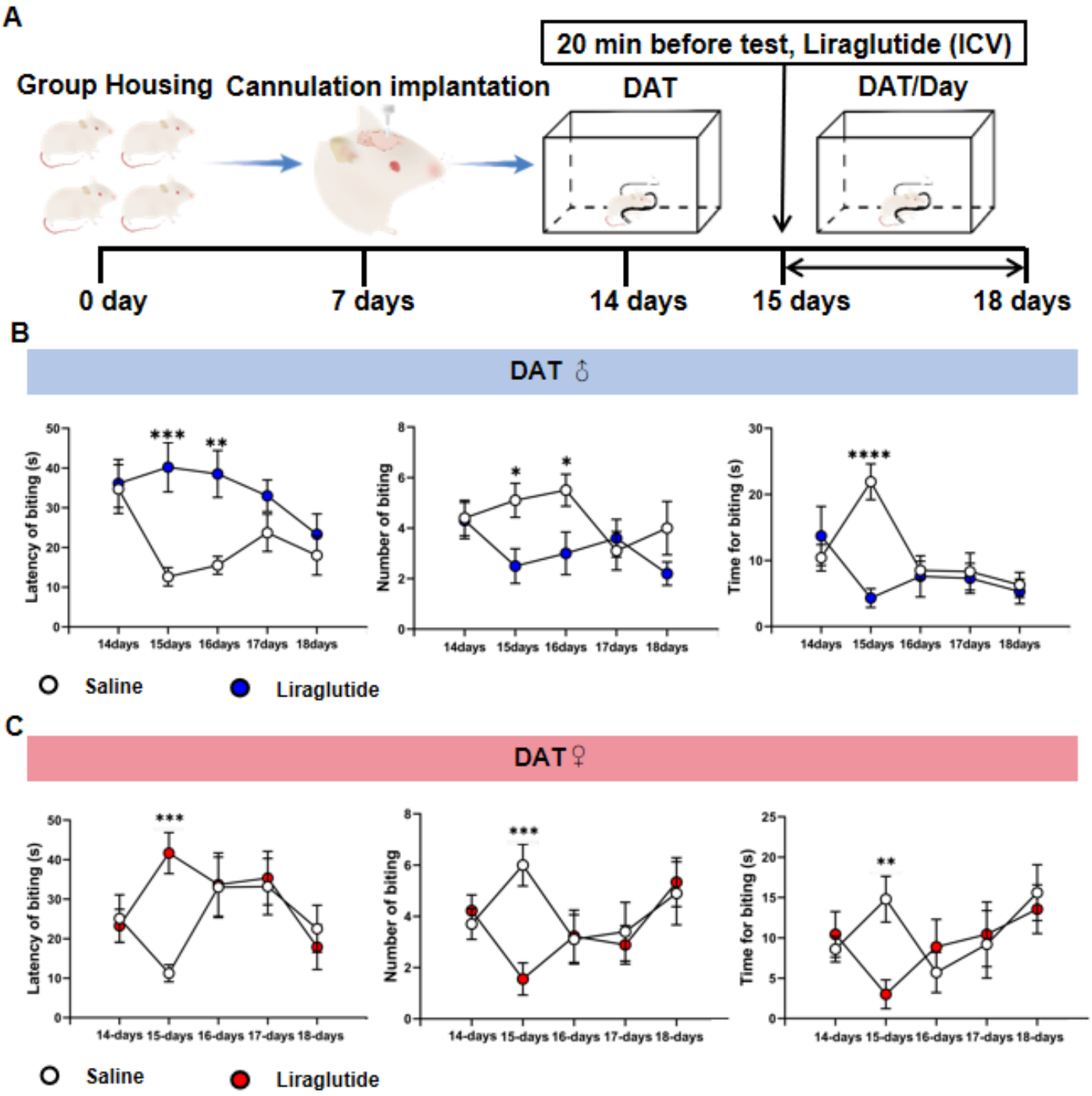
A
20 min before test, Liraglutide (ICV)
Group Housing
Cannulation implantation
DAT
DAT/Day
0 day
7 days
14 days
15 days
18 days
B
DAT ♂
Latency of biting (s)
Number of biting
Time for biting (s)
14days 15days 16days 17days 18days
Saline
Liraglutide
C
DAT ♀
Latency of biting (s)
Number of biting
Time for biting (s)
14-days 15-days 16-days 17-days 18-days
Saline
Liraglutide

Figure.S2

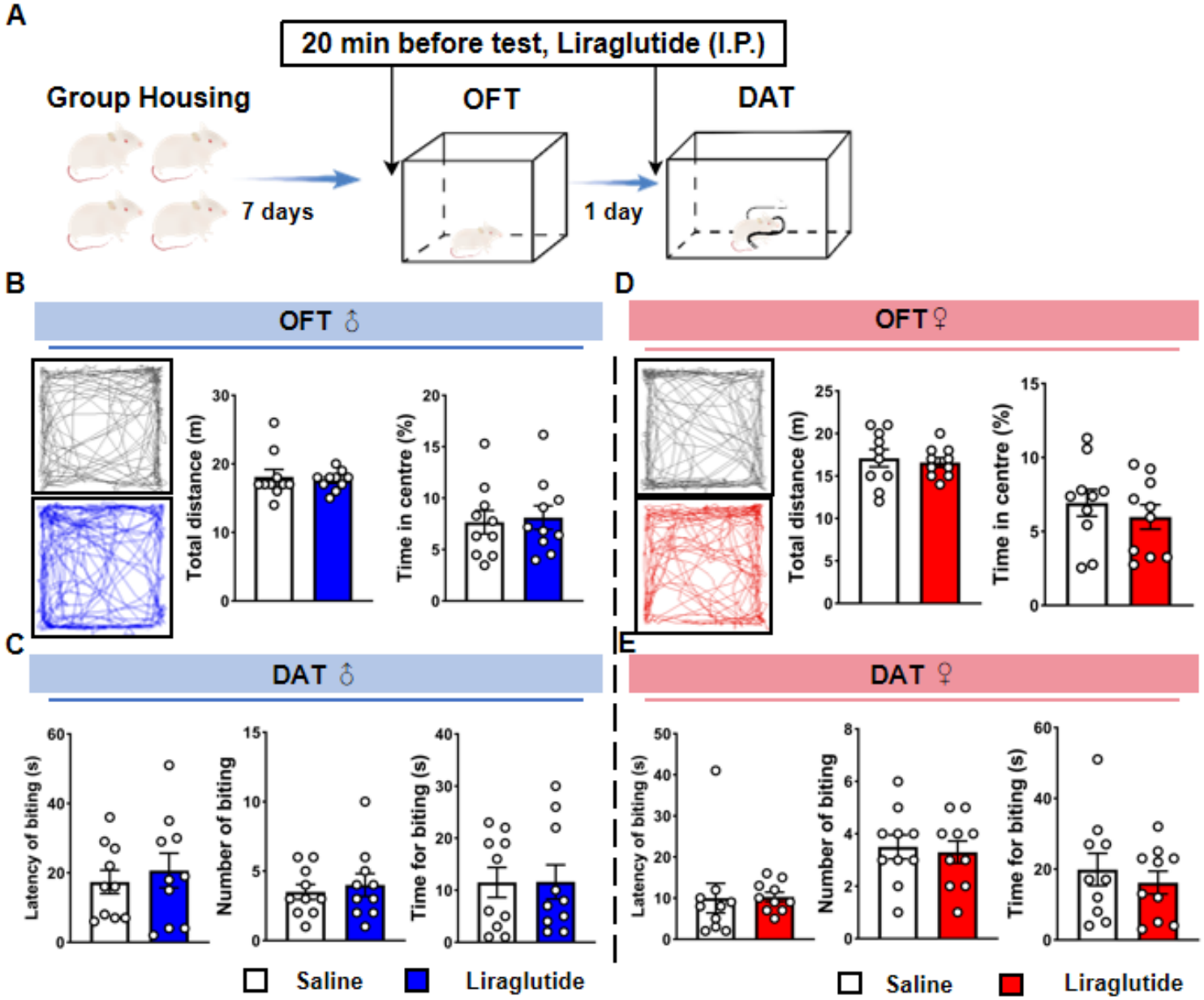
A
20 min before test, Liraglutide (I.P.)
Group Housing
OFT
DAT
7 days
1 day
B
OFT ♂
Total distance (m)
Time in centre (%)
C
DAT ♂
Latency of biting (s)
Number of biting
Time for biting (s)
D
OFT ♀
Total distance (m)
Time in centre (%)
E
DAT ♀
Latency of biting (s)
Number of biting
Time for biting (s)
Saline
Liraglutide
Saline
Liraglutide

Figure.S3

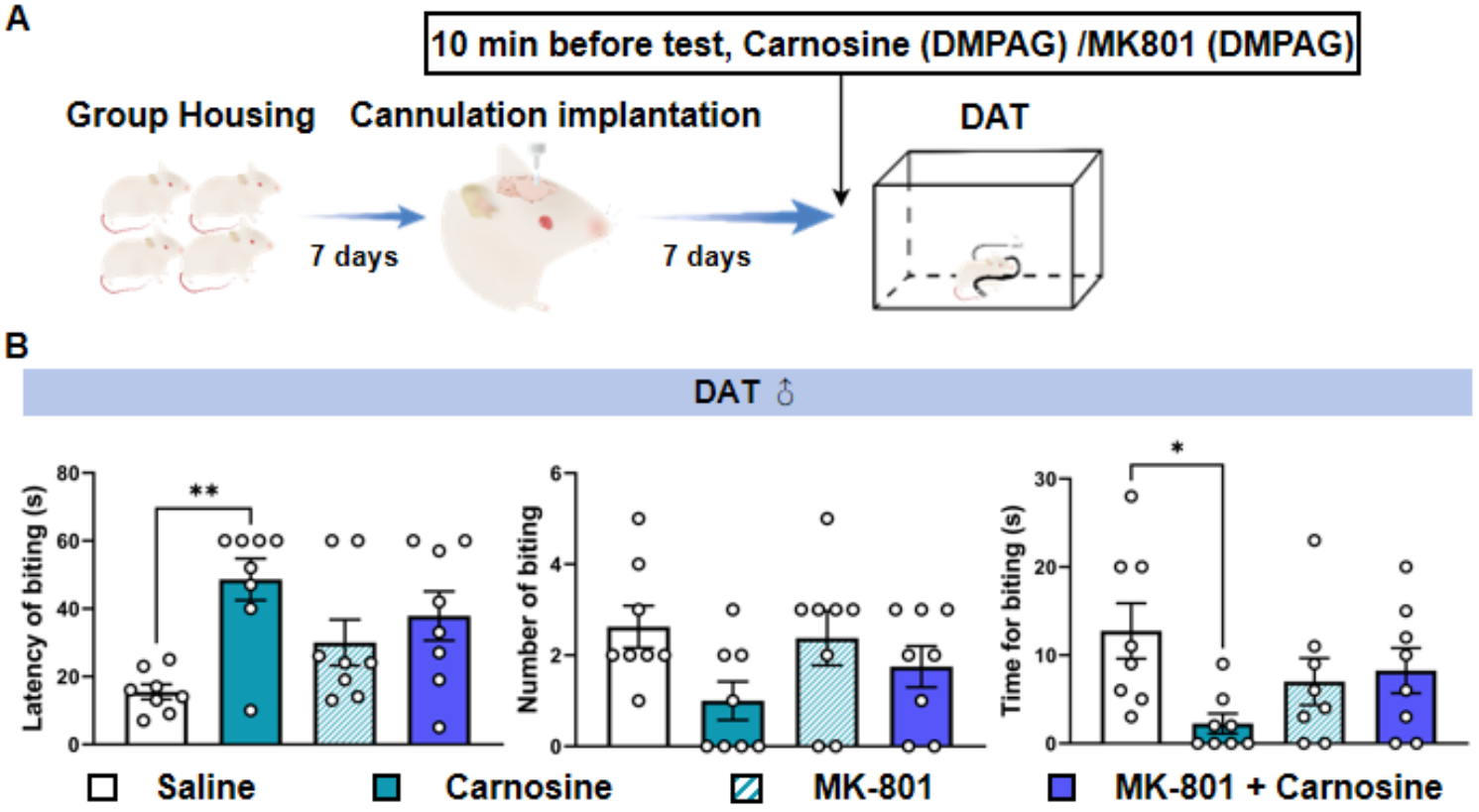
A
10 min before test, Carnosine (DMPAG) /MK801 (DMPAG)
Group Housing
Cannulation implantation
DAT
7 days
7 days
B
DAT ♂
Latency of biting (s)
Number of biting
Time for biting (s)
**
*
Saline
Carnosine
MK-801
MK-801 + Carnosine

Figure.S4

A