

SUPPLEMENTARY INFORMATION

Inhibition of the medial amygdala disrupts escalated aggression in lactating dams after repeated exposure to male intruders

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Experiment 1 Effect of repeated exposure to a male intruder in maternal aggression in dams and pup-sensitized virgin female mice

In contrast to dams, pup-sensitized females displayed socio-sexual investigation but not maternal aggression toward males. The aggressive behaviour of pup-sensitized females was very low (in fact, tending to 0), and the Friedman test for non-parametric samples did not reveal statistically significant differences between the mean ranks of the related groups ($\chi^2(2) = 0$, $p = 1$) in the total and mean duration of attacks (Table S1). Regarding socio-sexual investigation, neither anogenital approaches to males ($F_{2,6} = 0.33$, $p = 0.967$) (Table S1), nor body approaches ($F_{2,6} = 3.537$, $p = 0.097$) (Table S1) were significantly different across testing days. Thus, socio-sexual investigation of males did not change with repeated testing in pup-sensitized females.

Table S1. Quantification of the different socio-sexual behaviours displayed by males and pup-sensitized virgin females during the testing days. Data are represented as mean $\pm$ SEM. Postpartum day (PPD) (-) not measured.

	Total Duration attacks (s)			Anogenital approaches (s)			Body approaches (s)		
	PPD4	PPD5	PPD6	PPD4	PPD5	PPD6	PPD4	PPD5	PPD6
Pup-sensitized virgin females toward males	0,83 $\pm$ 0,83	0,01 $\pm$ 0,01	0,01 $\pm$ 0,01	7,39 $\pm$ 2,51	7,66 $\pm$ 2,42	7,44 $\pm$ 1,93	17,04 $\pm$ 4,39	29,53 $\pm$ 4,72	17,2 $\pm$ 5,94
Males toward pup-sensitized virgin females	-	-	-	32,53 $\pm$ 8,48	14,16 $\pm$ 4,33	32,83 $\pm$ 7,12	9,18 $\pm$ 2,15	12,48 $\pm$ 4,73	17,2 $\pm$ 5,94
Males toward Dams	-	-	-	0,14 $\pm$ 0,11	0,18 $\pm$ 0,18	2,25 $\pm$ 2,22	0	0	0

Males could spend little time displaying ano-genital approaches across testing days ($\chi^2(2) = 0,154$, $p = 0,926$) and could not initiate body approaches to lactating females, since they were extensively attacked (Figure 3 and Table S1). In the case of male anogenital approaches to pup-sensitized females, the repeated measures ANOVA revealed a significant effect of the factor DAY ($F_{2,6} = 7.485$, $p = 0.023$), but Bonferroni post-hoc did not show significant differences between the studied PPD ($p > 0.05$) (Table S1). In the case of males' approaches to the body of pup-sensitized females, the repeated measures ANOVA revealed a significant main effect of the factor DAY ($F_{2,6} = 0.845$, $p = 0.475$) but Bonferroni post-hoc did not show significant differences between the studied PPD ($p > 0.05$) (Table S1). In conclusion, the behaviour of males did not change significantly across testing days, and therefore the behavioural changes observed in females is unlikely to depend on how males behave.

Experiment 2: Effect of DREADD-induced inhibition of Me in maternal aggression

Table S2. Semiquantitative rating of the injection sites in the Me showing the extent of the DREADD infections in the different nuclei adjacent to the Me. ++++ very dense; + moderate to scarce; 0 not found. Anterior cortical amygdaloid nucleus (ACo), basomedial amygdaloid nucleus (BM), bed nucleus of the stria terminalis intraamygdaloid division (BSTIA), central amygdaloid nucleus (Ce), anterior medial amygdaloid nucleus (MeA), posteroventral medial amygdaloid nucleus (MePV) and posterodorsal medial amygdaloid nucleus (MePD), and supraoptic nucleus (SO).

TREATMENT	SUBJECT CODE	MeA	MePD	MePV	SO	ACo	BM	Ce	BSTIA
GROUP 1 CNO PPD4 VEH PPD5	1662	++++	++++	++++	++++	0	0	+	++++
	1873	++++	+	++++	++++	0	0	++++	++++
	1678	++++	++++	0	0	0	0	0	0
	1677	++++	++++	++++	++++	0	0	0	++++
	1680	++++	++++	++++	0	0	0	0	++++
	17105	++++	++++	++++	++++	++++	++++	+	0
	1794	++++	++++	++++	0	0	++++	++++	++++
GROUP 2 VEH PPD4 CNO PPD5	1665	++++	++++	++++	+	0	0		++++
	1667	++++	++++	++++	++++	+	0	++++	++++
	1669	++++	++++	++++	+	0	0	++++	++++
	1785	++++	0	0	0	++++	0	0	++++
	1788	++++	++++	0	++++	++++	++++	0	0
	1795	++++	++++	0	0	++++	0	0	0
	1709	++++	++++	++++	0	0	0	0	++++
	1786	++++	+	+	++++	++++	0	0	0

To test for the possible effect of CNO or vehicle injections, we added two groups of females without AAV injections, which received CNO injection in PPD4 and Vehicle in PPD5 (n=7) or Vehicle in PPD4 and CNO in PPD5 (n=8). For the total time devoted to aggression, we found a significant main effect of the factor DAY ($F_{1,16} = 38,47$, $p < 0,001$), but no significant main effect of TREATMENT ($F_{1,16} = 0,002$, $p > 0.6$) or DAY X TREATMENT ($F_{1,16} = 0,017$, $p > 0.6$) (Table S3). Similarly, for the latency to attack we found significant main effect of the factor DAY ($F_{1,16} = 15,21$, $p = 0.001$), but no significant main effect of TREATMENT ($F_{1,16} = 0,069$, $p > 0.6$) or DAY X TREATMENT interaction ($F_{1,16} = 0,003$, $p > 0.6$) (Table S3).

Table S3. Quantification of total duration of attacks and latency to attack displayed by lactating females injected with either vehicle or CNO in PPD4 and PPD5. Results reveal a significant increase of the total duration of attacks and a significant reduction of the latency to attack irrespective of the group. Data are represented as mean $\pm$ SEM. ** $p < 0.01$; *** $p < 0.001$

		Total Duration of Attacks (s)		Latency to Attack (s)	
		PPD4	PPD5	PPD4	PPD5
		CNO	VEH	VEH	CNO
GROUP 1		11,93 $\pm$ 4,62	31,67 $\pm$ 5,20***	43,82 $\pm$ 15,99	7,96 $\pm$ 2,21**
GROUP 2		12,91 $\pm$ 3,61	38,36 $\pm$ 7,41***	29,66 $\pm$ 3,89	21,99 $\pm$ 15,15**