

Dorsal raphe serotonin neurotransmission is required for the expression of nursing behavior and for pup survival

Aude Muzerelle<sup>1</sup>, Mariano Soiza-Reilly<sup>1,2</sup>, Cornelia Hainer<sup>3</sup>, Pierre-Louis Ruet<sup>1</sup>, Klaus-Peter Lesch<sup>4,5,6</sup>, Michael Bader<sup>3,7,8,9</sup>, Natalia Alenina<sup>3,7,10,11,\*</sup>, Sophie Scotto-Lomassese<sup>1,13,\*</sup> and Patricia Gaspar<sup>1,12, 13,\*</sup>

**Supplementary Figure 1. Normal maternal responsiveness in primiparous Pet1-KO mothers after mild stress**

(A) The number of retrieved pups (in %) was not different between Pet1-KO and control dams, despite a prior exposure to a mild stress (mean  $\pm$  SEM; MW U=7.5, p=0.18). (B) The latency to retrieve (in seconds) was not different between Pet1-KO and control dams, despite a prior exposure to a mild stress (mean  $\pm$  SEM; repeated-measures two-way ANOVA: genotype x pup number interaction  $F_{4,26}=1.438$ , p=0.25; Genotype main effect  $F_{1,9}=0.8936$ , p=0.37).

**Supplementary Figure 2. Comparison of the maternal activities at PPD0 from primiparous Pet1-KO and Tph2-KO mothers**

Histograms show the time dedicated to pup-oriented and non-pup-oriented activities of the primiparous Pet1-KO and Tph2-KO mothers compared to their respective controls (in % of total time). Presence in the nest was significantly reduced for the Tph2-KO mothers but not for the Pet1-KO mothers. When all the mothers were in the nest with all or only a fraction of their pups, no differences were found in pup licking and nest rearrangement activities between mutant and control mothers of the two mouse lines. By contrast, both Pet1-KO and Tph2-KO mothers spent significantly less time to pup nursing compared to controls. In addition, Pet1-KO mothers spent significantly more time to non-pup-oriented activities inside the nest, such as sleeping or self-grooming (MW U=17, p=0.04); \* p<0.05, \*\* p<0.01 (mean  $\pm$  SEM, Mann-Whitney test).

**Supplementary Figure 3. Maternal experience does not improve reproductive success and nursing behavior in Pet1-KO females**

Primiparous Pet1-KO and control (Pet1<sup>+/-</sup>) were mated to WT males. The number of litters analyzed was n=8 for Pet1-KO dams and n=10 for control dams. All litters were culled to 5 pups. (A) Survival of pups raised by Pet1-KO dams was strongly reduced; \*\*\*\* p<0.0001 (mean  $\pm$  SEM, Log-rank Mantel-Cox test). (B) Nest building score at PPD1 was similar between both groups of dams (mean  $\pm$  SEM, MW U=40, p>0.99). (C) No difference in the latency to retrieve the 5 pups into the nest was found between secondiparous Pet1-KO and control mothers (mean  $\pm$  SEM, two-way ANOVA: genotype x pup number interaction  $F_{4,54}=0.8489$ , p=0.50; Genotype main effect  $F_{1,15}=0.1101$ , p=0.74). (D-E) Time spent outside the nest did not differ between both groups of dams, but the Pet1-KO dams dedicated less time to nurse their pups; \* p<0.05 (mean  $\pm$  SEM, Mann-Whitney test).

**Supplementary Figure 4. Weight at birth and semi-quantitative scoring of the milk pouch**

(A) There are no differences in the weight of pups per litter at birth between Pet1-KO and control dams, irrespective of maternal experience (mean per litter  $\pm$  SEM; two-way ANOVA: Genotype x Maternal Experience interaction  $F_{1,11}=1.381$ , p=0.27; Genotype main effect  $F_{1,20}=0.01917$ , p=0.89; Maternal Experience main effect  $F_{1,11}=2.783$ , p=0.12). (B) Representative pictures of the semi-

quantitative milk pouch scoring from PPD1 pups. Left panel: score 1= pouch with little milk visible. Middle panel: score 2= visible milk pouch. Right panel: score 3= pouch full of milk. Image source: A. Muzerelle and S. Scotto-Lomassese (UMR-S 1270).

**Supplementary Figure 5. Maternal responsiveness of experienced DRN<sup>control</sup> and DRN<sup>TPH2-CKO</sup> mothers**

(A) Nest building score at PPD1 was similar between DRN<sup>TPH2-CKO</sup> and DRN<sup>control</sup> dams (mean  $\pm$  SEM, Mann-Whitney test  $U=62.5$ ,  $p=0.56$ ). (B) No difference in the latency to retrieve 5 pups into the nest was found between both groups (mean  $\pm$  SEM, repeated-measures two-way ANOVA: genotype x pup number interaction  $F_{4,87}=1.498$ ,  $p=0.21$ ; Genotype main effect  $F_{1,22}=0.5189$ ,  $p=0.48$ ). (C) No difference in the percent of time dedicated to pup nursing during 20 min observation was found between both groups (mean  $\pm$  SEM, Mann-Whitney test  $U=57$ ,  $p=0.34$ ). (D) The scatter plot (with best-fit regression line) shows a significant correlation between the number of TPH2+ cells in the DRN and the nursing time of experienced DRN<sup>TPH2-CKO</sup> (orange circle) and DRN<sup>control</sup> (green circle) dams (Spearman test).