Figures and figure supplements

Increased anxiety and decreased sociability induced by paternal deprivation involve the PVN-PrL OTergic pathway

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Figure 1. Effect of paternal deprivation on anxiety-like behavior and social preference in adult mandarin voles (n = 7). (A, B) Percentage of time in the central area. (C, D) Representative path and (E, F) total distance of mandarin voles in the open field test. *p<0.05; **p<0.01. Independent sample t-tests. (G, H) Percentage of time in the light area and (I, J) animal traces of mandarin voles in the light and dark box. *p<0.05; **p<0.01. Independent sample t-tests. Effect of PD on social preference in (K) males and (L) females. Error bars indicate SEM. **p<0.025 vs. object stimulus. ##p<0.025 vs. PC. Two-way ANOVA (factors: treatment x stimulus). PC, biparental care; PD, paternal deprivation.

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Figure 2. Effect of paternal deprivation on the percentage of c-fos/OT double-labeled neurons in the PVN in adult mandarin voles (n = 5). Voles were subjected to interaction with either (A) a juvenile voles or (B) a magic cube. (C, D) Double-immunohistochemical staining of c-fos (red) and OT (blue). Effect of PD on the percentage of neurons double-labeled for OT and c-fos in (E) males and (F) females. Error bars indicate SEM. **p<0.01 vs. object stimulus. **p<0.01 vs. PC. Two-way ANOVA (factors: treatment x stimulus). PC, biparental care; PD, paternal deprivation.

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Figure 2—figure supplement 1. Paternal deprivation diminishes social approach in (A) male and (B) female mandarin voles (n = 5). Error bars indicate SEM. **p<0.025 vs. Toy. ## p<0.025 vs. PC. PC, biparental care; PD, paternal deprivation.

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Figure 3. Effect of paternal deprivation on c-fos expression in the PrL and the NAc of adult mandarin voles (n = 5). (A) Voles were subjected to interaction with either a juvenile vole or a magic cube. (B) Immunohistochemical staining of c-fos (red) and 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) (blue). The effect of PD on c-fos expression in the PrL of (D) males and (E) females. (C) Images show c-fos immunoreactivity in the NAc shell and the NAc core. Effect of PD on c-fos expression in (F) males and (G) females of the NAc shell. Effect of PD on c-fos expression in the NAc core of (H) males and (I) females. Error bars indicate SEM. **p<0.01 vs. object stimulus. ##p<0.01 vs. PC. Two-way ANOVA (factors: treatment x stimulus). PC, biparental care; PD, paternal deprivation.

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**Figure 4.** Effect of paternal deprivation on PVN OT-IR neurons. (A) PC males, (B) PD males, (C) PC females and (D) PD females. 3V, 3rd ventricle. (E) Schematic drawing illustrating tissue in the PVN. (F) Quantification of OT-IR neurons in the PVN. Error bars indicate SEM. n = 4. **p<0.01 vs. PC. ***p<0.01 vs. male. Two-way ANOVA (factors: treatment × sex). PC, biparental care; PD, paternal deprivation; PVN, paraventricular nucleus.

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Figure 5. Effects of paternal deprivation on mesocorticolimbic. (A–B) OTR, (C–D) V1aR and (E–F) AVP immunoreactivity in male (n = 6) and female (n = 6) mandarin voles. Error bars indicate SEM. *p<0.05; **p<0.01. Independent sample t-tests. AVP, arginine vasopressin; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; OTR, oxytocin receptor; PC, biparental care; PD, paternal deprivation; PVN, paraventricular nucleus; V1aR, vasopressin 1a receptor.
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Figure 6. Effects of PrL OT administration on anxiety-like behavior and social preference in paternal deprivation mandarin voles. (A) Experimental schematics. (B) Histological representations of the microinjection site and (C, D) schematic diagrams showing the location of injector tips in the PrL. OT in the PrL is anxiolytic in both of sexes. (E, H) Percentage of time in the central area and (F, I) total distance in the open field test. One-way ANOVA. *p<0.05. OT in the PrL promotes a social preference in (G) males and (J) females. #p=0.0083 vs object stimulus. Two-way ANOVA (factors: treatment × sex). (Male: CSF – n = 6; 1 ng OT – n = 6; 10 ng OT – n = 6; 10 ng OT/10 ng OTA – n = 6; 10 ng OT/100 ng OTA – n = 5; 10 ng OT/10 ng V1aRA – n = 6. Female: CSF – n = 6; 1 ng OT – n = 6; 10 ng OT – n = 6; 1 ng OT/10 ng OTA – n = 5; 1 ng OT/100 ng OTA – n = 5; 10 ng OT/10 ng V1aRA – n = 6).

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Figure 7. Optogenetic stimulation of OT terminals rescued changes in emotion and social preference behavior that were induced by paternal deprivation. (A) Schematic drawing of the locations of rAAV-Ef1α-DIO-ChR2-mCherry plus rAAV-Oxytocin-Cre or rAAV-Ef1α-DIO-mCherry plus rAAV-Oxytocin-Cre injection into the right PVN and optic fiber implants. (B) Immunohistological image showing the targeting of fiber implants in the right PrL. Colocalization of ChR2-mCherry (red), OT neurons (green) and DAPI (blue) in the PVN of (C) males and (D) females. Confocal images of axonal mCherry signal in the PrL of (E) males and (F) females. (G) Images show expression of c-fos in the PrL after photostimulation. Quantification of c-fos in the PrL of (H) males (Ctrl: n = 4, ChR2: n = 5) and (I) females (Ctrl: n = 4, ChR2: n = 5) after photostimulation. Optogenetic activation of oxytocinergic fibers in the PrL increases social preference of (J) males (n = 6) and (K) females (n = 6). **p<0.01 vs. object stimulus. ## p<0.01 vs. Ctrl. Two-way ANOVA (factors: photostimulation treatment × stimulus type). Activation of PVN-PrL oxytocinergic projection significantly increased the percentage of time in the central area for both (L) males (n = 6) and (M) females (n = 6), but did not influence total distance traveled for (N) males (n = 6) or (O) females (n = 6). *p<0.05; **p<0.01. Independent sample t-tests. Error bars indicate SEM.

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Figure 7—figure supplement 1. Histology and immunostaining. The histology of CTB injecting into the right PrL of (A) male and (C) female voles. Immunostaining showing colocalization of OT neurons (green) and CTB (red) in the PVN of (B) male and (D) female voles.

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Figure 7—figure supplement 2. Immunostaining showing colocalization of mCherry (red) with OT (green) but not AVP (blue) in PVN neurons of (A) male and (B) female voles.

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**Figure 7—figure supplement 3.** Representative immunostaining allowing the visualization of (A) OT expression (green), (B) mCherry (red) and (C) DAPI (blue) in PVN neurons of PD voles. (D) Colocalization image including bands (A), (B) and (C). Arrowheads indicate the OT, mCherry and DAPI.

Quantification of OT–meCherry colocalization in (E) males (147/234 = 62.8%, from two voles) and (F) females (203/283 = 71.7%, from two voles).

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Figure 7—figure supplement 4. After activation of PVN-to-PrL OT terminals does not elicit long-lasting effects on anxiety-like behavior and social preference in PD voles (n = 6). The behavioral changes disappeared 8 hr after blue light photostimulation. Quantification of the percentage of time spent in the central area ((A): male; (B): female) and total distance traveled ((C): male; (D): female). **p<0.01. One-way ANOVA. Quantification of social preference ((E): male; (F): female). **p<0.01 vs. object stimulus. ## p<0.01 vs. Ctrl. Two-way ANOVA. Error bars indicate SEM.

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Figure 8. Optogenetic inhibition of OT terminals elicits anxiety-like behavior and attenuated social preference in naive voles. (A) Schematic drawing of the locations of rAAV-EF1α-DIO-eNpHR3.0-mCherry plus rAAV-Oxytocin-Cre or rAAV-EF1α-DIO-mCherry plus rAAV-Oxytocin-Cre injection bilaterally into the PVN and optic fiber implants. (B) Immunohistological image showing target of fiber implants in the bilateral PrL. Colocalization of eNpHR3.0-mCherry (red), OT neurons (green) and DAPI (blue) in the PVN of (C) males and (D) females. Confocal images of axonal mCherry signal in the PrL of (E) males and (F) females. (G) Images showing the expression of c-fos in the PrL after yellow light photostimulation. Quantification of c-fos in the PrL of (H) males (n = 4) and (I) females (n = 4) after photostimulation. **p<0.01. Independent sample t-tests. Optogenetic inhibition of oxytocinergic fibers in the PrL decreases the social preference of (J) males (n = 6) and (K) females (n = 6); p<0.025. Two-way ANOVA (factors: optogenetic inhibition treatment × stimulus type). Inhibition of PVN-PrL oxytocinergic projection significantly decreases the percentage of time spent in the central area for both (L) males (n = 6) and (M) females (n = 6), but did not influence total distance traveled by (N) males (n = 6) or (O) females (n = 6). **p<0.01. Independent sample t-tests. Error bars indicate SEM.

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Figure 8—figure supplement 1. Representative immunostaining of (A) OT expression (green), (B) mCherry (red) and (C) DAPI (blue) in PVN neurons of naive voles. (D) Colocalization image for bands (A), (B) and (C). Arrowheads indicate the OT, mCherry and DAPI. Quantification of OT/mCherry colocalization in (E) males (427/602 = 70.9%, from two voles) and (F) females (672/803 = 83.6%, from two voles).

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Figure 8—figure supplement 2. After inhibition of PVN-to-PrL OT terminals does not elicit long-lasting effects on anxiety-like behavior and social preference in naïve voles (n = 6). The behavioral changes (except social preference of male: within 8 hr) occur within 8–24 hr following yellow light photostimulation. Quantification of the percentage of time in the central area ((A): male; (B): female) and total distance traveled ((C): male; (D): female). **p<0.01. One-way ANOVA. Quantification of social preference ((E): male; (F): female). *p<0.05; **p<0.01 vs. object stimulus. Two-way ANOVA. Error bars indicate SEM.

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