Postnatal Fluoxetine-Evoked Anxiety Is Prevented by Concomitant 5-HT_{2A/C} Receptor Blockade, and Mimicked by Postnatal 5-HT_{2A/C} Receptor Stimulation

Supplementary Information

Table S1. Shown in the table is the list of primers used for quantitative polymerase chain reaction analysis of gene expression changes within the prefrontal cortex.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
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| Htr1a | F - GGACTCCGTGCACTAATGGG  
R - AGAGGAAGGTGCTTCTTTGAGTT |
| Htr2a | F - GGGCTCTCTGGTGACTGATT  
R - GTGACTGAGCCAGTGTTGGA |
| Htr2c | F - CTTCGTGCCATTCTTCATCC  
R - GAGCGTTCTCTTCCCTCACA |
| Arc  | F - GCCCAGACGATGATTCTA  
R - GACTCAGCCCTCTGGGAC |
| Hprt1 | F - GCAGACTTTGCTTTCCCTG  
R - GTCTGGCCTGTATCCAACACT |
Figure S1. Postnatal ketanserin (Ket) treatment blocks the emergence of postnatal fluoxetine (PNFlx)-evoked anxiety in adulthood on the open field test (OFT) and elevated plus maze (EPM). Shown is a schematic representation of the experimental paradigm (A). Control (Ctrl) and PNFlx pups that received either vehicle or Ket administration daily from P2 to P21 were assessed for behavior on the OFT (P90) and EPM (P100). PNFlx animals exhibited a significant decline in the number of rears on the OFT which was prevented in the PNFlx+Ket group (B). Total distance traveled in the OFT arena did not differ across groups (C). A significant decline was observed in the number of rears in PNFlx animals in the elevated plus maze (D), which was not significantly altered in the PNFlx+Ket group (D). Total distance traveled in the EPM did not differ between groups (E). Data are expressed as the mean ± SEM number of rears and total distance traveled in the OFT (n = 6-12 animals/group) and EPM (n = 7-12 animals/group). (*p < .05 as compared to Ctrl, $p < .05$ as compared to PNFlx, Two way ANOVA and Bonferroni post hoc test).
Figure S2. Postnatal 5-HT$_{2A}$ receptor blockade prevents both postnatal fluoxetine (PNFlx)-evoked anxiety and depressive behavior, whereas 5-HT$_{2C}$ receptor blockade prevents PNFlx-evoked anxiety, but not depressive behavior. Shown is a schematic representation of the treatment paradigms (A, E). Control (Ctrl) or PNFlx treated pups received either vehicle (Veh) or MDL/SB administration from P2 to P21 and were assessed for anxiety behavior on the open field test (OFT) (P90) and for depressive behavior on the forced swim test (FST) (P100). The PNFlx-evoked decline in the number of visits to the center of the OFT arena (B) was prevented by postnatal MDL treatment. Total distance traveled in the open field did not differ across groups (C). PNFlx animals exhibited a significant increase in the number of immobility events in the FST (D), which was prevented by postnatal MDL treatment. Number of visits to the center (F) and total distance moved (G) in the OFT arena were unaltered across groups in the PNFlx+SB
experiment. On the FST, PNFlx animals showed a significant increase in number of immobility events (H), which was not influenced by concomitant SB treatment. Data are the mean ± SEM of number of visits to the center, total distance traveled in the OFT arena and number of immobility events in the FST. OFT experiment: PNFlx-MDL: n = 7-13 animals/group, PNFlx-SB: n = 6-11 animals/group and FST experiment: PNFlx-PNMDL: n = 7-9 animals/group, PNFlx-SB: n = 7-11 animals/group. (*p < .05 as compared to Ctrl, $p < .05$ as compared to PNFlx, two way ANOVA and Bonferroni post hoc test).
Figure S3. Total distance moved in the open-field test (OFT) arena was unaltered between control (Ctrl) and postnatal DOI (PNDOI) groups. Data are the mean ± SEM for total distance traversed in the OFT ($n = 6-8$ animals/group).