Data Supplement for Dincheva et al., Effect of Early-Life Fluoxetine on Anxiety-Like Behaviors in BDNF Val66Met Mice. Am J Psychiatry (doi: 10.1176/appi.ajp.2017.15121592)

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Supplemental Methods

Western blot analysis

Dissected tissues were minced and lysed in lysis buffer (1x Tris buffered saline (TBS), 1% NP-40, 1% Triton-100, 1 mM PMSF, 10% glycerol, and protease inhibitor cocktail (Sigma)) for 30 minutes on ice. Lysates were further trituated using a 30 g needle, and supernatants collected following centrifugation at 14,000 rpm for 5 minutes. Following transfer, Western blots were developed using incubation with primary antibodies (anti-HA.11 antibody at 1:3000 dilution, Covance; anti-p75 antibody at 1:1000, R&D) then with HRP-conjugated secondary antibodies and developed with the ECL kit (Amersham). For the quantification, ImageJ software was used to analyze the Western blot bands, and statistical significance was determined by two- tailed Student's t- test. Results are expressed as the mean ± SD.

FIGURE S1. Effect of Early-life Fluoxetine on Fear Learning in BDNF$^{Met/Met}$ Mice

Freezing behavior was assessed for wild-type (Val/Val) and BDNF$^{Met/Met}$ (Met/Met) littermate mice treated with or without FLX at P21-42. Results are presented as means ±SD determined from the analysis of 8-15 mice per group. Two-way ANOVA of late trials with Bonferroni post hoc, ****P<0.0001(comparing water treated wild-type mice to fluoxetine treated BDNF$^{Met/Met}$ littermates); #P<0.0001 (comparing water treated wild-type mice to water treated BDNF$^{Met/Met}$ littermates).
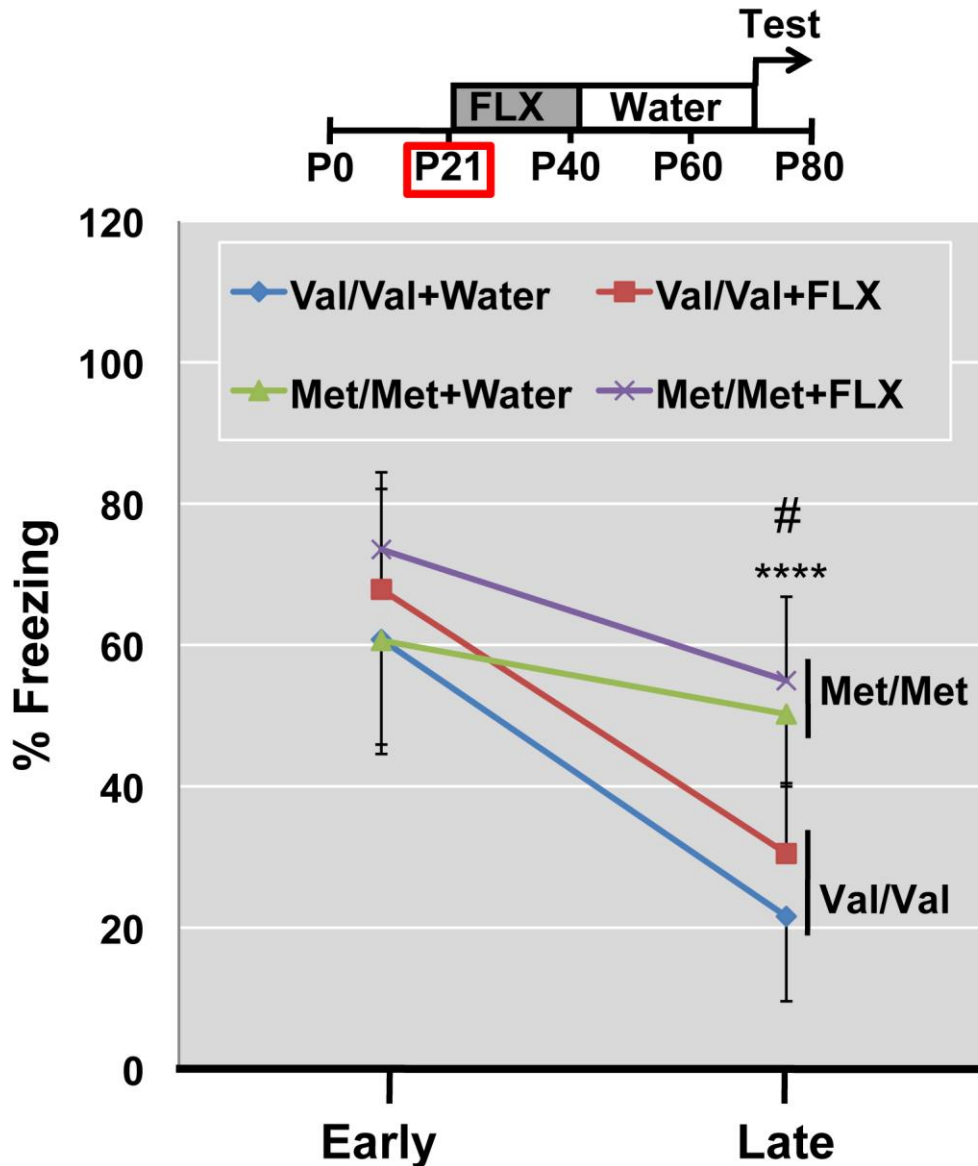
FIGURE S2. Effects of Fluoxetine on Hippocampal BDNF Levels

In Panel A, total BDNF protein levels were assessed using ELISA in BDNF-HA mice treated with or without fluoxetine at three developmental periods. Hippocampi were harvested on last day of treatment, except in the P21-42 group with a 3-week wash out. N=10 mice per group. Western blot analysis permits assessment of proBDNF and mature BDNF levels (Panel B) and p75 levels (Panel C) in hippocampi of mice expressing HA tagged wild-type BDNF treated with or without fluoxetine at P21-41, 40-61 or P60-81. Total BDNF protein content was assessed using ELISA (Panel D) in hippocampi of wild-type (Val/Val) mice and BDNF$^{Met/Met}$ (Met/Met) littermates treated with or without FLX P21-42. Hippocampi were harvested on last day of treatment. N=10 mice per group. All results are presented as means ±SD. *$P<0.05$, ***$P<0.001$, ****$P<0.0001$
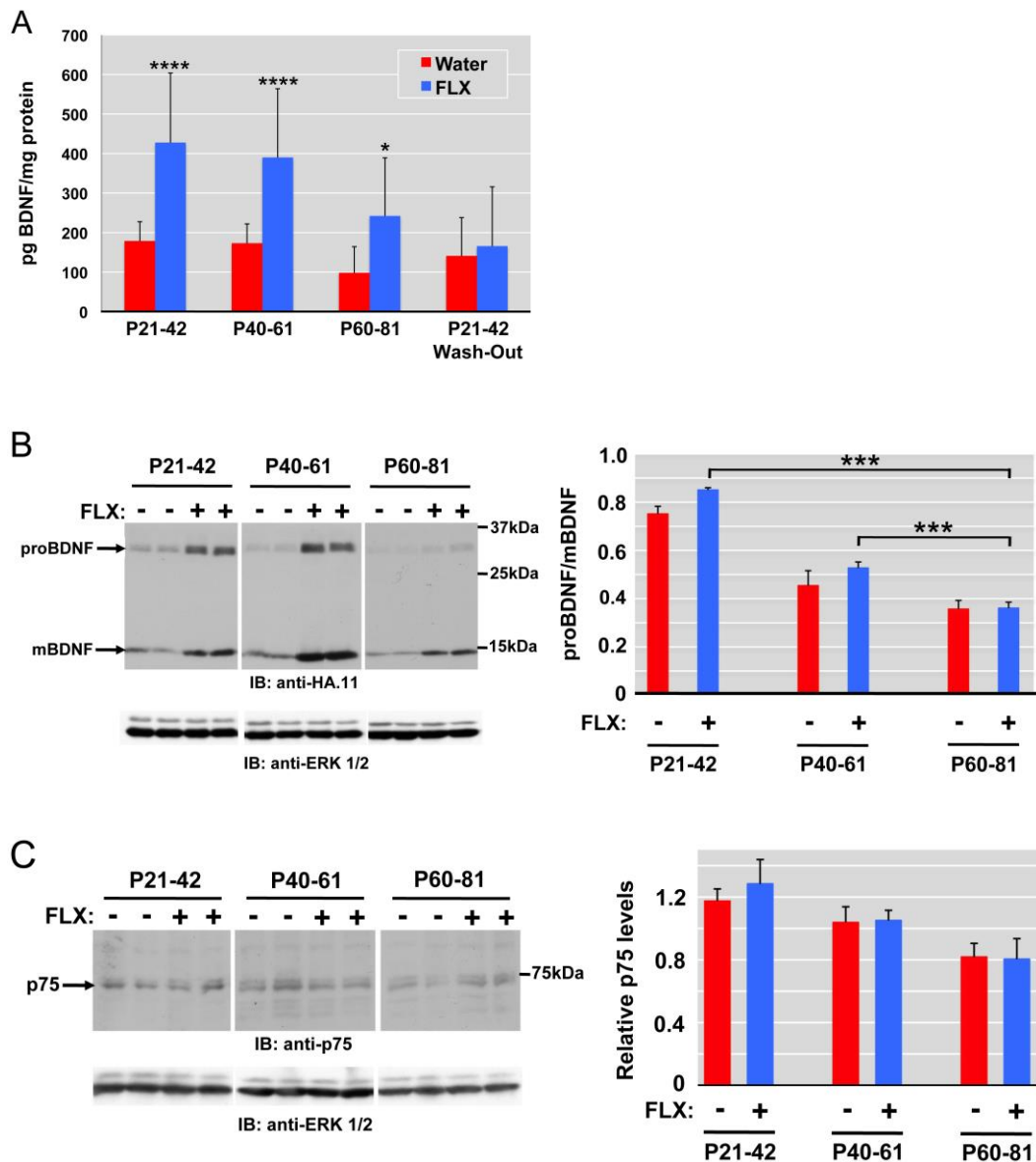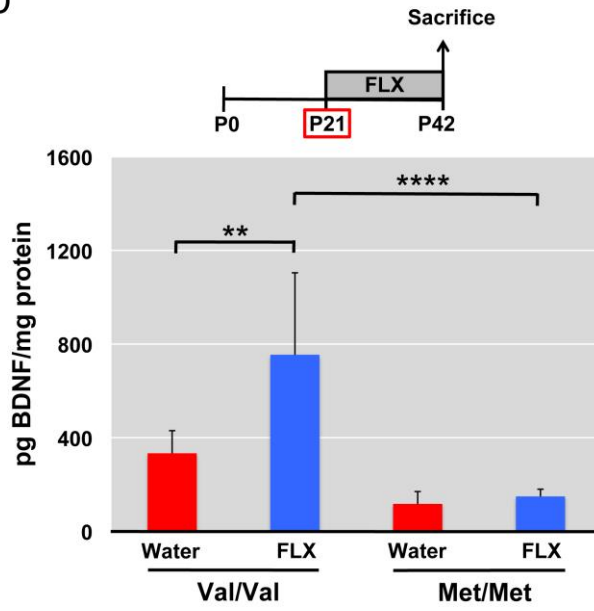
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FIGURE S3. Effect of Fluoxetine on Serotonergic Fiber Density and Cell Number

Panel A shows density of serotonin transporter (SERT) immunoreactive fibers in ventral CA1 (vCA1), which were analyzed in BDNF$^{Met/Met}$ mice and wild-type controls treated with or without fluoxetine at P21-42. Panel B shows density of serotonin transporter (SERT) immunoreactive fibers in infralimbic prefrontal cortex, which were analyzed in BDNF$^{Met/Met}$ mice and wild-type controls treated with or without fluoxetine at P60-81. Panel C shows serotonin immunoreactive cells in dorsal raphe nucleus of BDNF Val/Val and Met/Met mice treated with or without fluoxetine at P21-42. All results are presented as means ±SD determined from the analysis of 5 mice per group. *$P<0.05$, **$P<0.01$, ***$P<0.001$
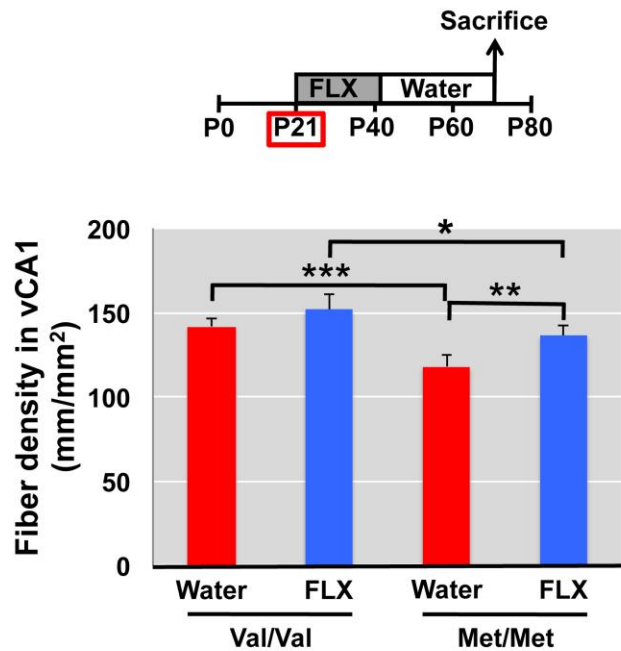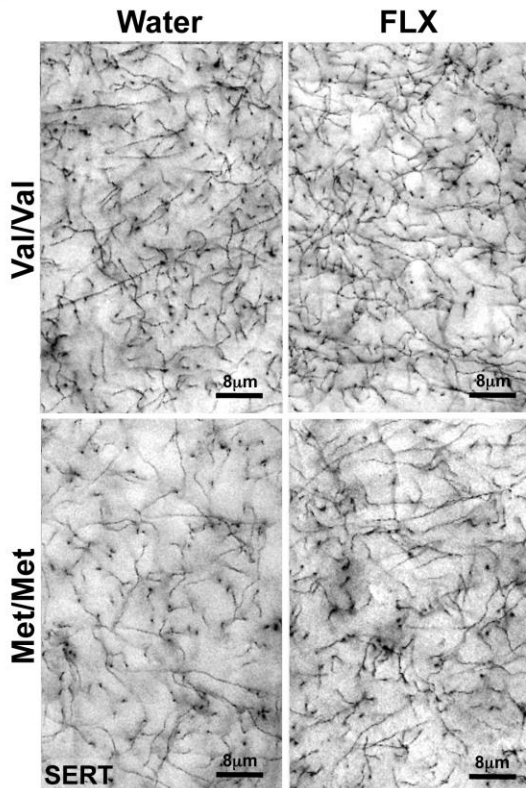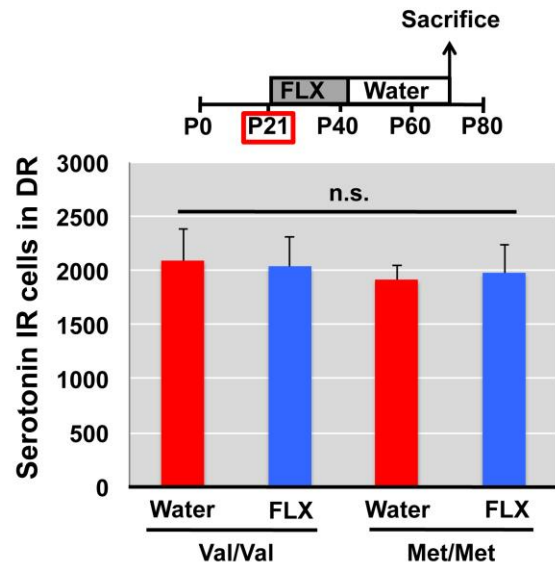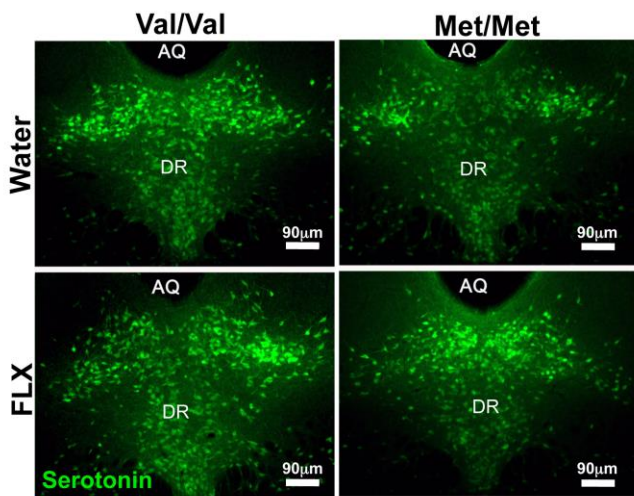
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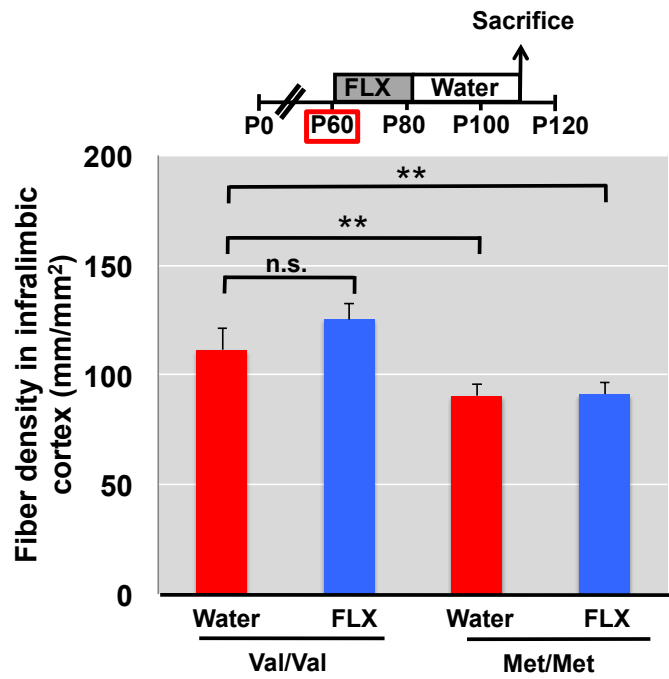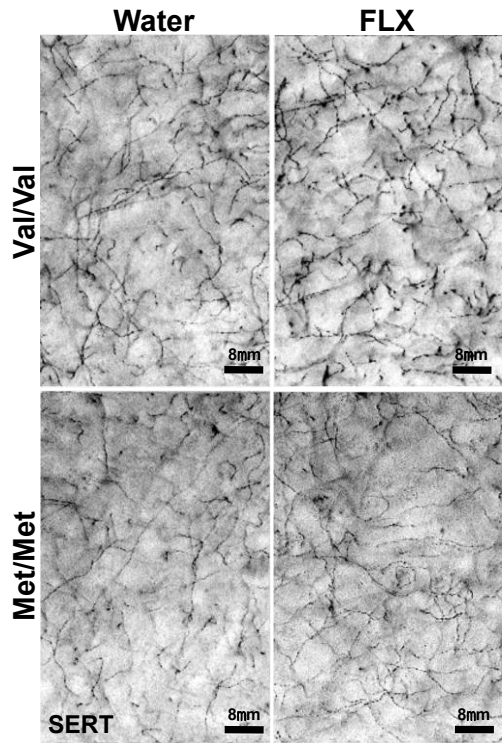
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FIGURE S4. Effect of Fluoxetine on S100B Expression

Panel A shows analysis of S100B immunoreactive cells in dorsal raphe of BDNF[Met/Met] mice (Met/Met) and wild-type controls (Val/Val) treated with or without fluoxetine at P60-81. Panel B shows analysis of S100B immunoreactive cells in dorsal hippocampus of BDNF[Met/Met] mice (Met/Met) and wild-type controls (Val/Val) treated with or without fluoxetine at P21-42. Results are presented as means ±SD determined from the analysis of 5 mice per group. ***$P<0.001$, ****$P<0.0001$
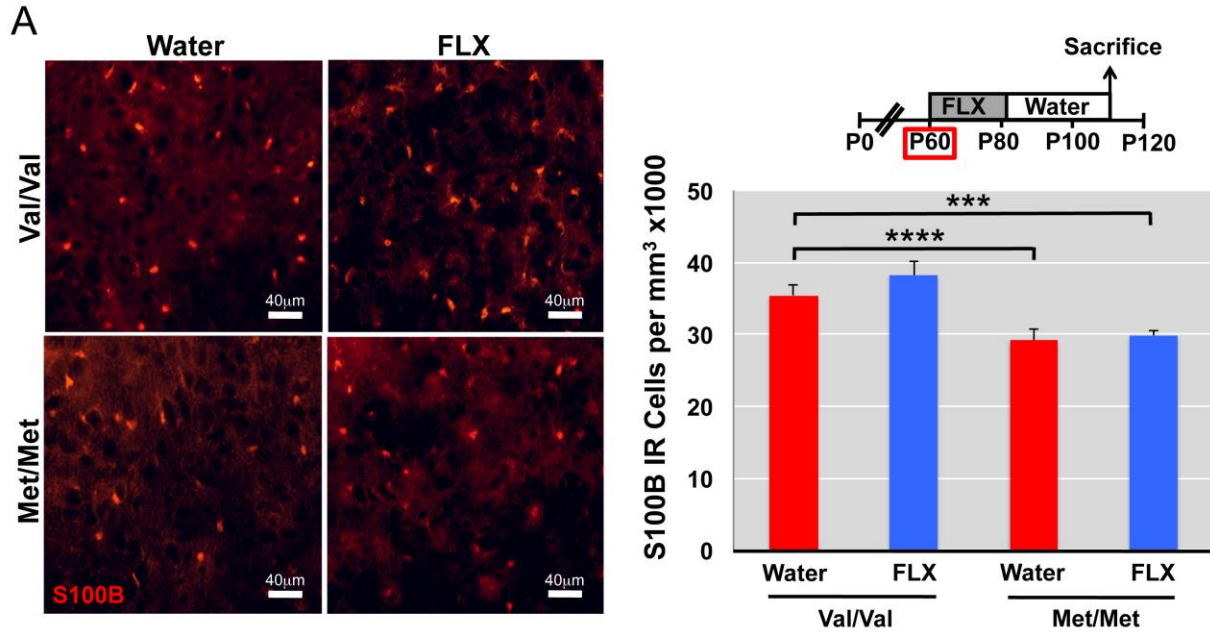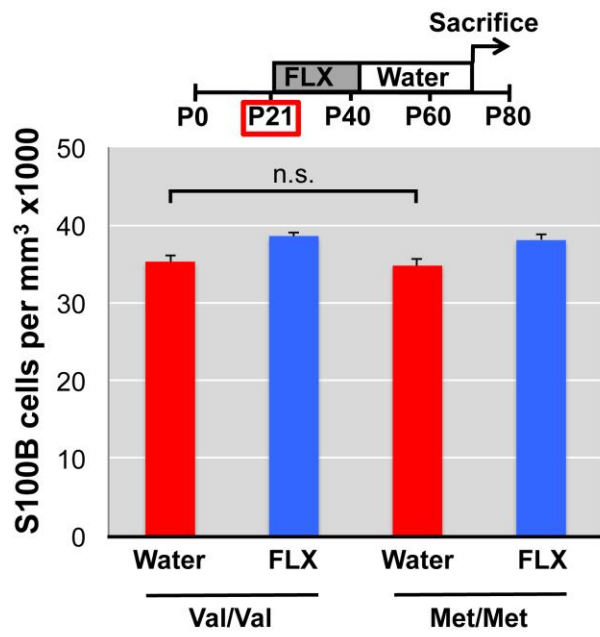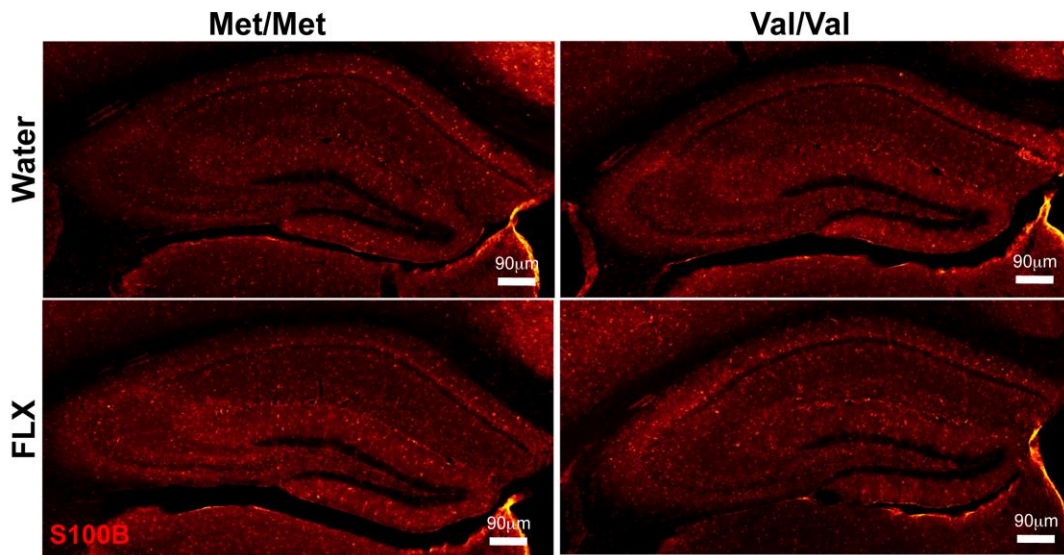
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TABLE S1. Effect of Age in Analyses of Adult Mice

Student's t-tests analysis comparing P21-42 vs P60-81 for water treated animals for S100B expression and 5HT fiber density and one-way ANOVA analysis comparing P21 vs P40 vs P60 for water treated animals.

Experiment	Statistical Analysis
S100B expression	BDNF$^{Val/Val}$ water at P21-42 vs P60-81 AVG: 34739.152; 35416.238 t=0.52961 P=0.610762 BDNF$^{Met/Met}$ water at P21-42 vs P60-81 AVG: 29121.494; 29183.928 t= 0.06348 P= 0.95094
5HT fiber density	BDNF$^{Val/Val}$ water at P21-42 vs P60-81 AVG 120.128; 111.346 t=0.52961 P=0.610762 BDNF$^{Met/Met}$ water at P21-42 vs P60-81 AVG: 81.968; 90.33 t=1.20577 P= 0.262356
Novelty induced hypophagia latency	BDNF$^{Val/Val}$ water at P21-42 vs P40-61 vs P60-81 AVG: 249.1; 116.05; 184.64 F(2,42)=1.94 P=0.1569 BDNF$^{Met/Met}$ water at P21-42 vs P40-61 vs P60-81 AVG: 540.86; 402.37; 448.31 F(2,29)=1.51 P=0.2373