

Beyond new neurons in the adult hippocampus: Imipramine acts as a pro-astrogliogenic factor and rescues cognitive impairments induced by stress exposure

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Supplementary Figures:

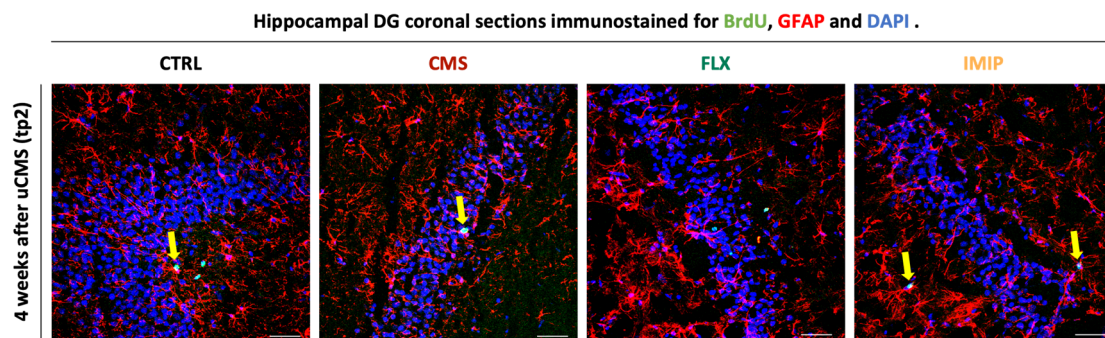


Figure S1. Representation of hippocampal DG coronal sections immunostained for bromodeoxyuridine (BrdU) (in green), glial fibrillary acidic protein (in red), and DAPI (in blue) for a visual understanding of newborn astrocytes (GFAP⁺BrdU⁺ cells). Images include the different conditions - control, SAL, fluoxetine, and imipramine treated animals – at time point 2. Scale bar: 50 μm. Abbreviations: DAPI, 4',6'-diamino-2-fenil-indol; BrdU, Bromodeoxyuridine; GFAP, Glial Fibrillary Acidic Protein; CTRL, non-stressed animals; SAL, animals exposed to uCMS and injected with saline; IMIP, animals exposed to uCMS and treated with imipramine; FLX, animals exposed to uCMS and treated with fluoxetine.

Representative GFAP⁺ processes skeleton

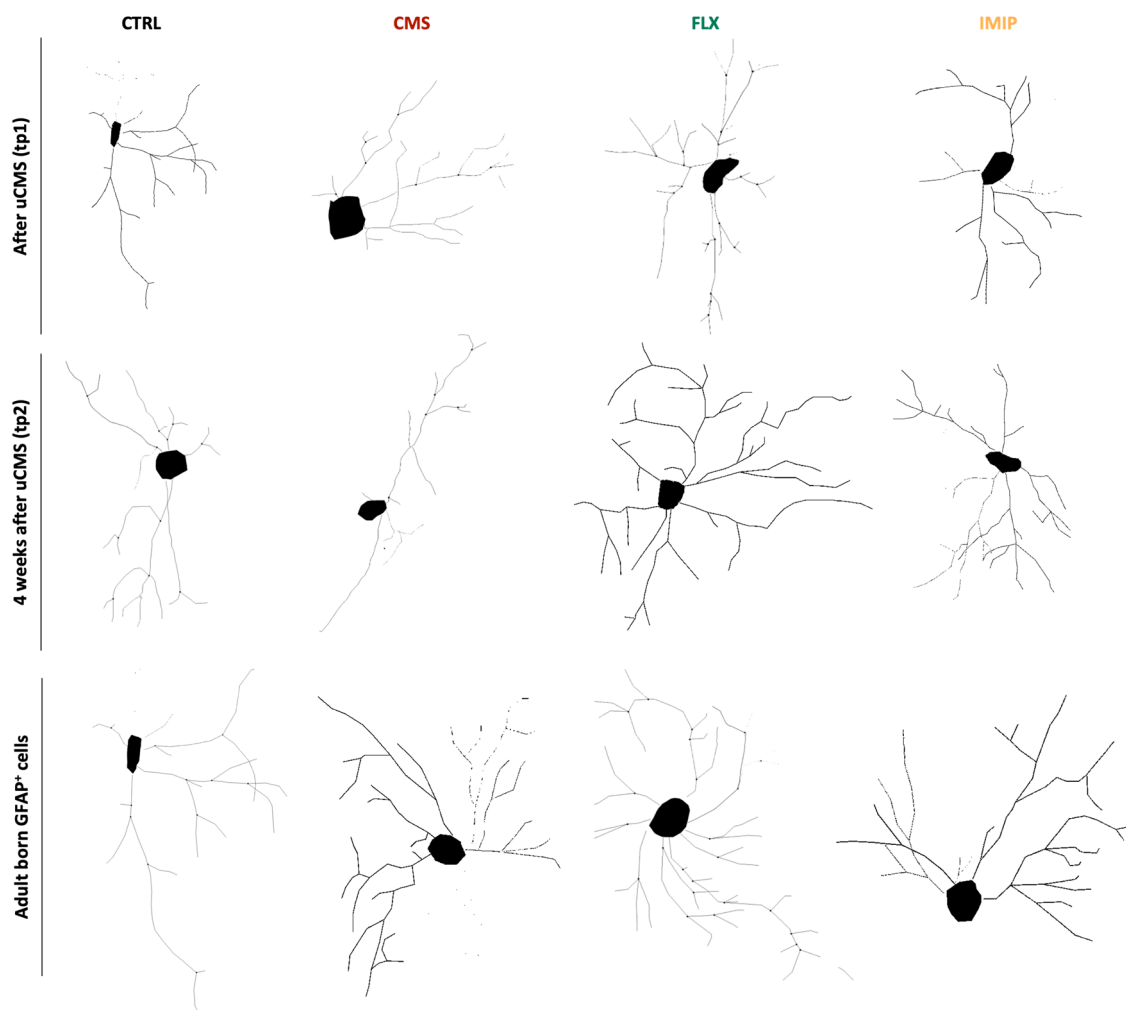


Figure S2. Representative 3D images of mature astrocytes analyzed with the Neurolucida software at tp1, tp2, as well as for adult-born astrocytes. Abbreviations: tp1, time point 1 (6 weeks; immediately after the end of the uCMS protocol); tp2, time point 2 (10 weeks; 4 weeks after the end of the uCMS protocol); GFAP, Glial Fibrillary Acidic Protein; CTRL, non-stressed animals; SAL, animals exposed to uCMS and injected with saline; IMIP, animals exposed to uCMS and treated with imipramine; FLX, animals exposed to uCMS and treated with fluoxetine.

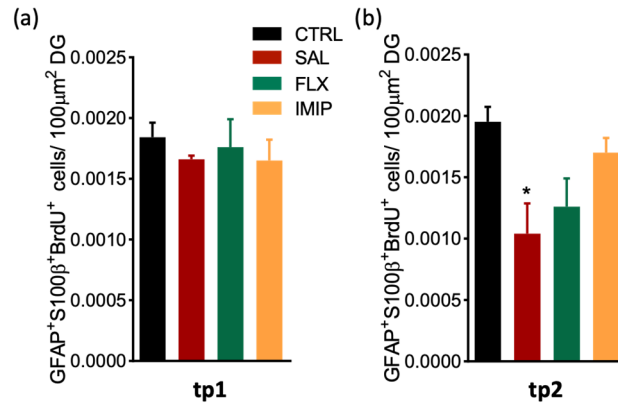


Figure S3. Longitudinal analysis of the number of GFAP⁺S100 β ⁺BrdU⁺ cells in the hippocampal dDG at tp1 (a) and at tp2 (b), after a six-week uCMS protocol that included a treatment with fluoxetine or imipramine. *Denotes the effect of uCMS analyzed by Student's t-test. Data are represented as mean \pm s.e.m. *P<0.05; Sample size: TP1: CTRL: 5-7; CMS: 5-7; FLX: 6-8; IMIP: 4-7; TP2: CTRL: 6-8; CMS: 6-9; FLX:6-8; IMIP: 6-8. Abbreviations: GFAP, Glial Fibrillary Acidic Protein; S100 β , S100 calcium-binding protein β ; CTRL, non-stressed animals; SAL, animals exposed to uCMS and injected with saline; FLX, animals exposed to uCMS and treated with fluoxetine; IMIP, animals exposed to uCMS and treated with imipramine; BrdU, Bromodeoxyuridine; tp1, time point 1 (6 weeks; immediately after the stress protocol cessation); tp2, time point 2 (10 weeks; 4 weeks after the stress protocol cessation).

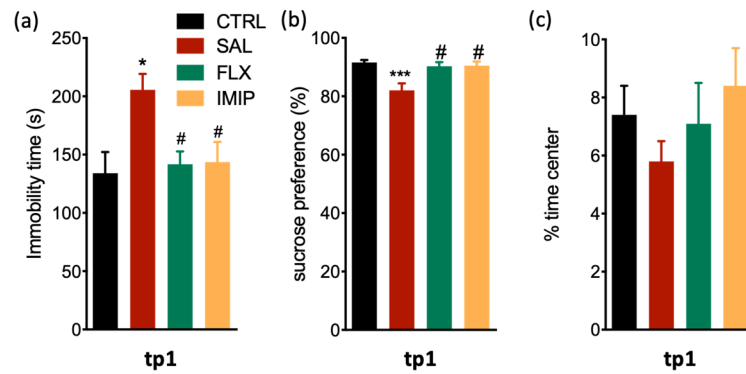


Figure S4: Emotional-behavior assessment immediately after the stress protocol for depressive-like phenotype validation. (a) Coping impaired behavior induced by chronic stress exposure was assessed by the forced swimming test (FST). (b) Anhedonic-like behavior induced by uCMS exposure was evaluated by the sucrose preference test (SPT). (c) Anxiety-like behavior was determined by the Open field test (OF). *Denotes the effect of uCMS analyzed by Student's *t*-test; #Denotes the effect of ADs, by comparison of treatment and SAL animals, analyzed by one-way ANOVA. Data are represented as mean \pm SEM. *, #*P* < 0.05, ****P* < 0.001; Sample size: TP1: CTRL: 10; CMS: 6; FLX: 8; IMIP: 8. Abbreviations: TP, time-point; CTRL, non-stressed animals; SAL, animals exposed to uCMS and injected with saline; FLX, animals exposed to uCMS and treated with fluoxetine; IMIP, animals exposed to uCMS and treated with imipramine; tp1, time point 1 (6 weeks; immediately after the stress protocol cessation).