Supplementary material

Statistical analysis.

In the experiments to evaluate the impact of different stressors during adolescence on rats as adults, both the body weight gain and locomotor response to amphetamine were analyzed using repeated measures 2-way ANOVA with stress exposure (naïve, RS, FS, or FS+RS) as the main independent factor and time as a repeated measurement. The EPM data, discrimination index in the NOR test and the parameters of DA activity measured (population activity, firing rate and burst activity) were analyzed by 1-way ANOVA. Additionally, we employed a 2-way ANOVA to evaluate DA activity across different VTA subregions using the stress exposure and the VTA subregions as main factors. To evaluate if a plPFC lesion would increase the susceptibility to stress, body weight gain and locomotor response to amphetamine were analyzed using repeated measures 3-way ANOVA with plPFC lesion (sham or lesion) and stress exposure (naïve or FS) as the main independent factors and time as a repeated measurement. The EPM data, discrimination index and the parameters of DA activity measured (population activity, firing rate and burst activity) were analyzed by 2-way ANOVA using the stress exposure and the plPFC lesion as main factors. Additionally, we employed a 3-way ANOVA to evaluate DA activity across different VTA subregions using plPFC lesion, stress exposure, and VTA subregions as main factors. For the effects of the exposure to the combination of FS+RS during adulthood, the body weight gain and locomotor response to amphetamine were analyzed using repeated measures 2-way ANOVA with stress exposure (naïve or FS+RS) as the main independent factor and time as a repeated measurement. The EPM data, discrimination index and the parameters of DA activity measured (population activity, firing rate and burst activity) were analyzed by Student's t test. Additionally, we employed a 2-way ANOVA to evaluate DA activity across

different VTA subregions using the stress exposure and the VTA subregions as main factors. Also, in the NOR test, acquisition and retention trial data were analyzed by repeated measures 2-way ANOVA with group as the main independent factor and the object (acquisition trail: familiar object placed in the left *vs.* right side of the arena; retention trial: novel *vs*. familiar object) as the repeated factor. Post hoc analysis was performed using the Tukey's test. All data were represented as mean ± SEM. Results of statistical tests with $P < 0.05$ were considered significant.

Figure Legends

Fig. S1 - Adolescent stress exposure (n = 10-12/group). (*A*) In the NOR test, no significant difference in time spent exploring the two identical objects in the acquisition trial was observed among groups. *(B)* In the retention trial, all groups explored the novel object significantly longer than the familiar object. However, this difference was markedly decreased in animals exposed to FS or to the combination of FS+RS. Data are presented as mean ± SEM. *** $P < 0.0001$; * $P < 0.05$.

Fig. S2 - Adolescent stress exposure. Evaluation of the activity of VTA DA neurons did not show any significant difference in average firing rate *(D-E)* and percentage of spikes fired in bursts *(F-G).* Number of active DA cell per group - naïve: 58; RS: 82; FS: 64; FS+RS: 107.

Fig. S3 - Evaluation of VTA DA neuron activity states. (A) DA neurons were sampled with a pre-determined grid pattern of six (3x2) to nine (3x3) tracks separated by 0.2mm. (B) Representative histology showing 3 electrode tracks. M, C and L indicate electrode tracks in the Medial, Central and Lateral VTA subregions, respectively.

Fig. S4 - Stress exposure in adulthood. (*A*) Adult male rats (n=12/group) were exposed to a combination of FS (daily, from PD65-74) and three RS sessions (PD65, PD66, and PD74). After that, animals were tested in the EPM (PD99), NOR test (PD100-101), and locomotor response to amphetamine (PD102-103). Extracellular recordings of VTA DA neurons started one week after the behavioral experiments (PD111-135). (*B*) FS+RS exposure during adulthood induced impairment in the body weight gain which was fully recovered 2 weeks after the end of stress. (*C-D*) No change in the exploration of the open and enclosed arms of the EPM was observed. (*E*) In the NOR test, no significant difference in time spent exploring the two identical objects in the acquisition trial was observed. *(F)* In the retention trial, similar to naïve animals, stressed animals explored the novel object significantly longer than the familiar object resulting in (*G*) no change in the discrimination index between groups. (*H*) FS+RS did not induce any change in the locomotor response to amphetamine (0.5 mg/kg; injection is indicated by the dashed line). Evaluation of the activity of VTA DA neurons did not show any significant difference in (I-J) DA population activity *(K-L)* average firing rate and *(M-N)* percentage of spikes fired in bursts (active DA cells/group – naïve: 78; FS+RS: 75). Electrophysiology data from 3 animals (2 naïve animals, 1 exposed to FS+RS) were excluded due to electrode misplacement. Data are presented as mean ± SEM. *** $P < 0.0001$; * $P < 0.05$.

Fig. S5 - Schematical drawings depicting the lesion boundaries of adolescent mPFC excitotoxic lesions. The coordinates refer to distance in mm anterior to bregma. Cg: cingulate; PL: prelimbic PFC; IL: infralimbic PFC.

Fig. S6 - plPFC disruption and adolescent stress exposure (n = 6-9/group). (*A*) In the NOR test, no significant difference in time spent exploring the two identical objects in the acquisition trial was observed among groups. *(B)* In the retention trial, all groups, except plPFC lesioned animals

exposed to FS, explored the novel object significantly longer than the familiar object. Also, the difference in time spent exploring novel *vs.* familiar object was markedly decreased in sham animals exposed to FS. Data are presented as mean ± SEM. *** $P < 0.0001$; * $P < 0.05$.

Fig. S7 - plPFC disruption and adolescent stress exposure. Evaluation of the activity of VTA DA neurons did not show any significant difference in average firing rate *(D-E)* and percentage of spikes fired in bursts *(F-G)*. Number of active DA cells per group – sham+naïve: 44; sham+FS: 70; plPFC lesion+naïve: 77; plPFC lesion+FS: 85).