Neuron, Volume *109*

Supplemental information

A transient developmental increase in prefrontal activity alters network maturation and causes cognitive dysfunction in adult mice

Sebastian H. Bitzenhofer, Jastyn A. Pöpplau, Mattia Chini, Annette Marquardt, and Ileana L. Hanganu-Opatz

Supplementary Information

Transient developmental increase of prefrontal activity alters network maturation and causes cognitive dysfunction in adult mice

Sebastian H. Bitzenhofer[1,#,*,§], Jastyn A. Pöpplau[1,#], Mattia Chini[1], Annette Marquardt[1] & Ileana L. Hanganu-Opatz[1,+,*]

[1] Institute of Developmental Neurophysiology, Center for Molecular Neurobiology, University Medical Center Hamburg-Eppendorf, 20251 Hamburg, Germany
[#] These authors contributed equally
[§] Current address: Center for Neural Circuits and Behavior, Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093, USA.
[+] Lead contact

* Corresponding authors: Ileana L. Hanganu-Opatz
hangop@zmnh.uni-hamburg.de
Falkenried 94, 20251 Hamburg, Germany

Sebastian Bitzenhofer
sbitzenhofer@ucsd.edu
9500 Gilman Dr., La Jolla, CA 92093, USA

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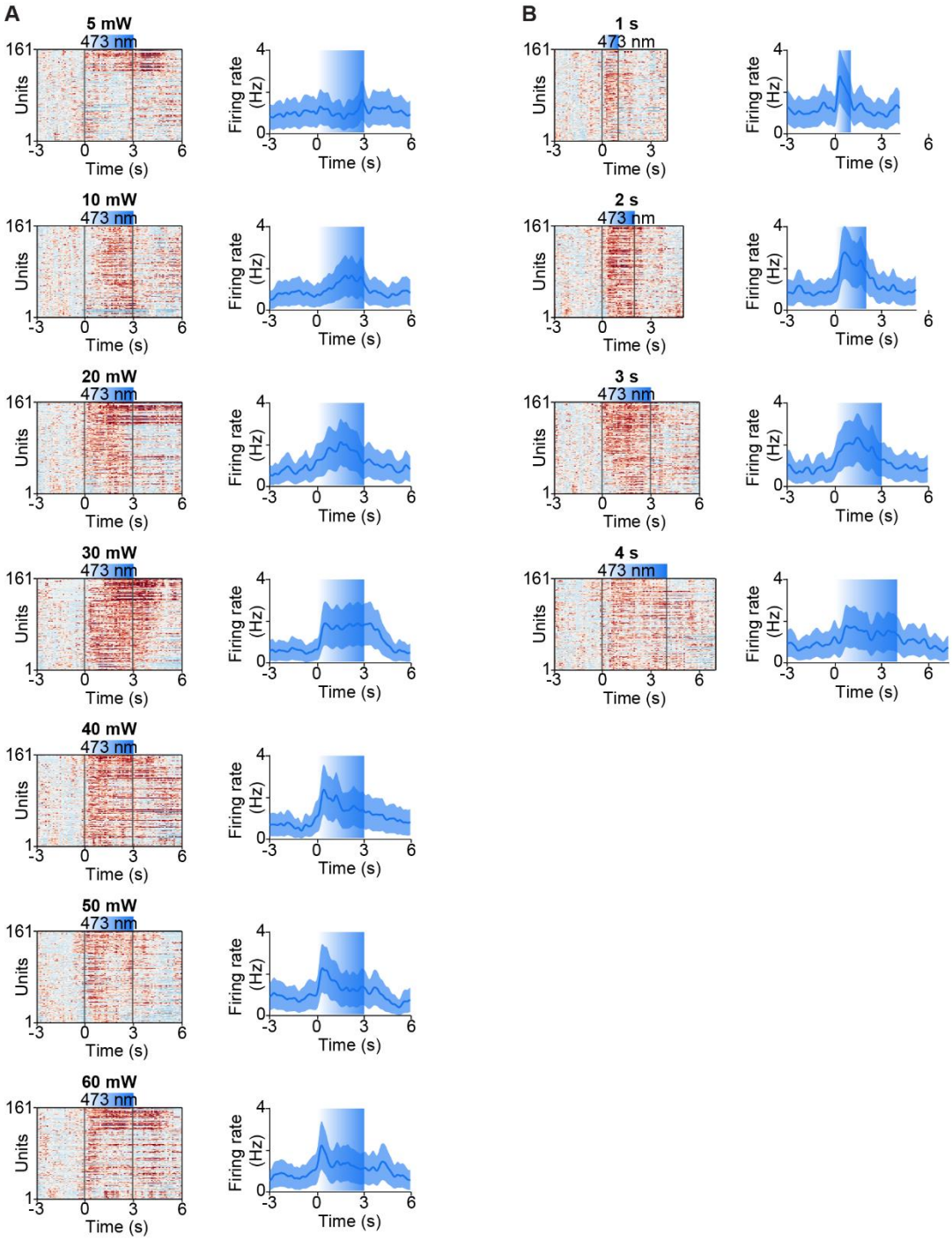

Figure S1. Related to Figure 1. Firing rates of L2/3 PYRs in the neonatal mPFC during light dose tests for transcranial stimulation.
(**A**) Firing rates of single units (n=161 units from 11 mice) in response to transcranial ramp light stimulation at P7-11 at different light intensities.
(**B**) Same as (A) for transcranial ramp light stimulation with different durations.
Data are presented as mean ± SEM.

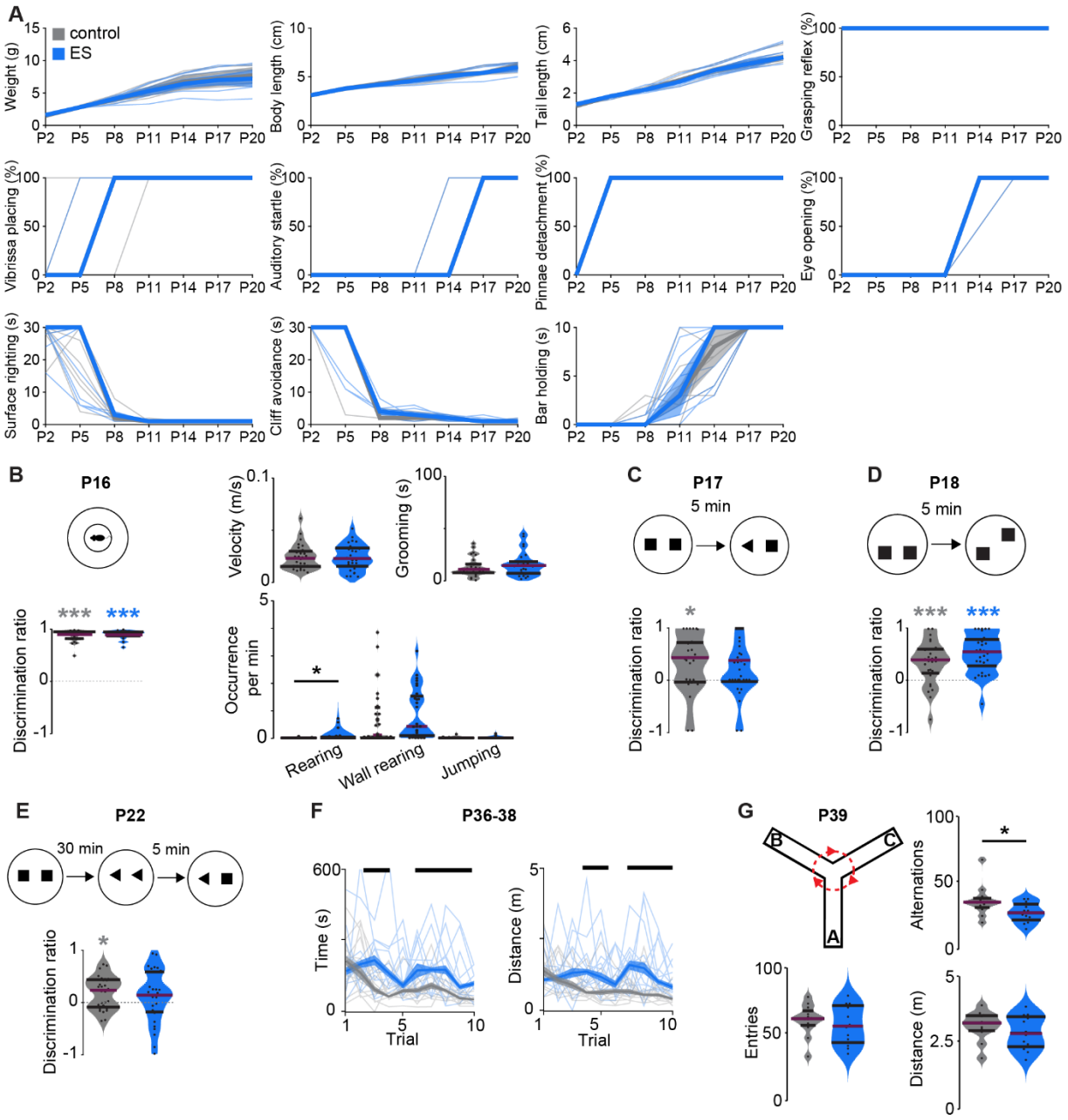

Figure S2. Related to Figure 2. Transient early stimulation impairs mPFC-dependent cognitive abilities but not reflex, motor and anxiety behavior of juvenile and young adult mice.

(**A**) Line plots displaying the age-dependence of developmental milestones for control (n=11) and ES (n=11) mice.

(**B**) Schematic of an open field task (top left) and violin plots displaying the discrimination ratio of time spend in border area vs. center area (bottom left), as well as the basic behavior (velocity, grooming, rearing, wall rearing, jumping) (right) for control (n=28) and ES (n=30) mice at P16. (Wilcoxon rank, discrimination ratio, control $p<0.001$, ES $p<0.001$, control-ES $p=0.809$).

(**C**) Schematic of NOR task and violin plot displaying the discrimination ratio of

interaction time with a novel vs. familiar object for control (n=28) and ES (n=30) mice at P17. (Wilcoxon rank, control p=0.018, ES p=0.157, control-ES p=0.177).
(**D**) Schematic of OLR task (top) and violin plot displaying the discrimination ratio of interaction time with an object in a novel vs. familiar location (bottom) for control (n=28) and ES (n=30) mice at P18. (Wilcoxon rank, control p<0.001, ES p<0.001, control-ES p=0.154).
(**E**) Schematic of RR task and violin plot displaying the discrimination ratio of interaction time with a less vs. more recent object for control (n=28) and ES (n=30) mice at P22. (Wilcoxon rank, control p=0.010, ES p=0.171, control-ES p=0.498).
(**F**) Line plots displaying time to completion and distance travelled for an 8-arm radial maze memory task over 10 trials on 3 consecutive days for control (n=12) and ES (n=12) mice at P36-38. (Kruskal-Wallis, time p<0.001).
(**G**) Schematic showing spontaneous alternation in a Y-maze as well as violin plots displaying quantified parameters (alternations, entries, distance) for control (n=12) and ES (n=12) mice at P39. (Wilcoxon rank, alternations, p=0.046).
Black lines and asterisks (* p<0.05, ** p<0.01, *** p<0.001) indicate significant differences. See Statistics table S1 for detailed statistics. For (A) data are presented as mean ± standard error of the mean (SEM). For (B-E), (G) data are presented as median with 25th and 75th percentile, shaded area represents the probability distribution of the variable. For (F) data are presented as median ± MAD.

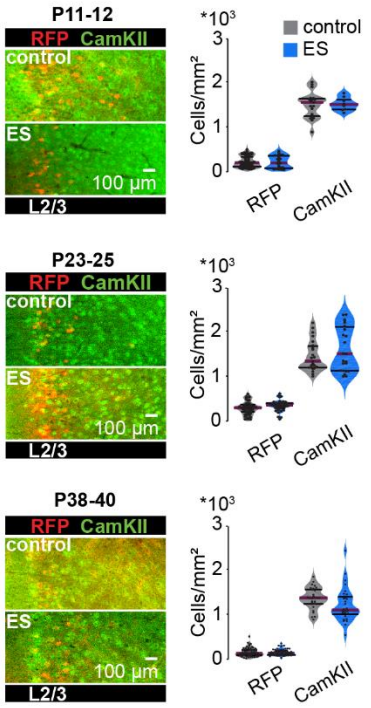

Figure S3. Related to Figure 3. Transient early stimulation does not alter the density of L2/3 PYRs.

Left, representative photographs displaying CaMKII immunostainings in the ChR2(ET/TC)-RFP-transfected mPFC of control and ES mice at P11-12 (control, RFP n=60 slices of 9 mice, CamKII n=19 slices of 6 mice; ES, RFP n=27 slices of 4 mice, CamKII n=9 slices of 2 mice), P23-25 (control, RFP n=47 slices of 5 mice, CamKII n=23 slices of 5 mice; ES, RFP n=43 slices of 5 mice, CamKII n=23 slices of 5 mice) and P38-40 (control, RFP n=65 slices of 5 mice, CamKII n=29 slices of 5 mice; ES, RFP n=62 slices of 5 mice, CamKII n=29 slices of 5 mice). Right, violin plots of RFP-expressing and CaMKII-positive neuronal density at different age groups. (LMEM, P11-12, RFP p=0.855, CamKII p=0.705, P23-25, RFP p=0.819, CamKII p=0.527, P38-40, RFP p=0.819, CamKII p=0.177).

Data are presented as median with 25th and 75th percentile, shaded area represents the probability distribution of the variable. See Statistics table S1 for detailed statistics.

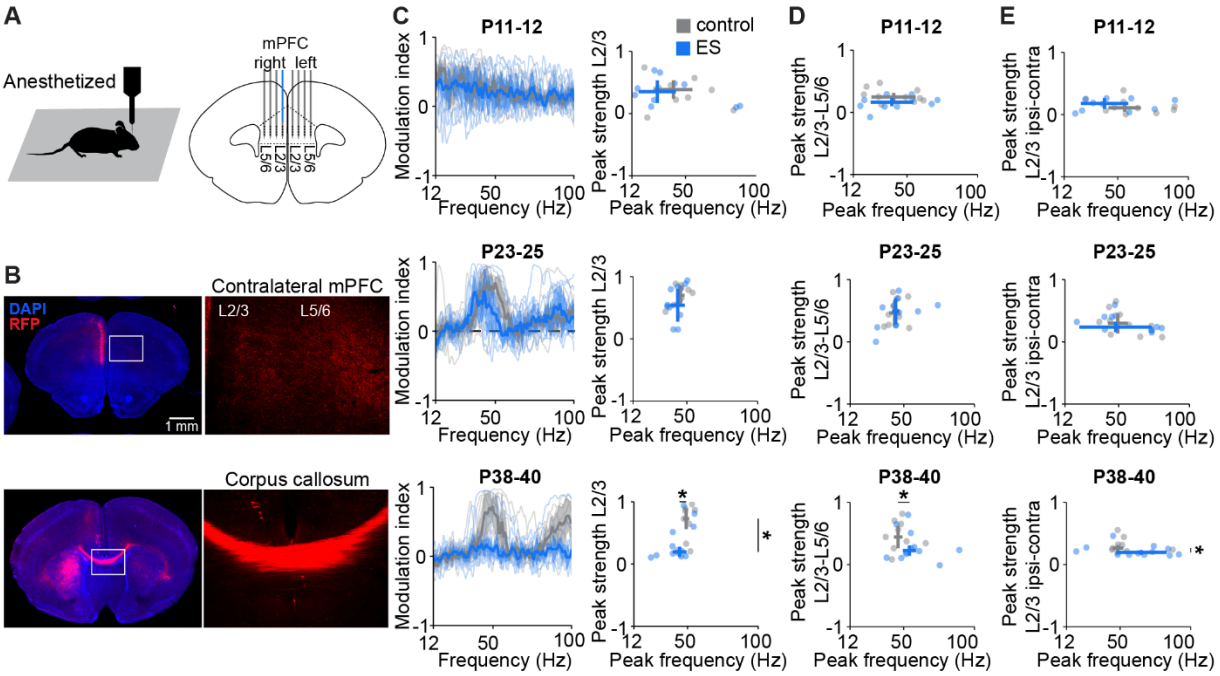

Figure S4. Related to Figure 5. Transient early stimulation impairs evoked intra- and interhemispheric synchrony in the adult mPFC.

(**A**) Schematic displaying bilateral multi-shank recordings in the mPFC of anesthetized mice.

(**B**) Representative photographs showing axonal projections of ChR2(ET/TC)-RFP-transfected L2/3 PYRs in coronal slices from a P10 mouse.

(**C**) Left, modulation index of LFP power in response to acute ramp light stimulation (473 nm, 3 s) for control and ES mice at P11-12 (control n=10 recordings, 10 mice, ES n=10 recordings, 10 mice), P23-25 (control n=10 recordings, 10 mice, ES n=11 recordings, 11 mice) and P38-40 (control n=9 recordings, 9 mice, ES n=12 recordings, 12 mice). Right, scatter plots displaying the peak strength and peak frequency of the power modulation index for control and ES mice. (Wilcoxon rank, P11-12, peak frequency p=0.520, peak strength p=0.909, P23-25, peak frequency p=0.290, peak strength p=0.459, P38-40, peak frequency p=0.039, peak strength p=0.025).

(**D**) Scatter plots displaying the peak strength and peak frequency of prefrontal L2/3-L5/6 coherence at different age. (Wilcoxon rank, P11-12, peak frequency p=1.000, peak strength p=0.053, P23-25, peak frequency p=0.943, peak strength p=0.915, P38-40, peak frequency p=0.042, peak strength p=0.069).

(**E**) Scatter plots displaying the peak strength and peak frequency of interhemispheric prefrontal L2/3-L2/3 coherence at different age. (Wilcoxon rank, P11-12, peak frequency p=0.212, peak strength p=0.623, P23-25, peak frequency p=0.832, peak strength p=0.915, P38-40, peak frequency p=0.270, peak strength p=0.036).

For (C, left) data are presented as mean ± SEM. For (C, right), (D), (E) data are presented as median ± MAD. Asterisks (* p<0.05, ** p<0.01, *** p<0.001) indicate significant differences. See Statistics table S1 for detailed statistics.

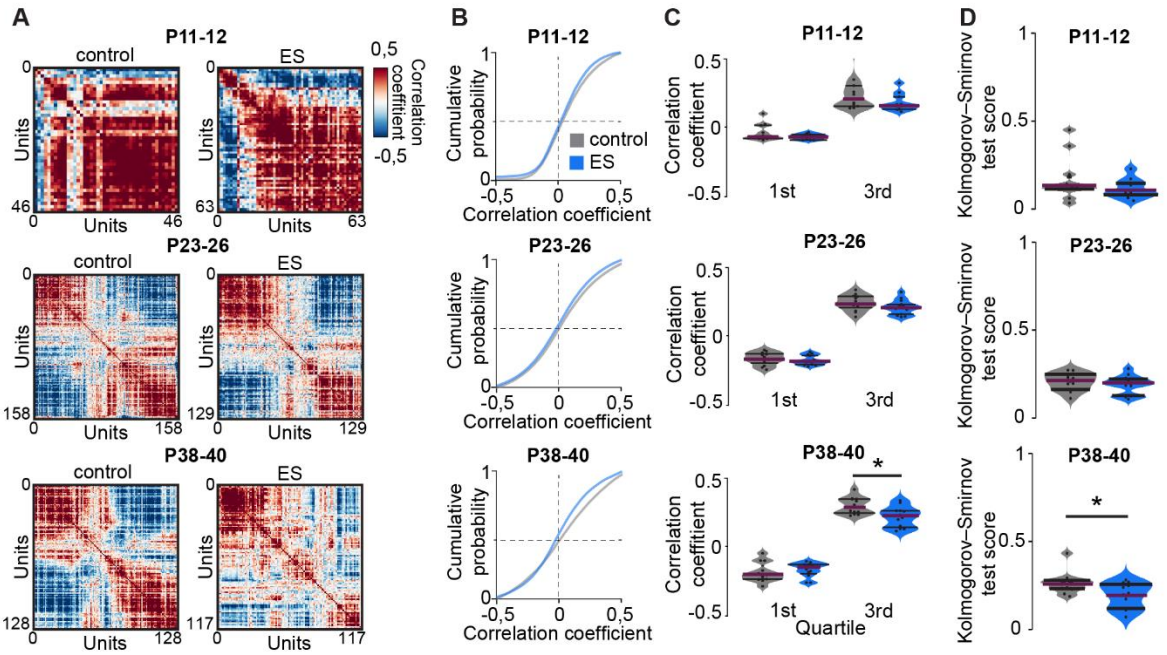

Figure S5. Related to Figure 5. Transient early stimulation reduces synchrony of highly correlated units in response to acute light stimulation in the adult mPFC.
(**A**) Representative pairwise correlations of L2/3 single units during acute ramp light stimulation (473 nm, 3 s) for a control (left) and ES mouse (right) at different developmental stages.
(**B**) Average cumulative density functions of pairwise correlations during acute ramp light stimulation for control and ES mice at P11-12 (control n=11 recordings, 11 mice, ES n=10 recordings, 10 mice), P23-26 (control n=13 recordings, 6 mice, ES n=14 recordings, 5 mice) and P38-40 (control n=12 recordings, 5 mice, ES n=12 recordings, 5 mice).
(**C**) Average intercept at 1st and 3rd quartile of correlation coefficients during acute ramp light stimulation for control and ES mice at different age. (Wilcoxon rank, P11-12, 1st quartile p=0.385, 3rd quartile p=0.162, LMEM, P23-26, 1st quartile p=0.470, 3rd quartile p=0.315, P38-40, 1st quartile p=0.537, 3rd quartile p=0.019).
(**D**) Kolmogorov-Smirnov test score of the distance between pre and stim cumulative density function of correlation coefficients for control and ES mice. (Wilcoxon rank, P11-12, p=0.418, LMEM, P23-26, p=0.631, P38-40, p=0.033).
For (C), (D) data are presented as median with 25th and 75th percentile, shaded area represents the probability distribution of the variable. Asterisks (* p<0.05, ** p<0.01, *** p<0.001) indicate significant differences. See Statistics table S1 for detailed statistics.

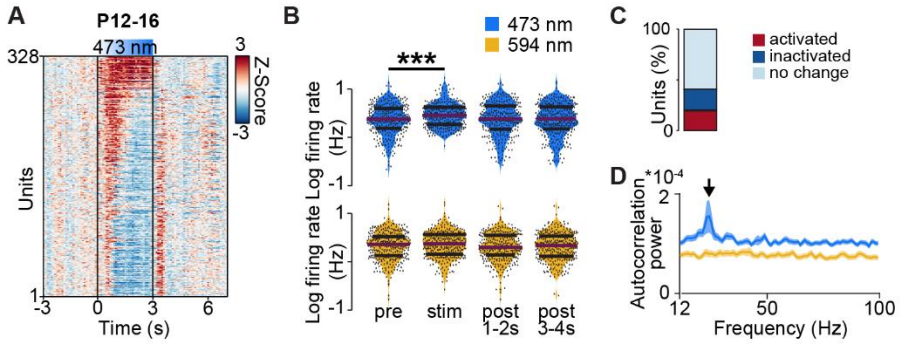

Figure S6. Related to Figure 6. Transcranial stimulation in the mPFC at P12-16.
(**A**) Firing rates of single units (n=328 units from 8 mice) in response to transcranial ramp light stimulation z-scored to pre-stimulation period at P12-16.
(**B**) Average single unit firing rate in response to ramp light stimulation at 473 nm and 594 nm averaged for P12-16 mice (n=328 units from 8 mice).
(**C**) Percent of significantly modulated units ($p<0.01$) during ramp light stimulation
(**D**) Power of single unit autocorrelations during ramp light stimulation at 473 nm and 594 nm averaged for P12-16 mice (n=328 units from 8 mice).
For (B) data are presented as median with 25th and 75th percentile, shaded area represents the probability distribution of the variable. For (D) data are presented as median ± median absolute deviation (MAD). Asterisks (* $p<0.05$, ** $p<0.01$, *** $p<0.001$) indicate significant differences. See Statistics table S1 for detailed statistics.

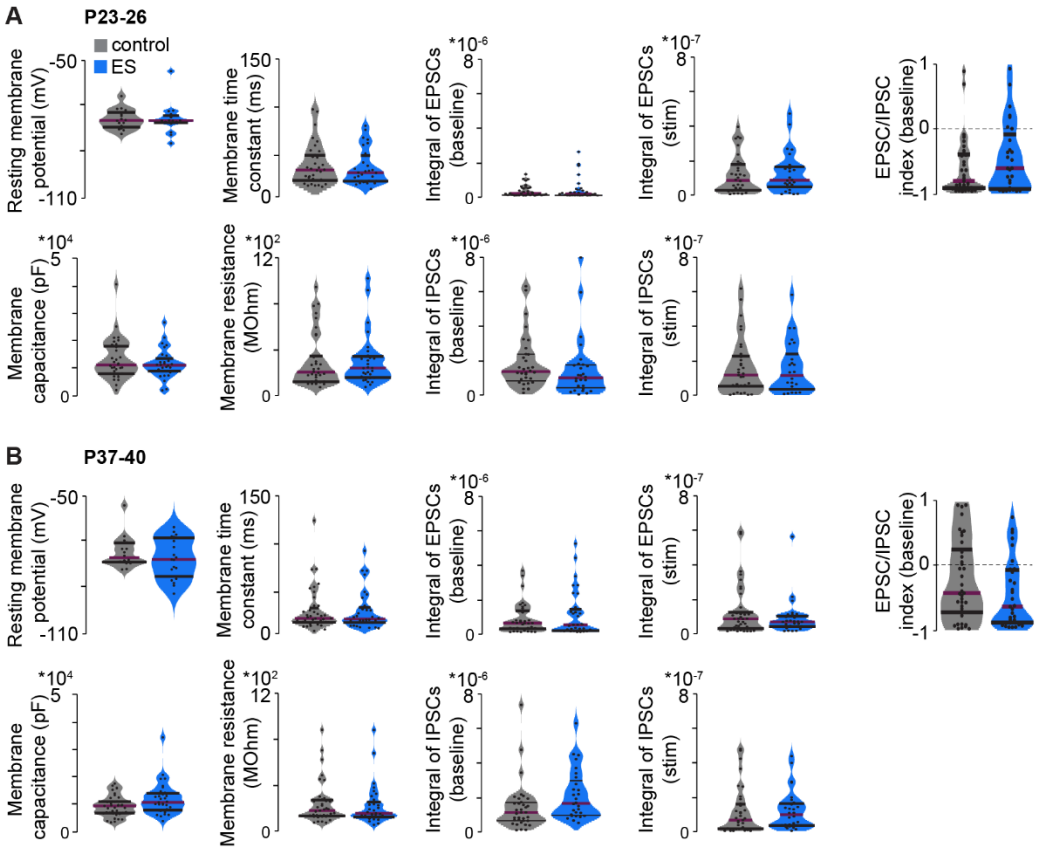

Figure S7. Related to Figure 7. Transient early stimulation does not alter the passive and active membrane properties of non-transfected L2/3 PYRs.
(**A**) Violin plots displaying passive and active membrane properties as well as properties of EPSCs and IPSCs induced by light stimulation in non-transfected L2/3 PYRs from control and ES mice at P23-26 (control n=35 neurons, 5 mice, ES n=30 neurons,5 mice). (LMEM, resting membrane potential p=0.545, membrane time constant p=0.426, EPSCs baseline p=0.743, EPSCs stim p=0.415 membrane capacitance p=0.218, membrane resistance p=0.564, IPSCs baseline p=0.234, IPSCs stim p=0.881, EPSC/IPSC index baseline p=0.218).
(**B**) Same as (A) for control and ES mice at P37-40 (control n=41 neurons, 6 mice, ES n=33 neurons, 4 mice). (LMEM, resting membrane potential p=0.526, membrane time constant p=0.907, EPSCs baseline p=0.339, EPSCs stim p=0.349 membrane capacitance p=0.304, membrane resistance p=0.436, IPSCs baseline p=0.332, IPSCs stim p=0.309, EPSC/IPSC index baseline p=0.402).
For (A), (B) data are presented as median with 25th and 75th percentile, shaded area represents the probability distribution of the variable. See Statistics table S1 for detailed statistics.

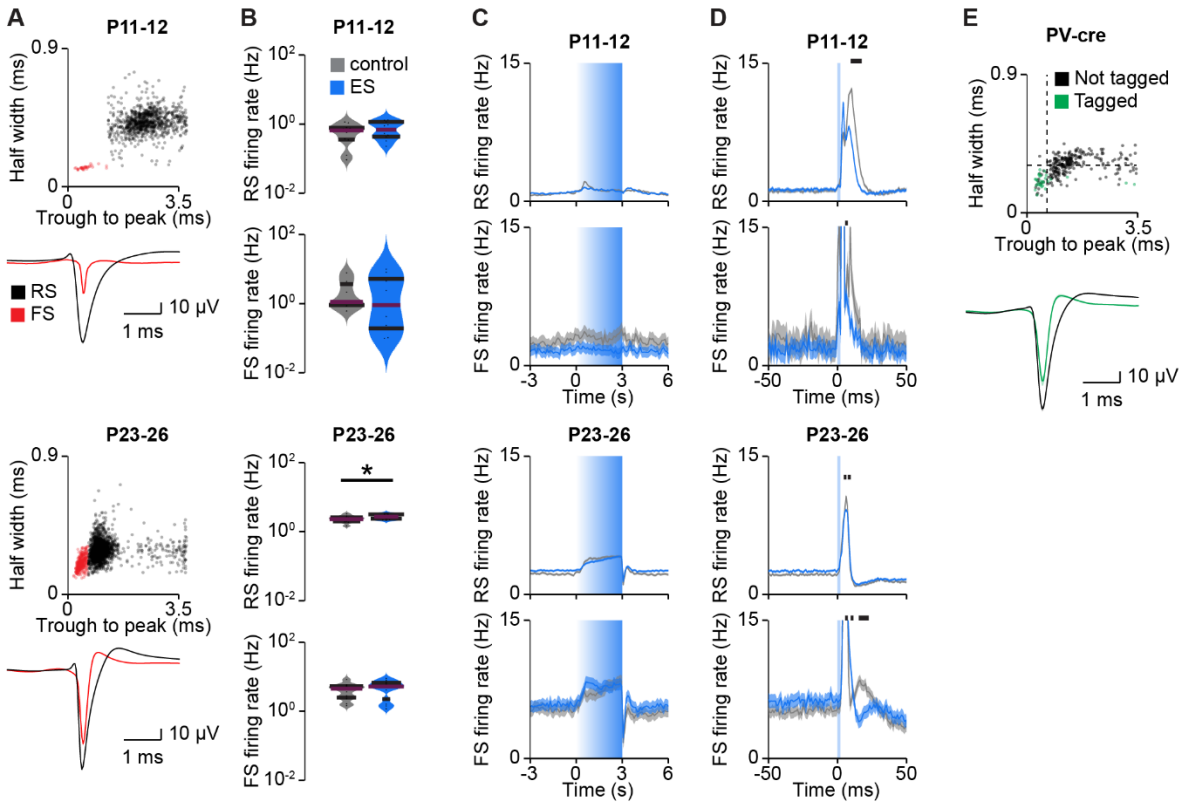

Figure S8. Related to Figure 8. Transient early stimulation alters inhibitory feedback in the mPFC.

(**A**) Scatter plots displaying half width and trough to peak duration (top) and average waveforms for RS and FS units (bottom) for control and ES mice at P11-12 (control 428 RS and 13 FS units, 11 mice, ES 475 RS and 22 FS units, 10 mice) and P23-26 (control 1140 RS and 185 FS units, 6 mice, ES 1220 RS and 141 FS units, 5 mice).

(**B**) Violin plots displaying spontaneous firing rate of RS and FS units for control and ES mice at P11-12 and P23-26. (Wilcoxon rank, P11-12, RS firing rate p=0.418, FS firing rate p=0.680, LMEM, P23-26, RS firing rate p=0.020, FS firing rate p=0.357).

(**C**) Average firing rate during acute ramp light stimulation (473 nm, 3 s) and percent of significantly modulated units for control and ES mice at P11-12 and P23-26. (LMEM, P11-12, RS firing rate p<0.001, FS firing rate p<0.001, P23-26, RS firing rate p<0.001, FS firing rate p<0.001).

(**D**) Average firing rate during acute pulse light stimulation (473 nm, 3 ms) for control and ES mice at P11-12 and P23-26. (LMEM, P11-12, RS firing rate p<0.001, FS firing rate p<0.001, P23-26, RS firing rate p<0.001, FS firing rate p<0.001).

(**E**) Scatter plots displaying half width and trough to peak duration (top) and average waveforms (bottom) for tagged (green) and not tagged (black) units of PV-cre mice expressing ChR2(ET/TC) in PV interneurons in the mPFC (3 mice P26, 5 mice P37-40).

For (B) data are presented as median with 25th and 75th percentile, shaded area represents the probability distribution of the variable. For (C), (D) data are presented as mean ± SEM. Black lines and asterisks (* p<0.05, ** p<0.01, *** p<0.001) indicate significant differences. See Statistics table S1 for detailed statistics.

Statistics table S2. Related to Figures 2 and S2. Statistics for comparison of male and female mice.

Behavioral test	Test	Groups	df	p-value
Open field Discrimination ratio	Linear mixed effect model (chi square)	control (28 mice (15 males, 13 females)) ES (30 mice (18 males, 12 females)) condition | sex	1	0.497 (χ^2=0.46)
Open field Velocity	Linear mixed effect model (chi square)	control (28 mice (15 males, 13 females)) ES (30 mice (18 males, 12 females)) condition | sex	1	1.000 (χ^2=0)
Open field Grooming	Linear mixed effect model (chi square)	control (28 mice (15 males, 13 females)) ES (30 mice (18 males, 12 females)) condition | sex	1	0.102 (χ^2=2.68)
Open field Rearing	Linear mixed effect model (chi square)	control (28 mice (15 males, 13 females)) ES (30 mice (18 males, 12 females)) condition | sex	1	0.101 (χ^2=2.67)
Open field Wall rearing	Linear mixed effect model (chi square)	control (28 mice (15 males, 13 females)) ES (30 mice (18 males, 12 females)) condition | sex	1	1.000 (χ^2=0)
Open field Jumping	Linear mixed effect model (chi square)	control (28 mice (15 males, 13 females)) ES (30 mice (18 males, 12 females)) condition | sex	1	1.000 (χ^2=0)
Novel object recognition Discrimination ratio	Linear mixed effect model (chi square)	control (28 mice (15 males, 13 females)) ES (30 mice (18 males, 12 females)) condition | sex	1	1.000 (χ^2=0)
Object location recognition Discrimination ratio	Linear mixed effect model (chi square)	control (28 mice (15 males, 13 females)) ES (30 mice (18 males, 12 females)) condition | sex	1	1.000 (χ^2=0)
Recency recognition Discrimination ratio	Linear mixed effect model (chi square)	control (28 mice (15 males, 13 females)) ES (30 mice (18 males, 12 females)) condition | sex	1	1.000 (χ^2=0)
Maternal interaction Discrimination ratio	Linear mixed effect model (chi square)	control (19 mice (9 males, 10 females)) ES (21 mice (9 males, 12 females)) condition | sex	1	1.000 (χ^2=0)
Spatial working memory Relative reference memory errors	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)
Spatial working memory Relative working memory errors	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)
Spatial working memory Time	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	0.713 (χ^2=0.14)
Spatial working memory Reference memory errors	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)

Spatial working memory Working memory errors	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)
Spatial working memory Distance	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)
Spatial working memory Time spent in baited arm	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	0.262 (χ^2=1.26)
Spatial working memory Time spent in unbaited arm	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)
Spontaneous alteration Entries	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)
Spontaneous alteration Distance	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)
Spontaneous alteration Alternations	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)
Spontaneous alteration % alternations	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)
Delayed non-match-to-sample task	Linear mixed effect model (chi square)	control (12 mice (8 males, 4 females)) ES (12 mice (8males, 4 females)) condition | sex	1	1.000 (χ^2=0)