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Supplemental information

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Supplementary Information for

Reorganization of adolescent prefrontal cortex circuitry is required for mouse cognitive maturation

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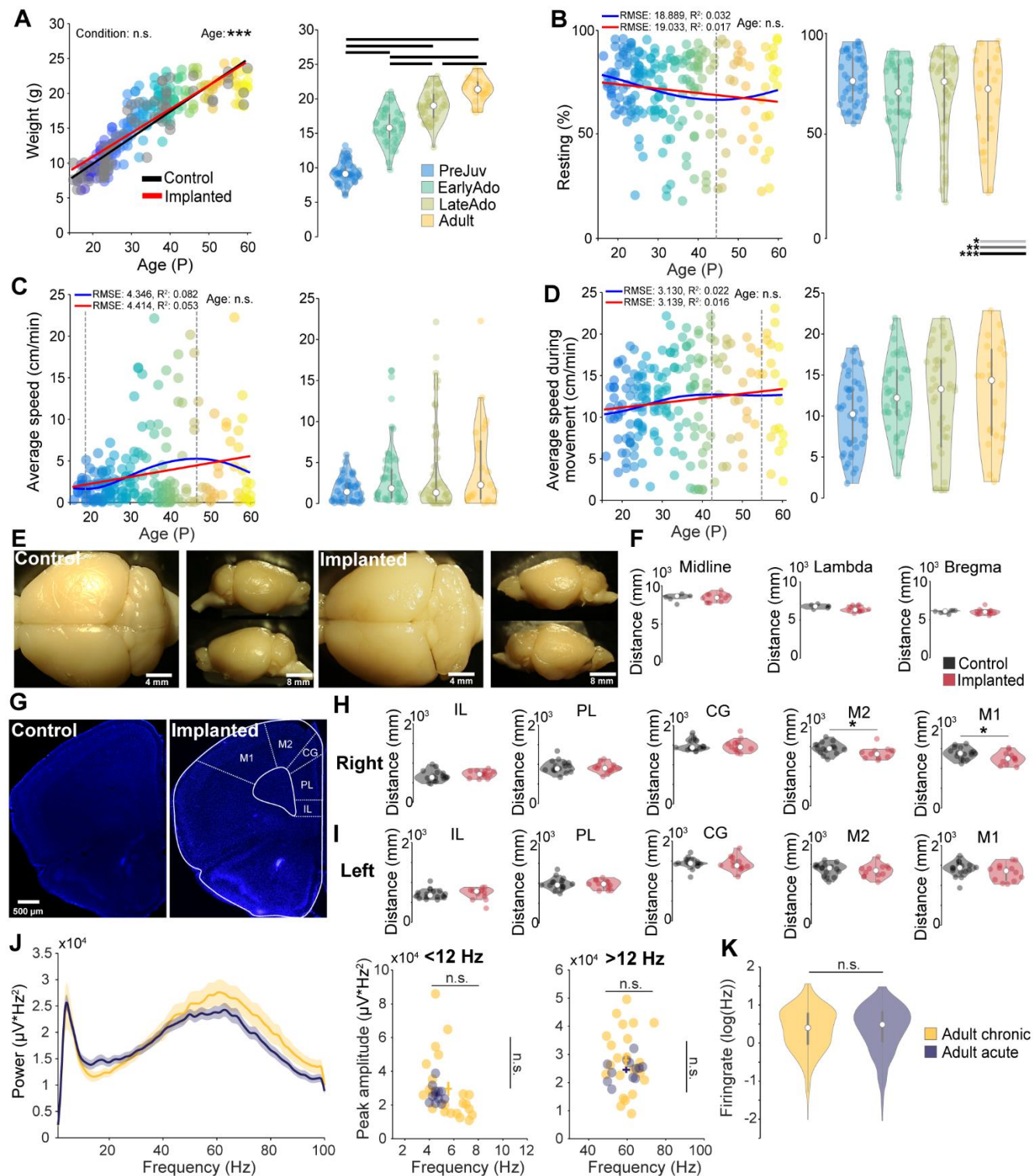


Fig. S1 related to Fig.1. Consequences of the chronic recording paradigm.

(A) Left, scatter plot displaying weight development of implanted and non-implanted (control) mice recorded from P16–60 (control: n=69 measurements, 69 mice; implanted: n=195 measurements, 26

mice). Right, violin plots displaying the same data as left for implanted mice when grouped by age (n=146 measurements, 26 mice).

(B) Left, scatter plot displaying relative resting time (given as %) during recordings of mice recorded from P16–60 (n=195 recordings, 26 mice). Right, violin plots displaying the same data as left when grouped by age (n=146 recordings, 26 mice). Data are fitted by a linear-regression (red line) and a cubic-spline (blue line) model. Dashed gray lines indicate present local minima and maxima of the spline fit.

(C) Same as (B) for average speed.

(D) Same as (B) for average speed during movement periods.

(E) Characteristic example photographs of perfused brains of a P60 Control (left, non-recorded) and Implanted (right, chronically recorded) mouse.

(F) Violin plots displaying brain length of midline (left), outer radius from lambda (middle), and outer radius from bregma (right) of Control and Implanted P54–60 mice (Control n=6 mice, Implanted n=12 mice).

(G) Photographs of characteristic DAPI-stained sections including the mPFC of a P60 Control (left) and Implanted (right) mouse.

(H) Violin plots displaying the cortical thickness for IL, PL, CG, motor cortex 1 (M1), and motor cortex 2 (M2) of the right hemisphere from Control and Implanted P54–60 mice (Control n=16 mice, Implanted n=12 mice).

(I) Same as (F) for the left hemisphere (Control n=16 mice, Implanted n=12 mice).

(J) Left, power spectra of LFP activity recorded in the PL from adult chronically investigated mice (Adult chronic, n=22 recordings, 11 mice) and mice that were recorded acutely (Adult acute, n=12 recordings, 6 mice) for 1-2 times between P53 and P60. Right, scatter plots of peak frequencies as a function of peak amplitudes for the 1–12 and 12–100 Hz activity, respectively.

(K) Violin plots displaying the firing rates of all recorded units (Adult chronic, n=691 units, 22 recordings, 11 mice; Adult acute, n= 290 units, 12 recordings, 6 mice)

For line plots in (J) data are presented as mean $\pm$ SEM. Data of violin plots are presented as median with 25th and 75th percentile. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also statistics table S1 for detailed statistics and recording summary table S3.

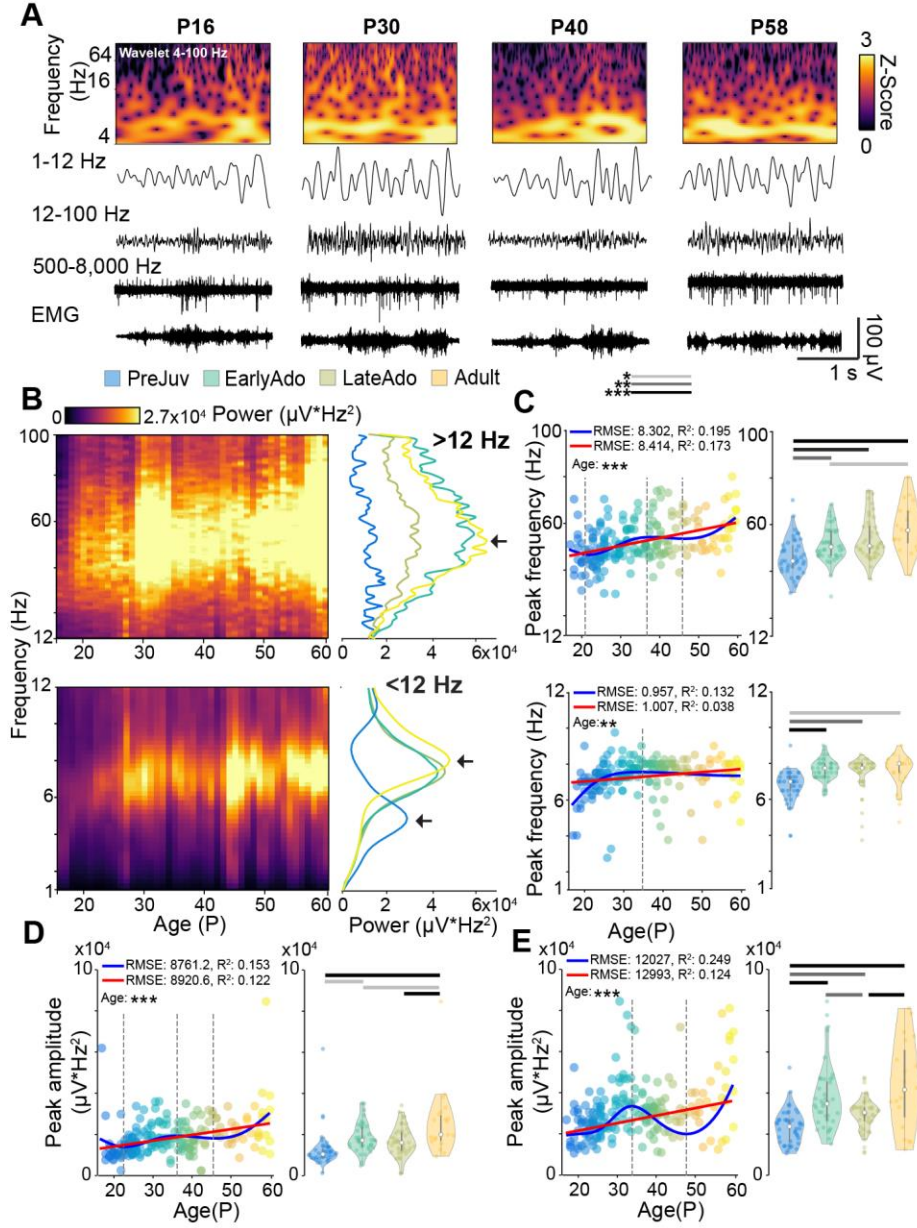


Fig. S2 related to Fig.1. Organization of prefrontal activity along late development during movement.

(A) Characteristic examples of LFP activity (middle) accompanied by color-coded wavelet spectra (top) as well as EMG (bottom) of a mouse during movement investigated at P16, P30, P40 and P58, respectively.

(B) Color-coded age-averaged power spectra (left, n=191 recordings, 26 mice) and example power spectra (right) during movement periods. Note the power peak within the 12–100 Hz band (top) and within the 1–12 Hz band (bottom).

(C) Left, scatter plot displaying detected peak frequencies within the 12–100 Hz band (top) and within the 1–12 Hz band (bottom) of mice chronically recorded from P16–60 (n=191 recordings, 26 mice). Data

are fitted by a linear-regression (red line) and a cubic-spline (blue line) model. Dashed gray lines indicate present local minima and maxima of the spline fit. Right, violin plots displaying the same data as left when grouped by age (n=143 recordings, 26 mice).

(D) Left, scatter plot displaying detected peak amplitudes (n=189 recordings, 26 mice) of peak frequencies (n=191 recordings, 26 mice) within the 1–12 Hz band of mice chronically recorded from P16–60. Data are fitted by a linear-regression (red line) and a cubic-spline (blue line) model. Dashed gray lines indicate present local minima and maxima of the spline fit. Right, violin plots displaying the same data as left when grouped by age (peak amplitudes, n=141 recordings, 26 mice; peak frequencies, n=143 recordings, 26 mice).

(E) Same as (D) for detected peak amplitudes of peak frequencies within the 12–100 Hz band (age, n=191 recordings, 26 mice; condition, n=143 recordings, 26 mice).

Data of violin plots are presented as median with 25th and 75th percentile. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also statistics table S1 for detailed statistics and recording summary table S3.

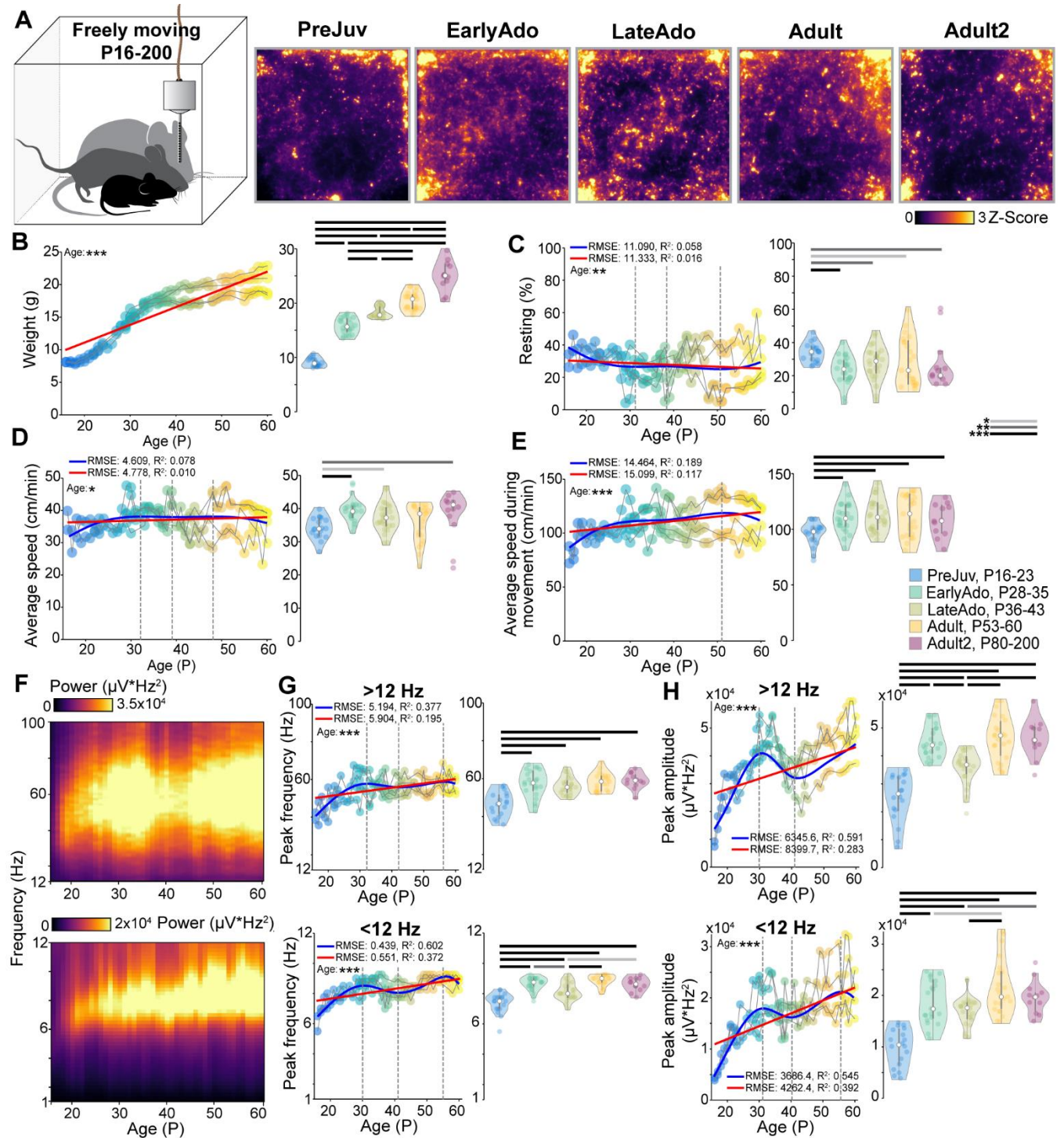


Fig. S3 related to Fig.1. Organization of prefrontal activity along late development during movement in freely moving mice.

(A) Left, schematic of the freely moving recording setup for mice chronically investigated from P16–200. Right, color-coded heatmaps of the time spent at different locations within the open-field arena for each age group.

(B) Left, scatter plot displaying the weight of mice chronically recorded from P16–60 (n=118 measurements, 4 mice). Right, violin plots displaying the data shown left when grouped by age together with additional values from P80–200 mice (n=100 measurements, 4 mice).

(C) Left, scatter plot displaying relative resting time (given as %) for mice chronically recorded from P16–60 (n=118 measurements, 4 mice). Data are fitted by a linear-regression (red line) and a cubic-spline (blue line) model. Dashed gray lines indicate present local minima and maxima of the spline fit. Right, violin plots displaying the data shown left when grouped by age together with additional values from P80–200 mice (n=100 measurements, 4 mice).

(D) Same as (C) for average speed.

(E) Same as (C) for average speed during movement periods.

(F) Color-coded age-averaged power spectra within 12–100 Hz band (top) and 1–12 Hz band (bottom) of mice chronically recorded from P16–60 (n=118 measurements, 4 mice).

(G) Left, scatter plot displaying detected peak frequencies within the 12–100 Hz (top) and 1–12 Hz (bottom) bands of mice chronically recorded from P16–60 (n=191 recordings, 26 mice). Data are fitted by a linear-regression (red line) and a cubic-spline (blue line) model. Dashed gray lines indicate present local minima and maxima of the spline fit. Right, violin plots displaying the data shown left when grouped by age together with additional values from P80–200 mice (n=100 measurements, 4 mice).

(H) Same as (G) for detected peak amplitudes of peak frequencies.

Gray lines indicate recordings from the same mouse. Data are presented as median with 25th and 75th percentile. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also statistics table S1 for detailed statistics and recording summary table S3.

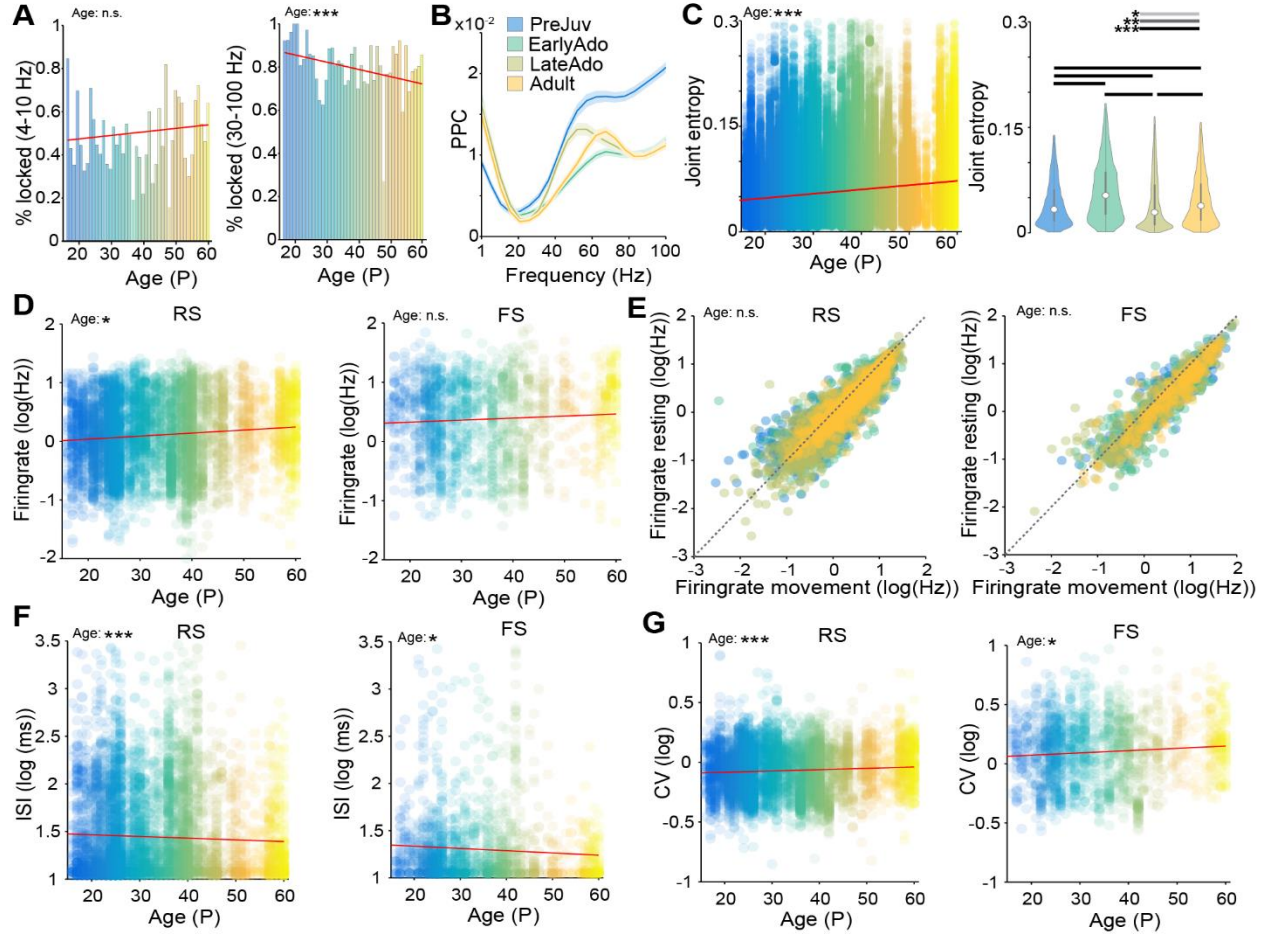


Fig. S4 related to Fig.2 and Fig.3. Dynamics of prefrontal spiking along late development.

(A) Bar plots displaying the percentage of single units significantly locked to theta (4-10 Hz, left) and gamma (30-100 Hz, right) phase for mice recorded from P16–60 (n=192 recordings, 26 mice).

(B) Line plots displaying PPC spectra of single units significantly locked either to the theta or gamma phase recorded from PreJuv, EarlyAdo, LateAdo and Adult mice (n=5096 units, 146 recordings, 26 mice).

(C) Left, scatter plot displaying joint entropy between spike trains recorded from P16-60 mice (n=211588 unit-pairs, 191 recordings, 26 mice). Right, violin plots displaying the same data as right when grouped by age (n=138677 unit-pairs, 146 recordings, 26 mice).

(D) Scatter plots displaying age as a function of firing rates of RS (left, n=5646 units, 191 recordings, 26 mice) and FS (right, n=1584 units, 181 recordings, 26 mice) units recorded from P16-60 mice.

(E) Scatter plots displaying the firing rates during movement as a function of the firing rates during resting periods of RS (left, n=5646 units, 191 recordings, 26 mice) and FS (right, n=1584 units, 181 recordings, 26 mice) units recorded from P16-60 mice.

(F) Same as (D) for ISI during active periods.

(G) Same as (D) for CV.

For line plots in (B) data are presented as mean $\pm$ SEM. Data of violin plots are presented as median with 25th and 75th percentile. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also statistics table S1 for detailed statistics and recording summary table S3.

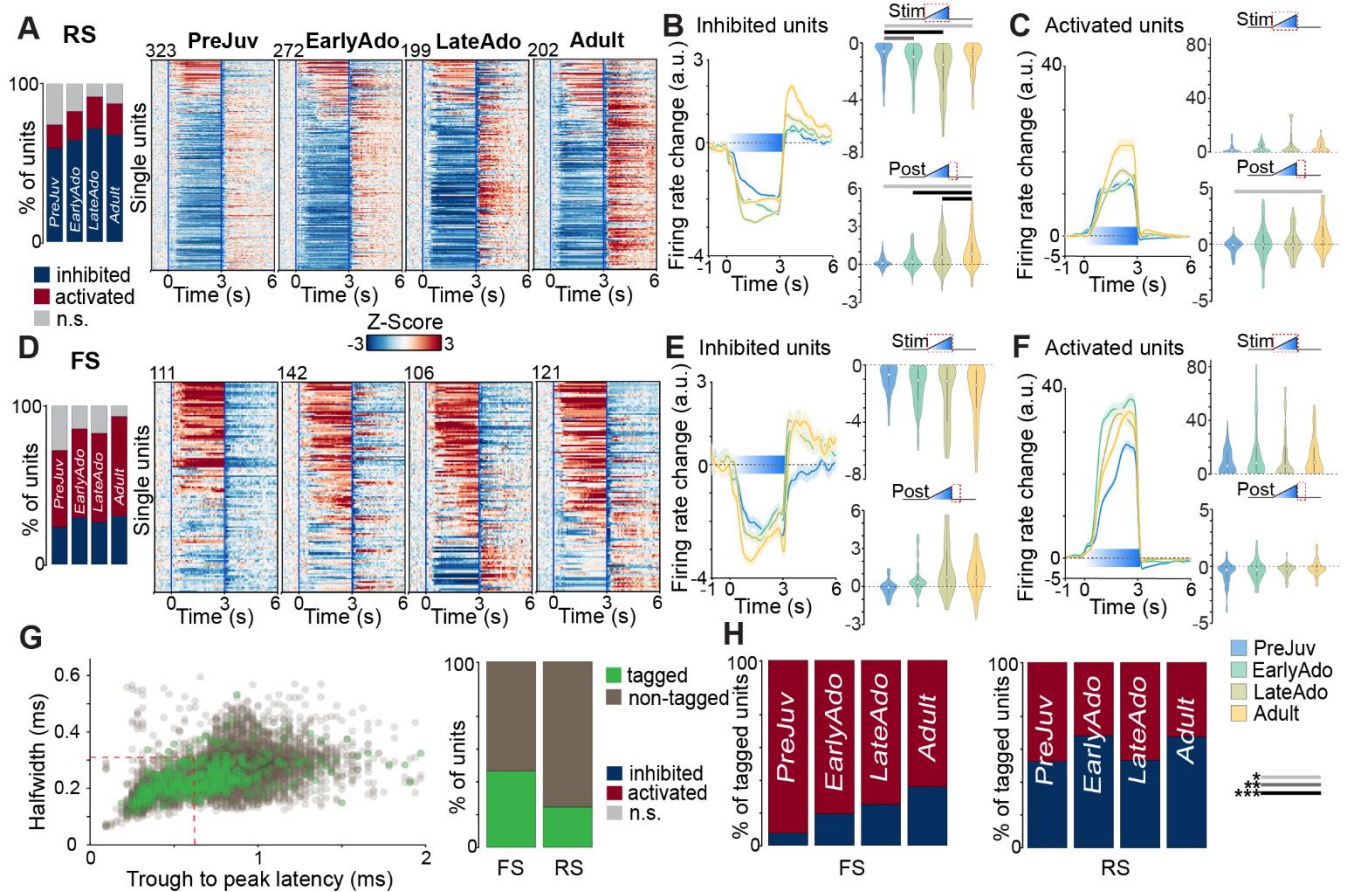


Fig. S5 related to Fig.4. The inhibitory effect of interneuronal firing on regular- and fast-spiking neurons in prefrontal circuits along late development.

(A) Left, bar graph displaying the percentage of significantly modulated RS units during ramped light stimulation. Right, color-coded raster plots displaying the z-score of firing rate change of recorded RS units for each age group.

(B) Line plots displaying firing rate change of inhibited RS units (n=765 units, 34 recordings, 6 mice) for each age group. Right, violin plots displaying the average firing rate change of inhibited RS units during (top) and post (bottom) 3 s-long ramped light stimulation.

(C) Same as (B) for activated RS units (n=231 units, 30 recordings, 6 mice) during (top) and post (bottom) ramped light stimulation.

(D) Same as (A) for FS units.

(E) Same as (B) for inhibited FS units (n=184 units, 30 recordings, 6 mice) during (top) and post (bottom) ramped light stimulation.

(F) Same as (B) for activated FS units (n=306 units, 34 recordings, 6 mice) during (top) and post (bottom) ramped light stimulation.

(G) Left, scatter plot displaying trough to peak latency as a function of halfwidth of all units recorded from P16–60 mice (n=7230 units, 192 recordings, 26 mice). Red dotted lines correspond to classification thresholds for RS (black) and FS (red) units. Green circles indicate photo-tagged units

after 3 ms-long pulsed light stimulation. Right, bar graph displaying the percentage of photo-tagged FS and RS units.

(H) Left, bar graph displaying the percentage of significantly modulated, photo-tagged FS units during ramped light stimulation. Right, same as left for RS units.

For line plots in (B), (C), (E), and (F) data are presented as mean $\pm$ SEM. Data of violin plots are presented as median with 25th and 75th percentile. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also statistics table S1 for detailed statistics and recording summary table S3.

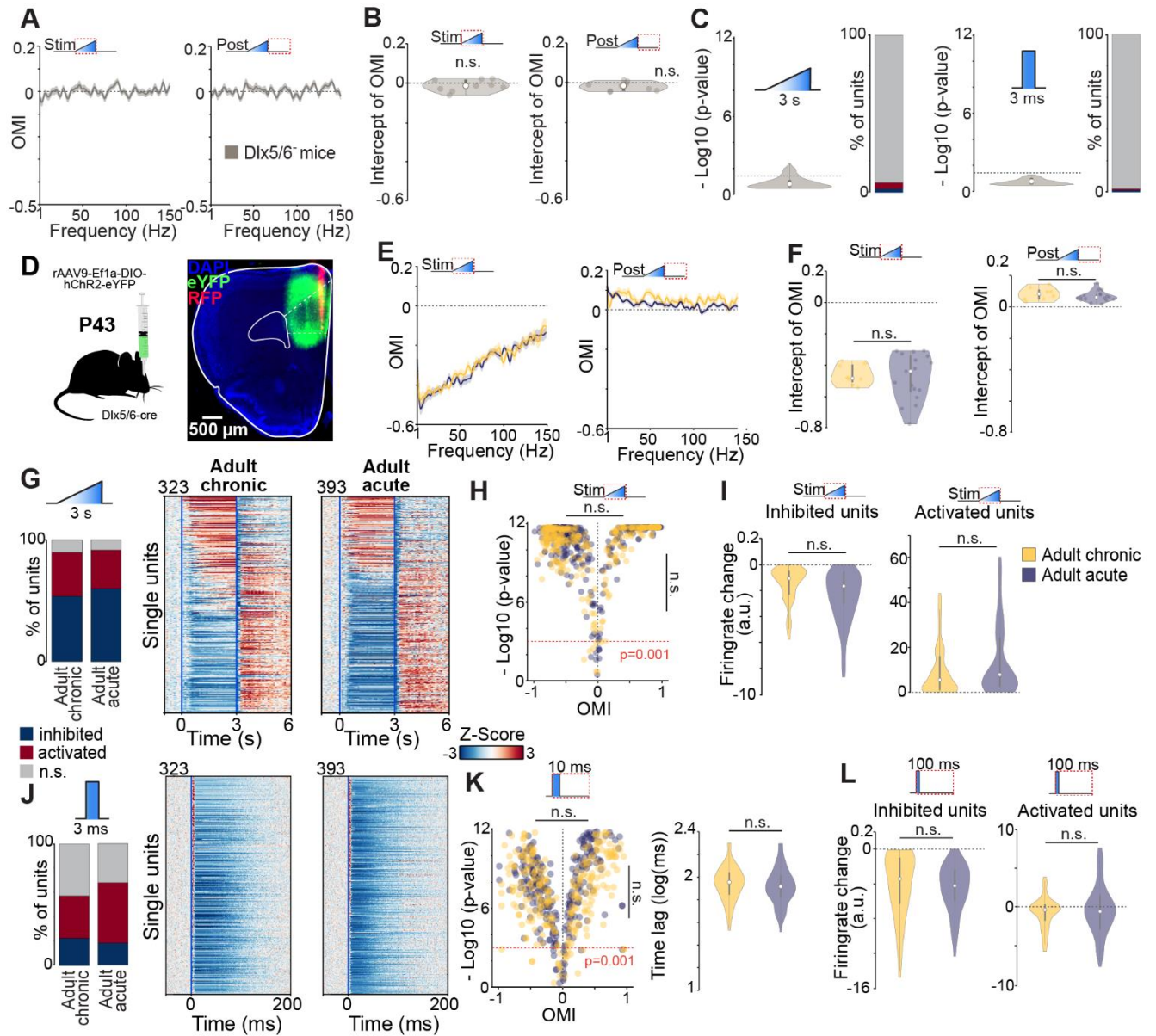


Fig. S6 related to Fig.4. Effects of optogenetic stimulation in Dlx 5/6-cre⁻ and young adult virus injected mice.

(A) OMI spectrum of LFP activity pre vs. during (left) and pre vs. post (right) ramped light stimulation of Dlx 5/6-cre⁻ mice (n=9 recordings, 3 mice).

(B) Violin plots displaying the intercept of individual OMI spectra during (left) and post (right) stimulation periods recorded from Dlx 5/6-cre⁻ mice (n=9 recordings, 3 mice).

(C) Left, violin plot displaying the p-value of firing rate change pre vs. during ramped light stimulation. Bar graph displaying the percentage of significantly activated and inhibited units during ramped light stimulation in Dlx 5/6-cre⁻ mice (n=492 units, 9 recordings, 3 mice). Right, same as left for effects 10 ms after 3 ms square pulse stimulation (n=492 units, 9 recordings, 3 mice).

(D) Left, drawing illustrating viral targeting of interneurons in mPFC of P43 mice and the viral construct. Right, coronal section from a P60 eYFP-expressing *Dlx5/6-cre⁺* mouse.

(E) OMI spectrum of LFP activity pre vs. during (left) and pre vs. post (right) ramped light stimulation of P53–60 *Dlx 5/6-cre⁺* mice virally injected either at P5 (Adult chronic, n=8 recordings, 5 mice) or at P43 (Adult acute, n=17 recordings, 6 mice).

(F) Violin plots displaying the intercept of individual OMI spectra during (left) and post (right) stimulation periods recorded from each group.

(G) Left, bar graph displaying the percentage of significantly modulated units. Right, color-coded raster plots displaying the z-score of firing rate change of all recorded single units for each group.

(H) Volcano plot displaying the OMI as a function of the p-value of firing rate change for Adult chronic (n=323 units, 8 recordings, 5 mice) and Adult acute (n=393 units, 17 recordings, 6 mice) mice.

(I) Violin plots displaying the average firing rate change of inhibited (left; Adult chronic, n=190 units, 8 recordings, 5 mice; Adult acute, n=256 units, 17 recordings, 6 mice) and activated (left; Adult chronic, n=133 units, 8 recordings, 5 mice; Adult acute, n=137 units, 17 recordings, 6 mice) units during 3s of ramped light stimulation.

(J) Same as (G) for effects 10 ms after 3 ms square pulse stimulation.

(K) Left, same as (H) for effects 10 ms after 3 ms square pulse stimulation. Right, violin plots displaying the post-stimulus time that single units require to reach pre-stimulus firing rates for Adult chronic (n=323 units, 8 recordings, 5 mice) and Adult acute (n=393 units, 17 recordings, 6 mice) mice.

(L) Same as (I) for firing rate changes of inhibited (left; Adult chronic, n=190 units, 8 recordings, 5 mice; Adult acute, n=256 units, 17 recordings, 6 mice) and activated (left; Adult chronic, n=133 units, 8 recordings, 5 mice; Adult acute, n=137 units, 17 recordings, 6 mice) units during 100ms after 3 ms square pulse stimulation.

For line plots in (A) and (E) data are presented as mean $\pm$ SEM. Data of violin plots are presented as median with 25th and 75th percentile. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also statistics table S1 for detailed statistics.

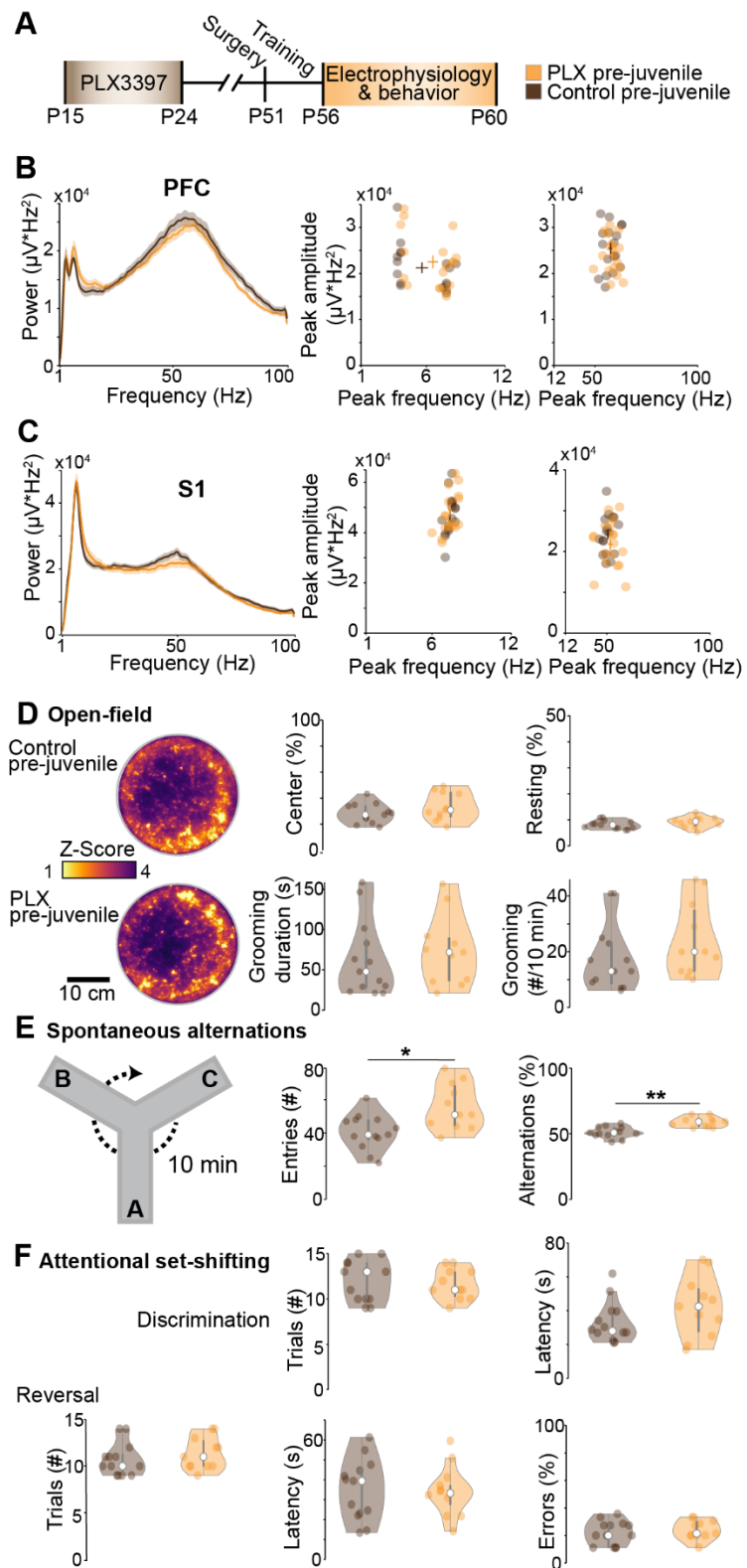


Fig. S7 related to Fig.6 and Fig.7. Network activity and behavioral abilities after pre-juvenile microglia manipulation.

(A) Schematic of the experimental protocol for microglia manipulation at pre-juvenile age.

(B) Left, power spectra of LFP activity recorded in the PL of P56–60 Control and pre-juvenile PLX mice. Right, scatter plot of peak frequencies as a function of peak amplitudes for the 1-12 and 12-100 Hz bands, respectively (n=18 mice, 35 recordings).

(C) Same as (B) for LFP activity recorded in the S1 (n=18 mice, 35 recordings).

(D) Color-coded heatmaps of the time spent at different locations within the open-field arena by Control (top) and pre-juvenile PLX (bottom) mice. Top right, violin plots displaying relative time spent in the center (given as %, left) and relative resting-time (given as %, right) in the open-field arena (n=24 mice). Bottom right, violin plots displaying grooming duration (left) and grooming frequency (right) (n=24 mice).

(E) Violin plots displaying the number of entries (left) and percentage of alternations (right) in the Y-maze (n=24 mice).

(F) Top, violin plots displaying number of trials (left) and average trial latency (right) required by each mouse to reach criterium (n=24 mice). Bottom, violin plots displaying number of trials (left), average trial latency (middle), and total errors (given as %, right) required by each mouse to reach criterium (n=24 mice).

For line plots in (B) and (C) data are presented as mean $\pm$ SEM. Data of violin plots are presented as median with 25th and 75th percentile. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also Statistics table S1 for detailed statistics.

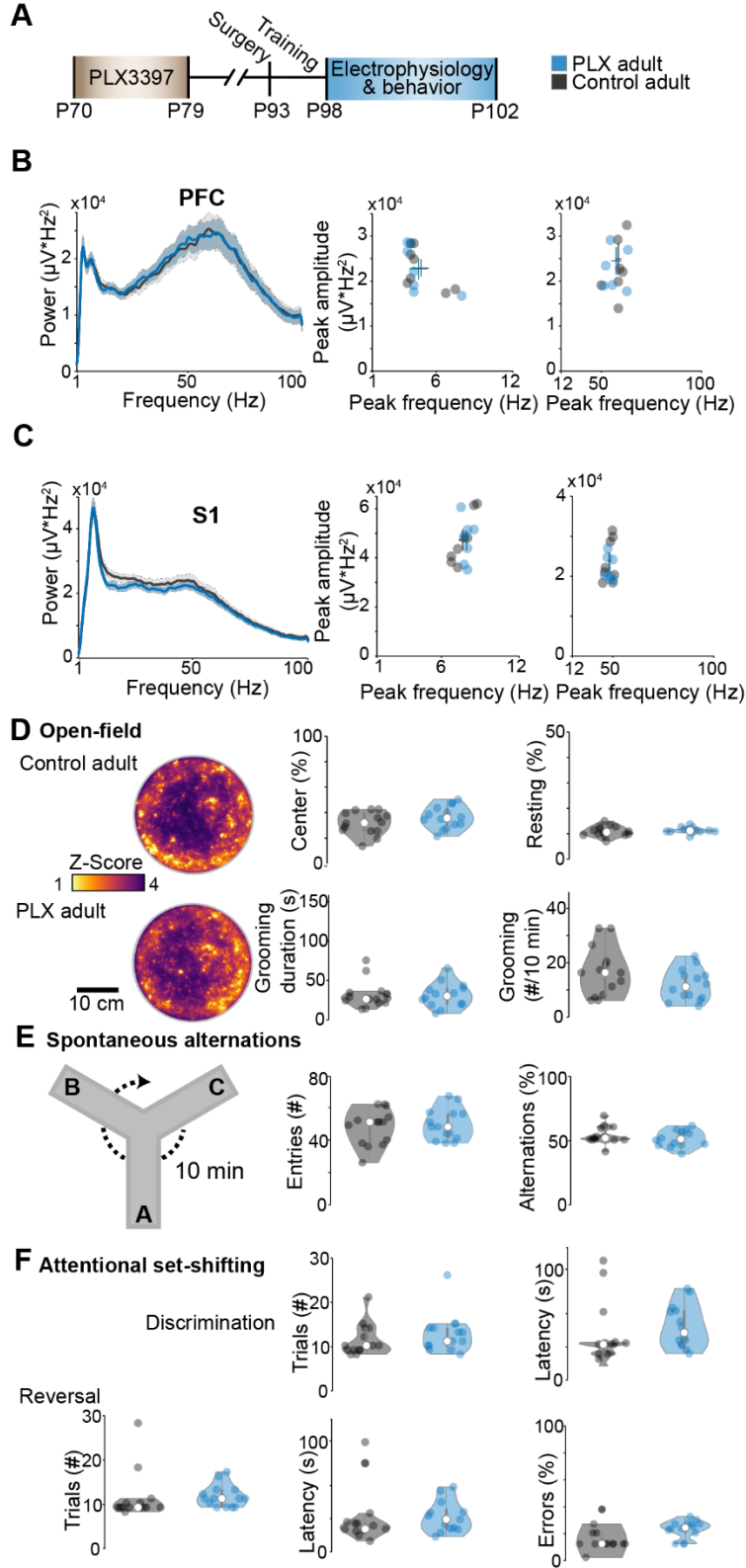


Fig. S8 related to Fig.6 and Fig.7. Network activity and behavioral abilities after adult microglia manipulation.

(A) Schematic of the experimental protocol for microglia manipulation at adult age.

(B) Left, power spectra of LFP activity recorded in the PL of P98–102 Control and adult PLX mice. Right, scatter plot of peak frequencies as a function of peak amplitudes for the 1-12 and 12-100 Hz bands, respectively (n=8 mice, 15 recordings).

(C) Same as (B) for LFP activity recorded in the S1 (n=8 mice, 15 recordings).

(D) Color-coded heatmaps of the time spent at different locations within the open-field arena by Control (top) and adult PLX (bottom) mice. Top right, violin plots displaying relative time spent in the center of the open-field arena (given as %, left) and relative resting-time (given as %, right) in the open-field arena (n=28 mice). Bottom right, violin plots displaying grooming duration (left) and grooming frequency (right) (n=28 mice).

(E) Violin plots displaying the number of entries (left) and percentage of alternations (right) in the Y-maze (n= 28 mice).

(F) Top, violin plots displaying number of trials (left) and average trial latency (right) required by each mouse to reach criterium (n=28 mice). Bottom, violin plots displaying number of trials (left), average trial latency (middle), and total errors (given as %, right) required by each mouse to reach criterium (n=28 mice).

For line plots in (B) and (C) data are presented as mean $\pm$ SEM. Data of violin plots are presented as median with 25th and 75th percentile. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. See also Statistics table S1 for detailed statistics.