Behavioral and electrophysiological effects of enhancing activity of layer 5 pyramidal neurons during early postnatal development

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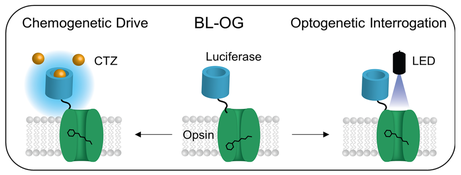
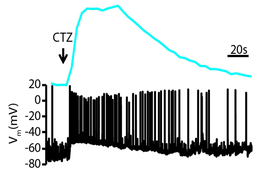
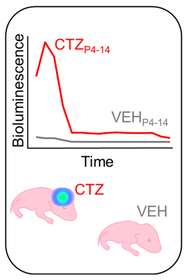
BL-OG enables genetically targeted excitation

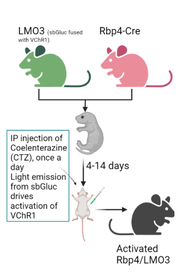
Patterns of neuronal activity during early development are believed to guide the assembly of neural circuits. Thus, alterations of neuronal activity during postnatal time windows may cause structural and functional changes that persist into adulthood. We used bioluminescent optogenetics (BL-OG) to hyperexcite pyramidal neurons during critical periods of postnatal development (P4-14), then observed behavioral and circuit changes in adults.





When a cell expressing the excitatory luminopsin LMO3 is treated with the luciferase substrate CTZ, the luciferase (sbGluc) emits light. This opens the channelrhodopsin (VChR1) to elicit spiking of the neuron.





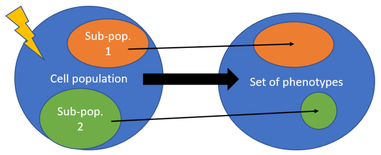
In our previous research, Emx-Cre/LSL-LMO3 mice were given daily injections of CTZ during P4-14 to hyperexcite pyramidal neurons throughout the entire cortex.

Pan-cortical hyperexcitation of pyramidal cells early in development led to circuit and behavioral phenotypes resembling established ASD models (Medendorp et al., 2021):

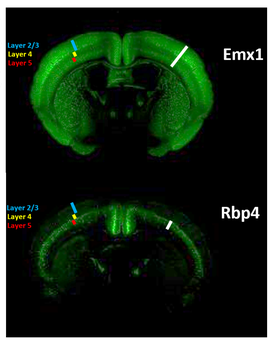
- Increased grooming
- Decreased social interaction
- PFC: decreased baseline firing and increased excitability of pyramidal cells with LED stimulation, impaired recruitment of FS cells

In general, we hypothesize that narrowing down the population of cells that are hyperexcited in early development will similarly narrow down the set of phenotypes that are altered in adulthood.

Targeting Emx1-expressing cells restricted the population of interest to excitatory pyramidal neurons, but this population is highly distributed throughout the brain.



To test whether the effects of developmental hyperexcitation in pyramidal cells can be further specified anatomically, we gave daily injections of CTZ to Rbp4-Cre/LSL-LMO3 during P4-14. In these mice, early stimulation with CTZ is highly selective for neocortical L5 pyramidal cells.

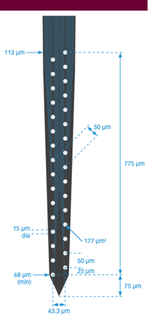


Layer 5 was identified as a region of interest due to its roles in cortical computation and relevance to neuropsychiatric disorders including ASD:

- Integration of signals from higher cortical layers
- Projection to subcortical regions including the thalamus and striatum
- Top-down control of behavior (Naka & Adesnik, 2016)
- Point of convergence between causal mechanisms that produce phenotypes associated with ASD (Brumback et al., 2017)

Methods

- Social approach, social novelty, and novel object exploration videos were processed using DeepLabCut for pose estimation (Mathis et al., 2018). Jitter was managed by application of a median filter before analysis.
- *In vivo* electrophysiological recordings were obtained using a Blackrock Neurotech acquisition system and 32-channel NeuroNexus polytrode probe (right) with 50 µm electrode spacing, at a sampling rate of 30 kHz.
- Kilosort (Pachitariu et al., 2016) and Phy were used for spike sorting.
- For LFP analysis, data were downsampled to 1000 Hz by saving the median in 30-sample intervals. Line noise was removed by Zapline-Plus (Klug & Kloosterman, 2022). Whisker stimulation artifacts were visually identified and removed using the FastICA algorithm in a custom GUI, following a bandpass filter from 4 to 121 Hz that improved the decomposition. Power spectral density was estimated using the multitaper method in all analyses (Bokil et al., 2010).

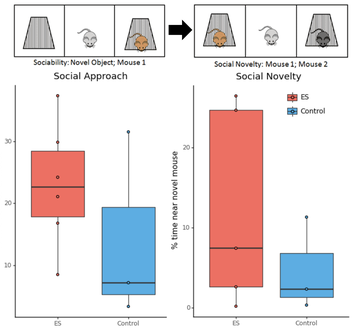


Early postnatal hyperexcitation of L5 pyramidal neurons leads to selective deficits in motor learning

Social interaction results inconclusive

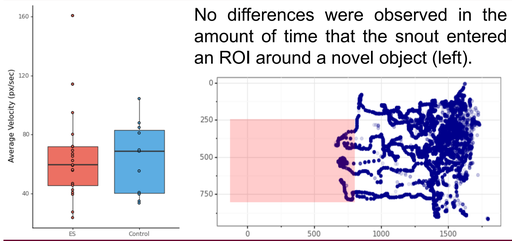
Social interaction was quantified as the amount of time the snout was located inside an ROI drawn around the novel mouse (left).

In our prior study, developmental hyperexcitation of all pyramidal cells led to decreased social interaction in adulthood.



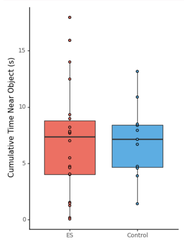
Developmental hyperexcitation restricted to L5 pyramidal neurons appeared to *increase* interaction in a social approach test (left) and preference for a novel mouse (right), but these results are limited by a small sample size. Other approaches such as relative head angle will also be tested.

No difference in exploratory behavior



No differences were observed in the amount of time that the snout entered an ROI around a novel object (left).

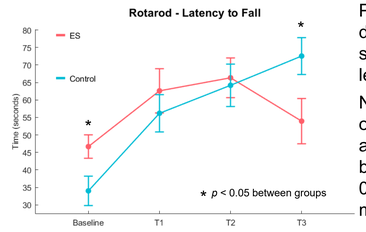
No difference in average velocity



In our prior Emx-Cre/LSL-LMO3 study, widespread early developmental hyperexcitation of pyramidal neurons led to hypolocomotion in adulthood.

No differences were observed in the average velocity of mice during the novel object test (left) when hyperexcitation was restricted to L5. However, open field data should be analyzed to examine locomotion.

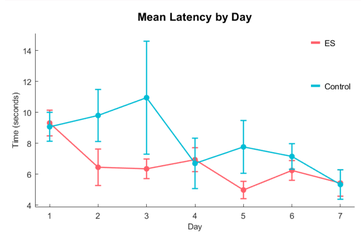
Deficits in motor learning, but not coordination



Pyramidal hyperexcitation in L5 during early development led to a significant impairment in motor learning (RMANOVA, $p = 0.0083$).

No general motor deficit was observed; in fact, the ES group appeared to perform significantly better than controls at baseline ($p = 0.0253$, Bonferroni correction for multiple comparisons).

No deficits in spatial memory or swimming ability

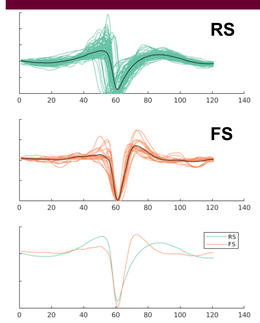


Developmentally hyperexcited mice performed similarly to controls in the water T-maze test, suggesting that spatial memory is unaffected by early pyramidal stimulation in L5.

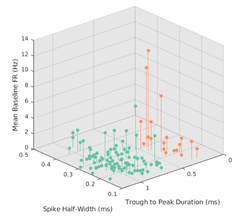
Additionally, this provides further evidence that motor coordination is intact: a generalized motor deficit would appear as increased latency to swim to the platform.

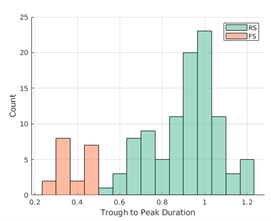
Inverse effects on evoked responses to LED and whisker stimulation in barrel cortex

Cell type classification

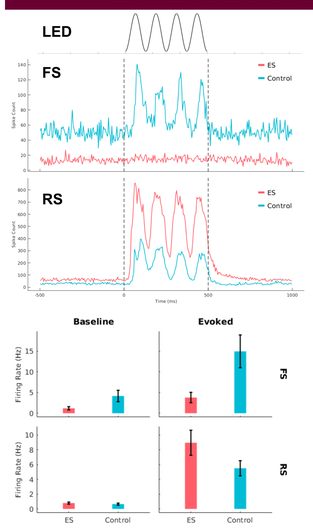


Units were identified using Kilosort (Pachitariu et al., 2016) and Phy, then classified as fast-spiking (FS) if the mean waveform trough-to-peak duration did not exceed 500 µs and otherwise as regular-spiking (RS). In our data, this represented the clearest dividing line between clusters of units across multiple metrics (center).



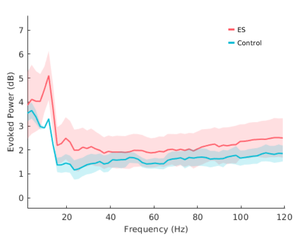


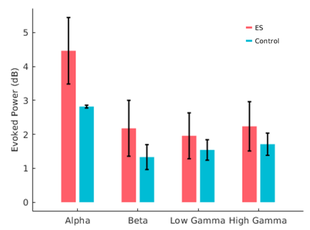
Enhanced excitatory response to LED stimulation



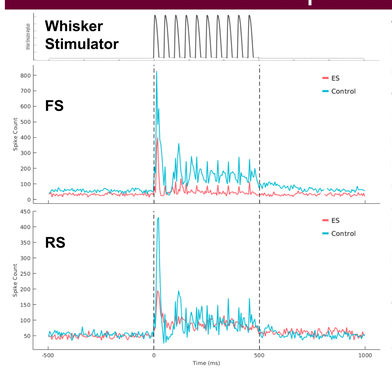
We repeated the paradigm of our previous Emx1-Cre/LSL-LMO3 study by directly stimulating the LMO3-expressing pyramidal cells in anesthetized mice, but this time in barrel cortex rather than PFC. 500ms windows of LED stimulation led to similar results. PSTHs (upper left) show near-zero recruitment of FS cells and overactivation of RS cells during LED stimulation in the treated group, along with lower baseline activity in both cell types. This is reflected in their firing rates (lower left, error bars ± SEM).

Lower right and center plots show evoked LFP power during the 500 ms stimulation window, decibel normalized to the 500 ms leading up to stimulation. In Rbp4 ES mice, LED stimulation caused a large increase in alpha power that was not seen in higher frequency bands. This is the opposite of our prior observations from the Emx1 ES PFC, where evoked gamma power was enhanced and evoked alpha power was reduced.





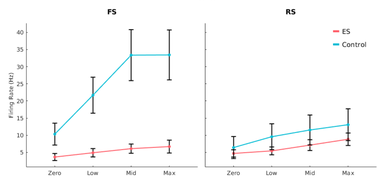
Diminished response to whisker stimulation



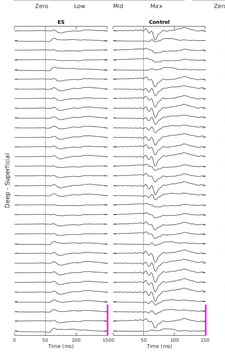
To expand our investigation from direct stimulation into a sensory system, we recorded from barrel cortex in anesthetized mice during repeated trials of whisker stimulation at three intensities.

Interestingly, hyperexcitation of pyramidal cells in L5 during early development led to a lower response to whisker stimulation in RS cells, conflicting with our prediction based on LED stimulation findings that the treated mice would be hyperresponsive to sensory input.

Left: PSTHs showing FS and RS activity during max stimulation trials. The response to each deflection is greatly reduced in the treated group. However, RS cells still show an increase in sustained firing whereas FS cells do not.

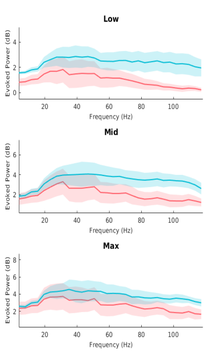


Left: firing rate response curve of FS and RS cells to increasing intensities of whisker stimulation (zero, low, mid, and max) during the first 100 ms. The greatest difference is in FS activity, with FS cells in the Rbp4 ES group showing only a minimal response at all intensities. Reduced baseline FS activity is demonstrated again during the "zero intensity" trials.



Right: evoked PSD across all channels during the first 100 ms of whisker stimulation, decibel normalized to the 100 ms period leading up to each stimulation. Rbp4 ES mice show a reduced response overall; the difference from the control group is greater with increasing frequency and at lower stimulation intensity.

Left: mean LFP traces confirm that the amplitude of the evoked potential corresponding to the first whisker deflection is greatly diminished in Rbp4 ES mice. Electrical artifacts from the whisker stimulator were removed via ICA (see Methods).



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