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## **Supplemental Information**

### **Layer 3 Pyramidal Cells in the Medial Entorhinal Cortex Orchestrate Up-Down States and Entrain the Deep Layers Differentially**

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### **Layer 3 pyramidal cells in the medial entorhinal cortex orchestrate up-down states and entrain the deep layers differentially**

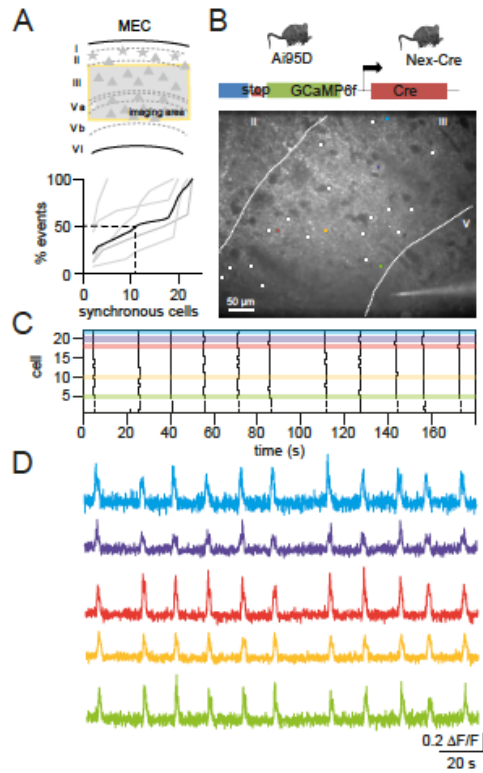
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#### **Inventory of Supplemental Information**

The Supplemental Information contains the following items:

**Supplementary Figures S1 - S5**

## MEC population imaging



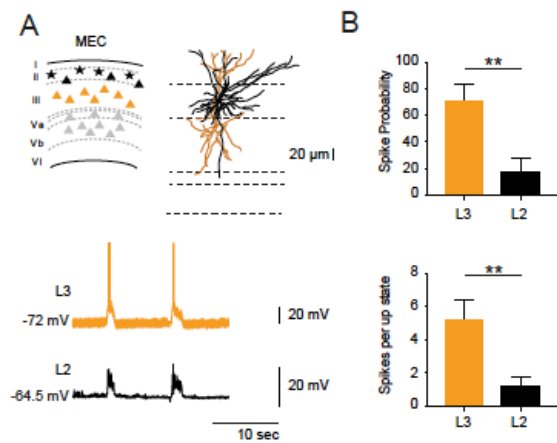
**Figure S1 (related to Figure 1): Calcium imaging for synchronous activity.**

(A) Top: *In vitro* calcium imaging was performed with a 20x objective over a field of view marked with the yellow box that included L3. Bottom: Cumulative distribution plot of the number of synchronous cells in all recorded events (n = 5 slices, 20-33 cells per slice). Grey lines are individual experiments and black line is the average.

(B) Top: Ai95D mice were crossbred with Nex-Cre animals for GCaMP6f expression in pyramidal cells only. Averaged fluorescence image of a time series. Markers in the fluorescence image correspond to analyzed cells.

(C) Raster plot of up state onsets of individual cells labelled in (B)

(D) Temporally aligned fluorescence traces of the color labeled cells in (B) and (C).



**Figure S2 (related to Figure 1): L3 pyramidal cells are more active than L2 stellate cells during up down states.**

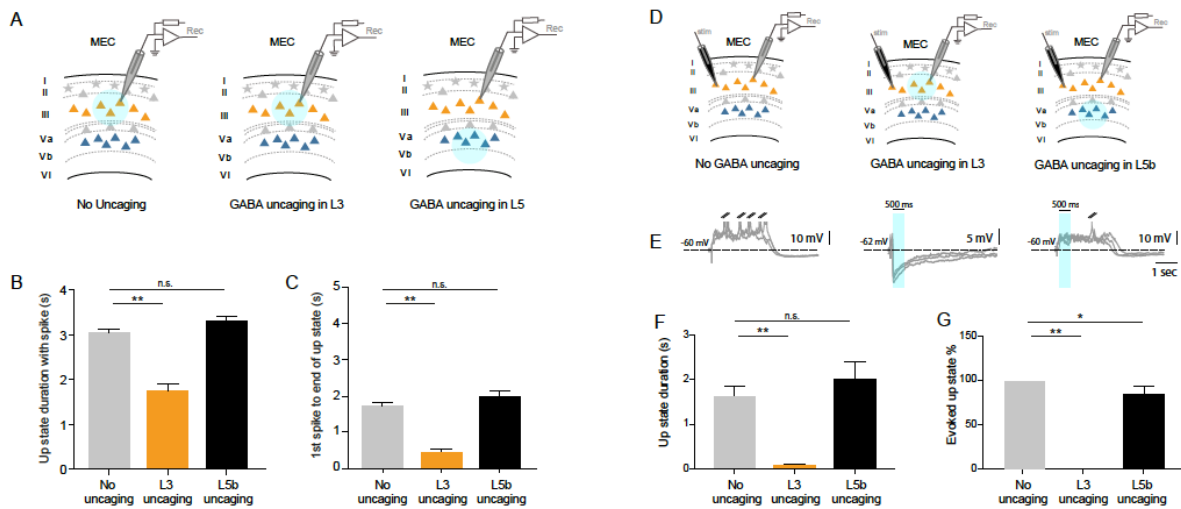
(A) L3 pyramidal cells (in orange) were patched simultaneously with L2 stellate cells (in black). Upper trace is from the L3 pyramidal cell and lower trace is from the L2 stellate cell depicted in biocytin reconstructions.

(B) Upper panel: Spike probability (Up states with spikes / total number of up states) was significantly different between L3 and L2 cells. Lower panel: Spikes per up state was significantly higher in L3 pyramidal cells compared to L2 stellate cells ( $n = 9$  cells for L3 and 10 cells for L2).

Data are presented as mean  $\pm$  SEM

Closed-loop optical suppression of spontaneous up states is layer specific

Suppression of evoked up states is layer specific



**Figure S3 (related to Figure 2): Suppression of up states is layer specific in the MEC.**

Hyperpolarizing L5 cells had no effect on up-down states (UDS) in the MEC in contrast to hyperpolarization of L3 cells, suggesting that suppression of UDS in the MEC is layer specific.

(A) Schematic of closed-loop GABA uncaging experiments for spontaneously occurring up states. As soon as an up state with spikes was detected, GABA was uncaged using LED-emitted blue light (488 nm) for 500ms either in L3 (middle panel) or L5 (right panel). No uncaging served as control (left panel).

(B) Duration of up states was reduced when GABA was uncaged in L3 ( $n = 41$  up states from 5 cells), while GABA uncaging in L5 ( $n = 47$  up states from 5 cells) had no effect compared to no uncaging control ( $n = 84$  up states from 5 cells).

(C) Time from 1st spike (when GABA was uncaged) to the end of up state was reduced upon GABA uncaging in L3 ( $n = 41$  up states from 5 cells), while GABA uncaging in L5 ( $n = 47$  up states from 5 cells) had no effect compared to no uncaging control ( $n = 84$  up states from 5 cells).

(D) Schematic of GABA uncaging experiments for evoked up states. 100ms after an evoked up state, GABA was uncaged for 500ms using blue light (488 nm) either in L3 (middle panel) or L5 (right panel) with no uncaging as control (left panel).

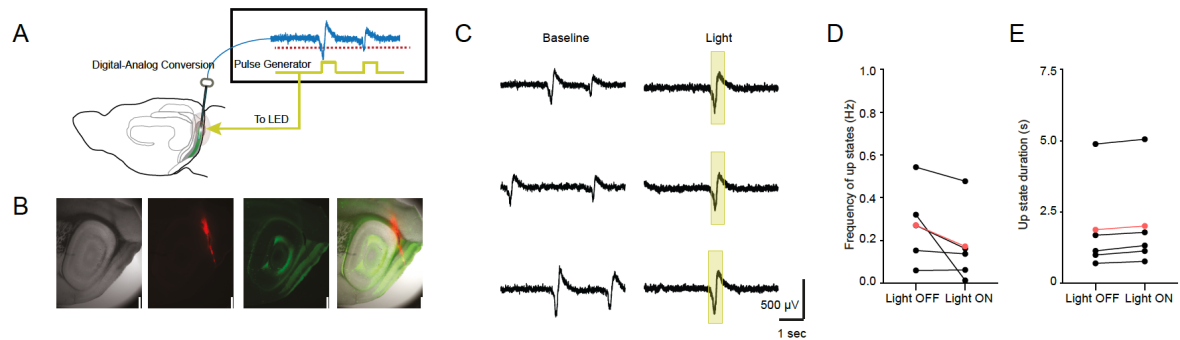
(E) Example traces for the 3 conditions above (action potentials are clipped).

(F) Duration of up states was reduced when GABA was uncaged in L3 ( $n = 20$  up states from 4 cells), while GABA uncaging in L5 ( $n = 12$  up states from 3 cells) had no effect compared to no uncaging control ( $n = 21$  up states from 5 cells).

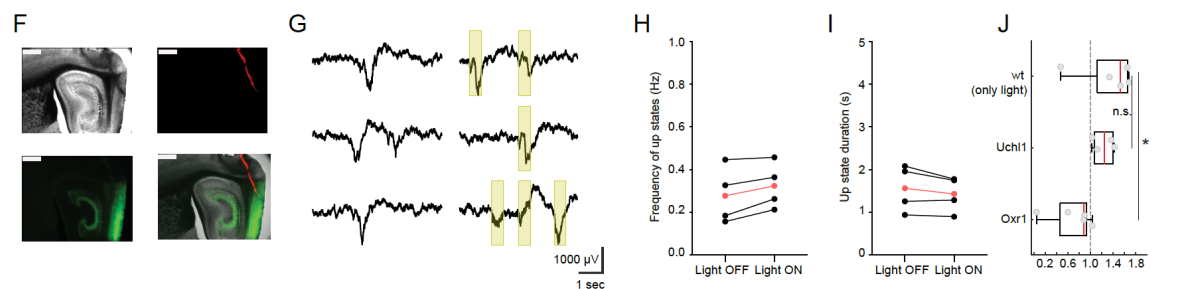
(G) Percentage of evoked up states were completely suppressed by GABA uncaging in L3 ( $n = 4$  cells) while it had a mild suppression of evoked up states when GABA was uncaged in L5 ( $n = 3$  cells) when compared to no uncaging control ( $n = 5$  cells).

Data are presented as mean  $\pm$  SEM

Suppression of L3 pyramidal cell activity (Oxr1-Cre x Ai40D) reduces the frequency of up states in the MEC



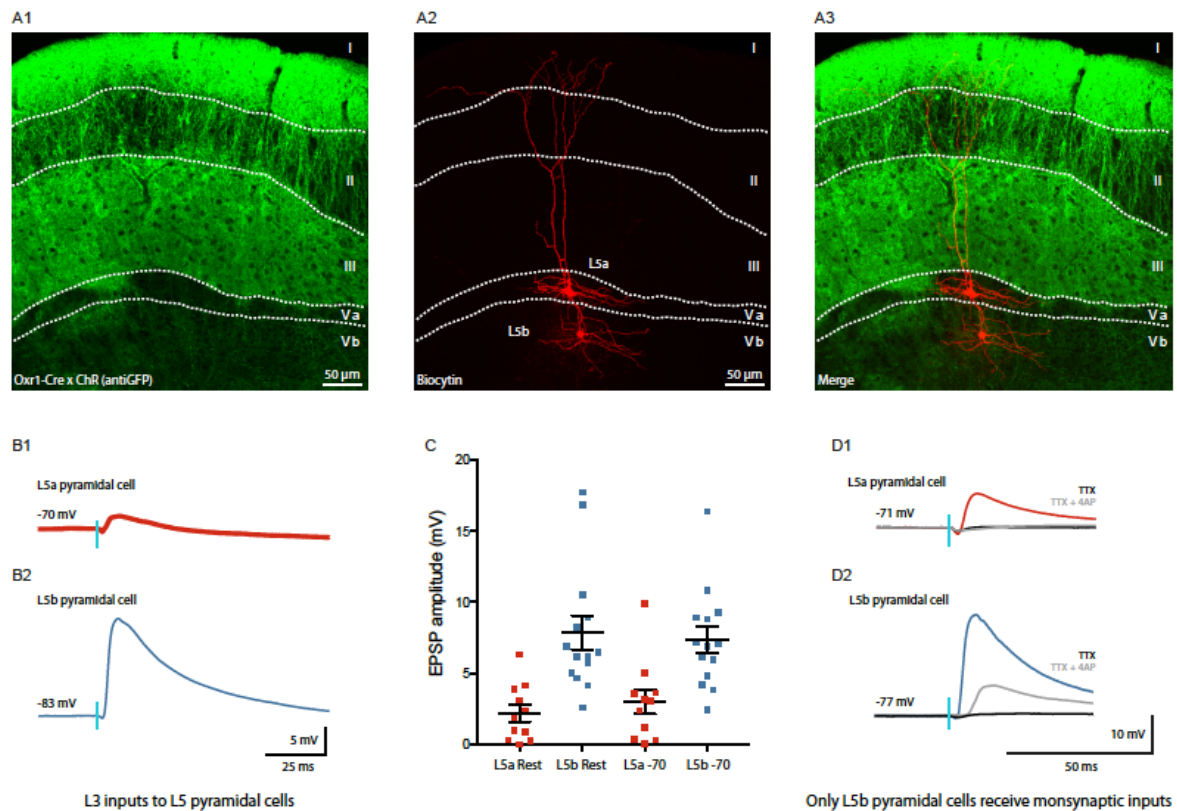
Suppression of L2 stellate cell activity (Uchl1-Cre x AAV-NpHr) does not affect the frequency of up states in the MEC



**Figure S4 (related to Figure 3): Suppression of L3 pyramidal cell activity in L3 (Oxr1-Cre x Ai40D) reduces the frequency of up states in the MEC.**

(A) Schematic showing activation of LED when LFP crosses threshold using a closed-loop system.  
 (B) Example sagittal section showing recording site in L3 of Oxr1-Cre x Ai40D mouse showing Dil trace from silicon probe (left), Oxr1+ ArchT expressing fibers (middle) and overlay (right). Scale bar = 500  $\mu$ m.  
 (C) Example traces showing 5 second excerpts from LFP in baseline (light OFF, left) and during stimulation period (light ON, right). Yellow rectangles show LED illumination during detected up states.  
 (D) Average up state frequency in 10 min light OFF and light ON periods in Oxr1-Cre x Ai40D mice (Hz). Black lines represent individual recordings, red line shows the group average (n = 5 recordings from 5 animals).  
 (E) Average up state duration in 10 min light OFF and light ON periods in Oxr1-Cre x Ai40D mice (ms). Black lines represent individual recordings, red line is the group average (n = 5 recordings from 5 animals).  
 (F) Example sagittal section showing recording site in L2 of Uchl1-Cre x AAV- eNpHR3.0-EGFP mouse showing Dil trace from silicon probe (right), halorhodopsin-expressing cells (lower left) and overlay (lower right). Scale bar = 750  $\mu$ m.  
 (G) Example traces showing 5-second excerpts from LFP in baseline (light OFF, left) and during stimulation period (light ON, right). Yellow rectangles show LED illumination during detected up states.  
 (H) Average up state frequency in 10 min light OFF and light ON periods in Uchl1-Cre x AAV- eNpHR3.0-EGFP mice (Hz). Black lines represent individual recordings; Red line is the group average (n = 4 recordings from 3 animals).  
 (I) Average upstate duration in 10 min light OFF and light ON periods in Uchl1-Cre x AAV- eNpHR3.0-EGFP mice (ms). Black lines represent individual recordings, red line is the group average (n = 4 recordings from 3 animals).  
 (J) Comparison of up state frequency normalized to baseline (light OFF periods) frequency in wildtype (top), Uchl1-Cre x AAV eNpHR3.0-EGFP (middle) and Oxr1-Cre x Ai40D mice (bottom). Red lines indicate the median of each group, box edges show 25th and 75th percentile, respectively. Whiskers extend to most extreme data points (n = 5, 4, and 5 recordings from 4, 3, and 5 animals, respectively).

Data are presented as mean  $\pm$  SEM



**Figure S5 (related to Figure 5): Mapping L3 inputs to L5 pyramidal cells using the Oxr1-Cre x ChR mice.**

(A1) Distribution of ChR expressing fibers from Oxr1 positive L3 pyramidal cells in the MEC.

(A2) L5a and L5b pyramidal cells filled with biocytin were recorded from and L3 inputs mapped onto them.

(A3) Merge of A1 and A2.

(B1) L3 input (2ms blue LED in current clamp) onto L5a pyramidal cell at resting membrane potential. An average of 10 traces is shown.

(B2) Same as in B1 but for L5b pyramidal cell.

(C) Overview of input amplitudes onto L5a (n = 11 cells) and L5b (n = 14 cells) pyramidal cells recorded at resting membrane potential and at -70mV.

(D1) Test of monosynaptic input onto L5a and in (D2) L5b pyramidal cell using the TTX-4AP approach. An average of 10 traces is shown. L5b pyramidal cells receive monosynaptic input from L3 Oxr1 positive pyramidal cells.

Data are presented as mean  $\pm$  SEM