Supplementary Materials for

Characterizing brain stage-dependent pupil dynamics based on lateral hypothalamic activity

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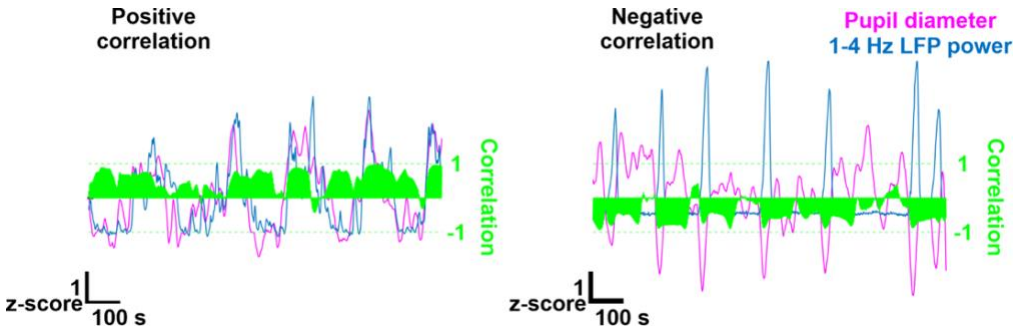

Fig. S1. Examples of the positive and negative correlation between pupil dynamics (pink trace) and LFP delta (1-4 Hz) band power (blue trace) fluctuations in the LH during 15 minutes of resting state. The green plots represent how the correlation between LH delta power and pupil size changed over 15 minutes of recording, calculated with a 60 seconds window for every 2 seconds. The green dash lines represent correlation coefficient at -1 and 1 respectively.

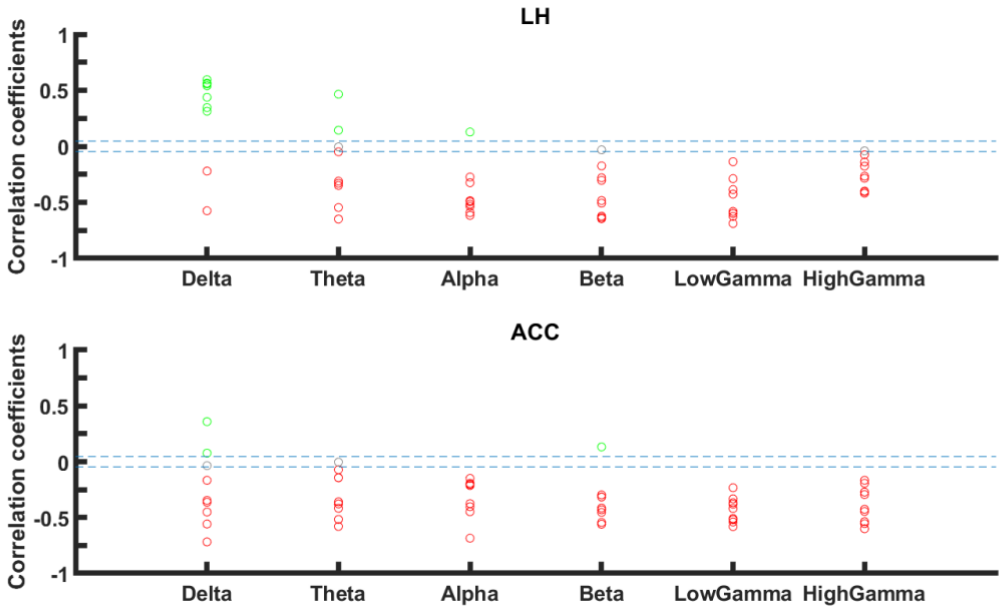

Fig. S2. Correlation coefficients between pupil dynamics and power of all LFP bands in LH (top) and ACC (bottom) from a single representative animal. Each marker represents positive (green), no (gray), or negative (red) correlation from every single trial. Thresholds of positive (0.046) or negative (-0.046) correlation are shown as blue dashed lines.

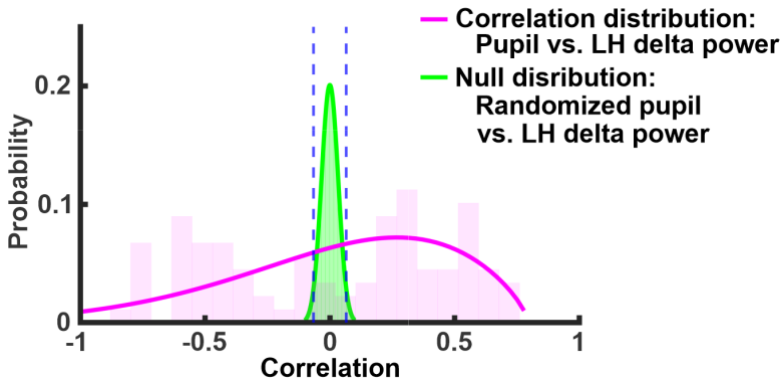

Fig. S3. Determination of the threshold for positive and negative correlations between electrophysiology signals and pupil fluctuation. The pupil signals were first randomized using randperm function in Matlab. Using these randomized pupil signals together with 1-4 Hz LFP power, the correlation coefficient was calculated, and the permutation was conducted with 10,000 times of repetition across 61 trials. The 95 % confidence interval of correlation coefficients from this permutation test was between -0.046 and 0.046, indicated with dashed blue lines. The distribution of correlation coefficients between randomized pupil and LH delta power is in green and the distribution of correlation coefficients between pupil and LH delta power is in pink (same as Fig.1e left).

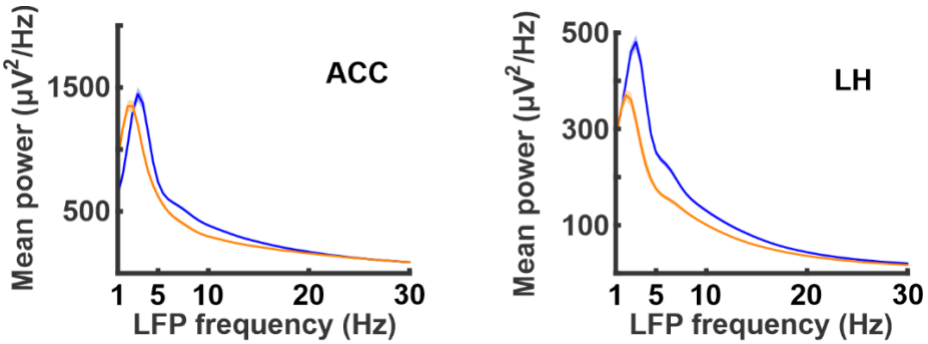

Fig. S4. Power spectral density of the ACC (**left**) and LH (**right**) between 1 Hz and 30 Hz range in [+] pupil × LH LFP (δ) cases (blue line) and [-] pupil × LH LFP (δ) cases (orange line). Standard errors are represented with a shading area. The peak frequency in both LH and ACC is at 3 Hz in LFP-Group 2 (blue; [+] pupil × LH LFP (δ) cases) but at 2 Hz in LFP-Group 1 (orange; [-] pupil × LH LFP (δ) cases).

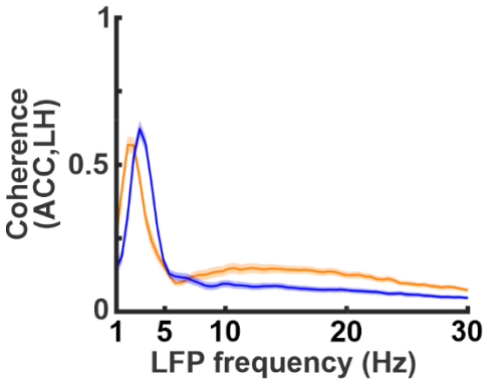

Fig. S5. Magnitude squared coherence (MSC) between the LH and ACC in 1Hz to 30 Hz range. The highest coherence occurred at 3 Hz in LFP-Group 2 (blue; [+] pupil × LH LFP (δ) cases) but at 2 Hz in LFP-Group 1 (orange; [-] pupil × LH LFP (δ) cases).

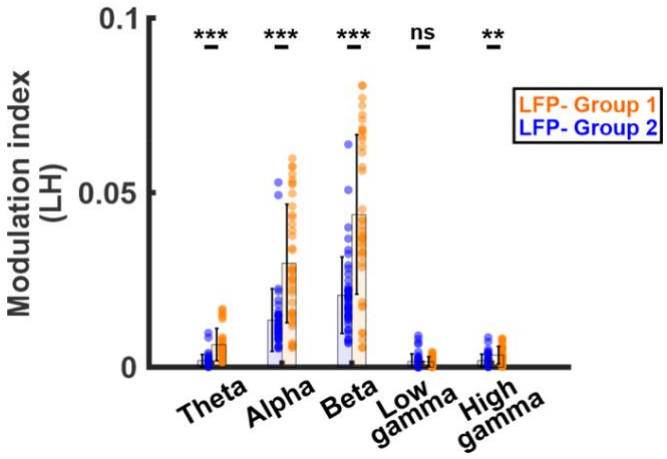

Fig. S6. Phase-amplitude coupling using the modulation index at the LH between the phase of delta (1-4 Hz) band and amplitude of other frequency bands. The modulation index in LFP-Group 1 (orange) is higher than LFP-Group 2 (blue) on theta, alpha, beta, and high gamma bands. Error bars indicate the standard deviation. *Note*: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

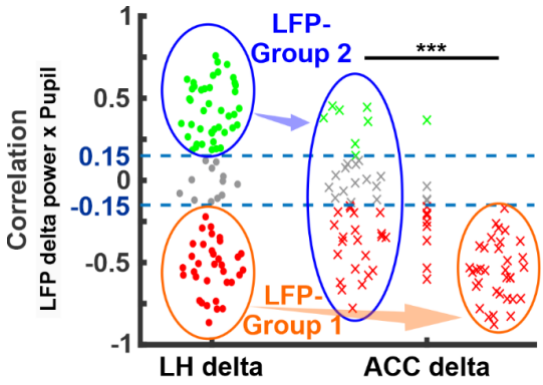

Fig. S7. Categorization of correlation coefficients in the ACC between LFP delta power fluctuation and pupil dynamics when LH delta power fluctuation has a negative (LFP-Group 1, orange) or positive (LFP-Group 2, blue) correlation with pupil dynamics. In LFP-Group 1, ACC delta power fluctuation and pupil size fluctuation show a negative correlation (correlation coefficient < -0.15) in all trials. In LFP-Group 2, correlations of ACC delta power fluctuation and pupil dynamics diversify to a great extent. Note: [+]: correlation coefficient > 0.15, [0]: -0.15 < correlation coefficient < 0.15, [-]: correlation coefficient < -0.15.

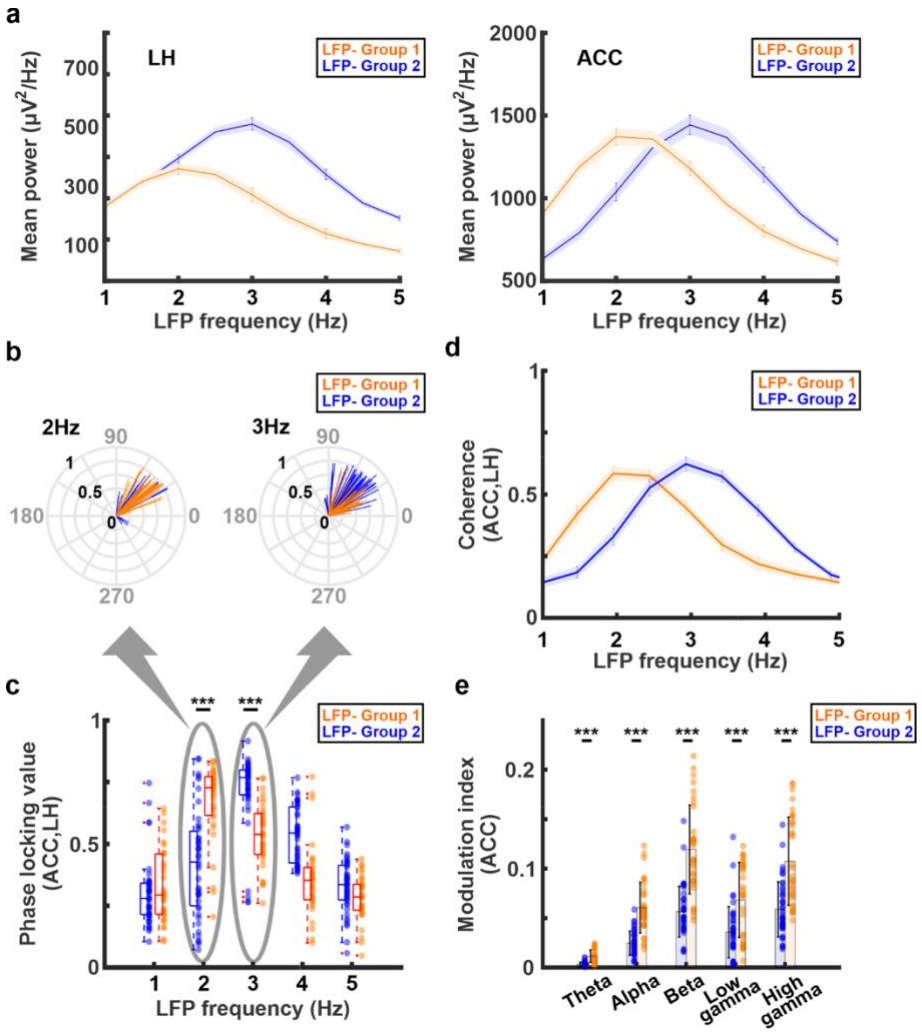

Fig. S8. Comparison of power spectral density and cross frequency analyses between LFP-Group 1 and 2, categorized by using correlation coefficients of 0.15 as the threshold of being positive and negative correlations. a) Power spectrum density of the LH (left) and ACC (right) in LFP-Group 2 (blue line) and LFP-Group 1 (orange line). The shaded areas represent the standard error of the mean. The peak frequency in both LH and ACC is at 3 Hz in LFP-Group 2 (blue line) but at 2Hz in LFP-Group 1 (orange line). b) The radial plots of the trial-specific phase angles ([degrees], grey) and phase-locking value (black) between LH and ACC at 2 Hz and 3 Hz in LFP-Group 1 (orange) and LFP-Group 2 (blue). c) Phase locking value between the LH and ACC in both groups. The highest phase synchrony occurred at 3 Hz in LFP-Group 2 (blue) but at 2 Hz in LFP-Group 1 (orange). d) Magnitude squared coherence between LH and ACC. The highest coherence occurred at 3 Hz in LFP-Group 2 (blue) but at 2 Hz in LFP-Group 1 (orange). The shaded areas represent the standard error of the mean. e) Phase-amplitude coupling using the modulation index (MI) at the ACC between the phase of delta (1-4 Hz) band and amplitude of other frequency bands. The MI in LFP-Group 1 (orange) is higher than LFP-Group 2 (blue) on all frequency bands. Error bars indicate the standard deviation. Note: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

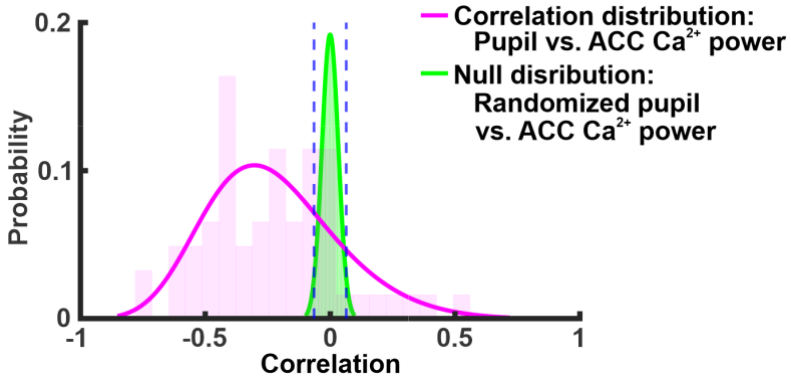

Fig. S9. Determination of the threshold for positive and negative correlations between Ca^{2+} signals and pupil fluctuation. The correlation coefficient was calculated between randomized pupil signals and 1-4 Hz Ca^{2+} power, and then permutation was conducted with 10,000 times of repetition across 61 trials. The 95 % confidence interval of correlation coefficients from this permutation test was between -0.064 and 0.064, indicated with dashed blue lines. The distribution of correlation coefficients between randomized pupil and ACC 1-4 Hz Ca^{2+} power is in green and the distribution of correlation coefficients between pupil and ACC 1-4Hz Ca^{2+} power is in pink (same as Fig.3d).

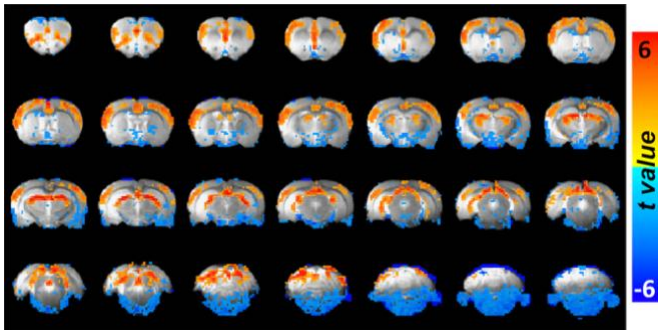

Fig. S10. a) Averaged correlation maps between ACC 1-4 Hz Ca^{2+} power and each voxel of fMRI signals from all trials (n=61). The overlay shows voxels with t-value < -2.25 and t-value > 2.25.

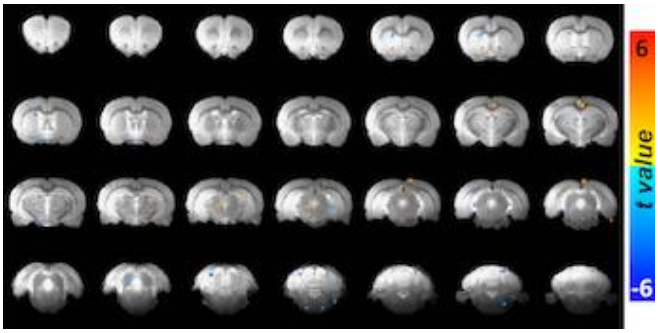

Fig. S10. b) Averaged correlation maps between ACC 1-4 Hz Ca^{2+} power and each voxel of fMRI signals when ACC 1-4 Hz Ca^{2+} power and pupil dynamics are not negatively correlated (n=19). The overlay shows voxels with t-value < -2.25 and t-value > 2.25.

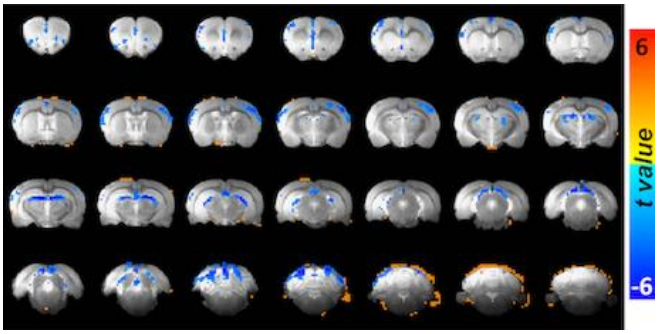

Fig. S11. Correlation maps between each fMRI voxel and pupil dynamics from all trials (n = 61). The overlay shows voxels with t-value < -2.54 and t-value > 2.54.

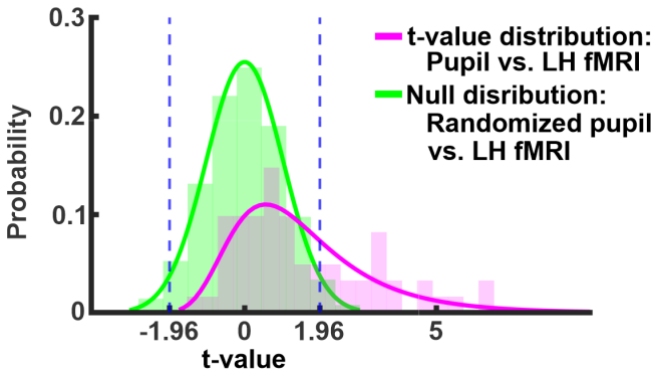

Fig. S12. Determination of the threshold for positive and negative correlation between LH fMRI signals and pupil fluctuation. The pupil signals were first randomized, and t-values between this randomized pupil signal and all LH fMRI voxels (AP = -3.2 mm) were calculated. Then, permutation was conducted with 10,000 repetitions across 61 trials. The 95 % confidence interval of t-values from this permutation test was between -1.96 and 1.96 (indicated with dashed blue line). The distribution of t-values between randomized pupil and LH fMRI is in green and the distribution between pupil and LH fMRI is in pink (same as Fig.4b).

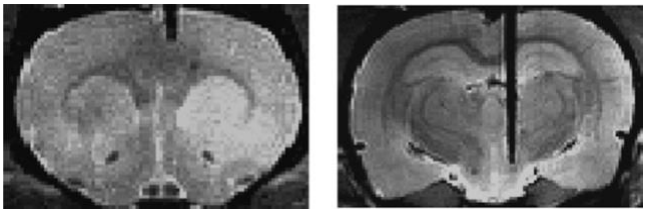

Fig. S13. The location of the electrodes in the ACC (left) and LH (right) from a representative animal, imaged by anatomical MR imaging.

Tables

Table S1. Mean and standard deviation of phase locking value in LFP-Group 1 and 2.

	Mean		SD	
Phase locking value (ACC vs LH)	State #1	State #2	State #1	State #2
1 Hz	0.3388	0.3027	0.1536	0.1291
2 Hz	0.6596	0.4279	0.1723	0.2136
3 Hz	0.5426	0.7119	0.1393	0.1535
4 Hz	0.3724	0.5322	0.1622	0.1267
5 Hz	0.2799	0.3257	0.0977	0.1097

Table S2. Mean and standard deviation of modulation index at the ACC in LFP-Group 1 and 2.

	Mean		SD	
Modulation index	State #1	State #2	State #1	State #2
Delta phase x theta amplitude	0.0114	0.0035	0.0061	0.0019
Delta phase x alpha amplitude	0.0592	0.0247	0.0255	0.0117
Delta phase x beta amplitude	0.1201	0.0564	0.0466	0.0248
Delta phase x low gamma amplitude	0.0701	0.0361	0.0376	0.0251
Delta phase x high gamma amplitude	0.1087	0.0591	0.0443	0.0272

Table S3. Mean and standard deviation of modulation index at the LH in LFP-Group 1 and 2.

	Mean		SD	
Modulation index	State #1	State #2	State #1	State #2
Delta phase x theta amplitude	0.0065	0.0019	0.0046	0.0018
Delta phase x alpha amplitude	0.0298	0.0136	0.0170	0.0090
Delta phase x beta amplitude	0.0438	0.0207	0.0228	0.0109
Delta phase x low gamma amplitude	0.0016	0.0017	0.0014	0.0022
Delta phase x high gamma amplitude	0.0035	0.0019	0.0025	0.0018