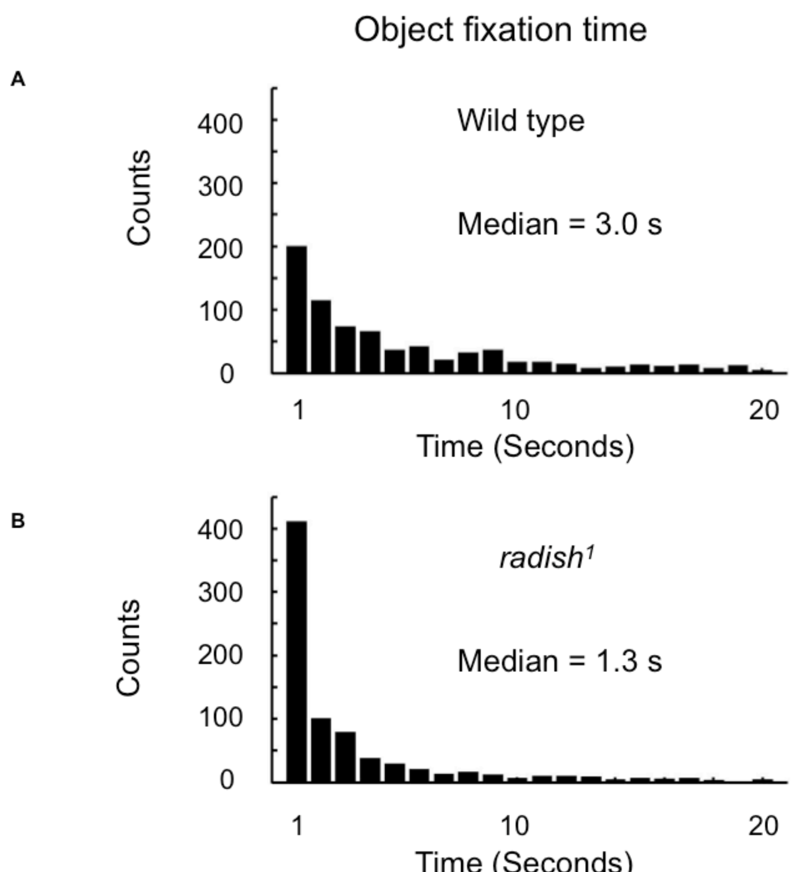
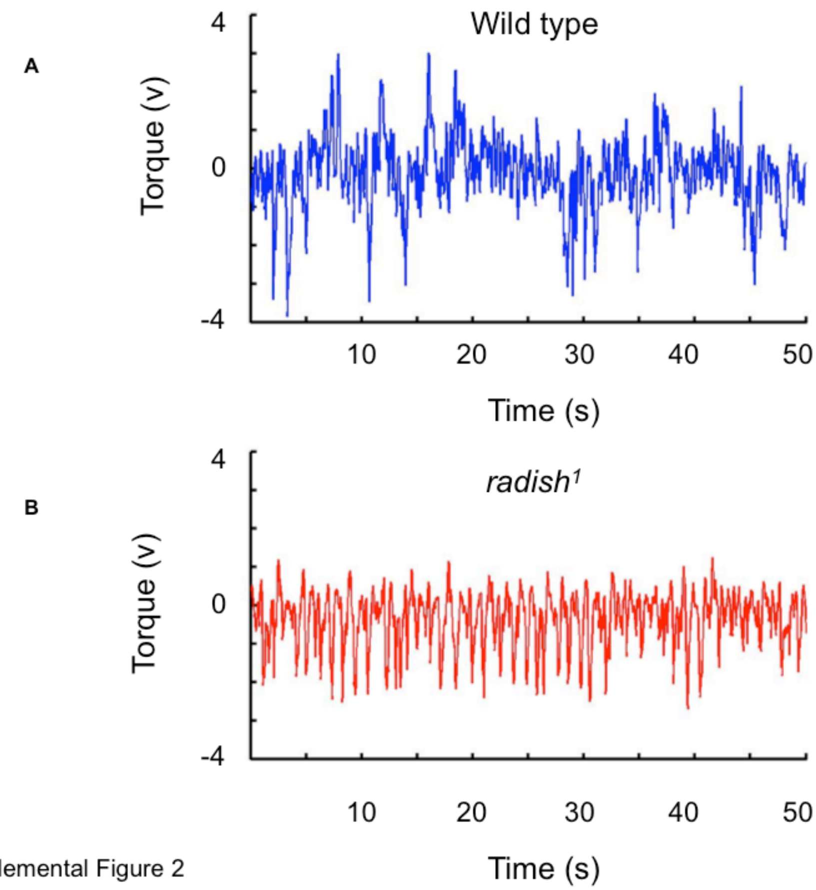
Supplemental Material

Supplemental Figure 1. Frequency histogram for fixation times in the tethered flight arena (see Methods). Counts for each time bin are combined for all flies within a genotype; median fixation time is indicated. **A** wild type n=25 **B** *radish*[1] n=24.



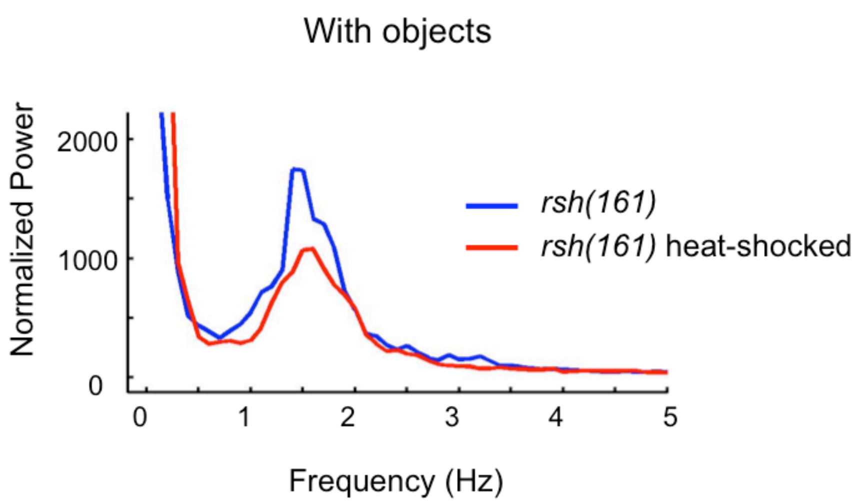
Supplemental Figure 1

Supplemental Figure 2. Tethered flight paradigm **A** Torques (in volts) caused by changes in wing-beat behavior for 50 s of tethered flight in a sample wild-type fly. **B** Torques behavior for 50 s of tethered flight in a sample *radish[1]* fly.



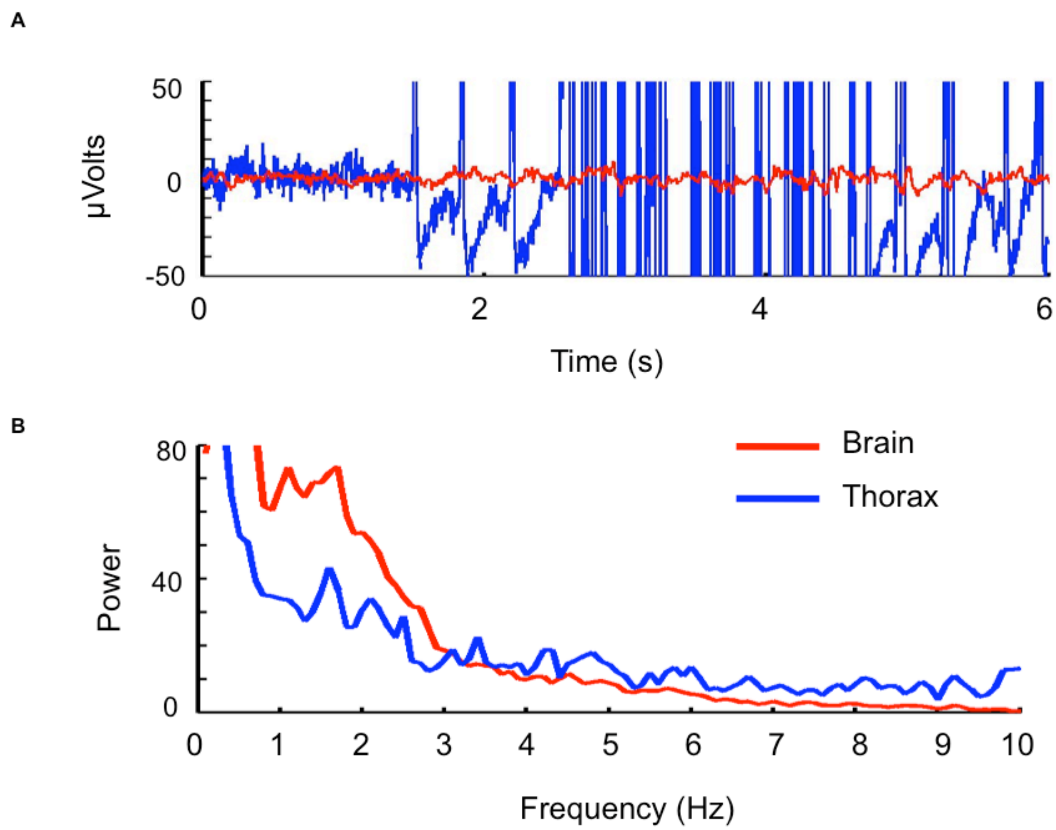
Supplemental Figure 2

Supplemental Figure 3. Average power spectra between 0 and 5 Hz for non heat-shocked *hs-rsh(161)* (blue line, n=4) and heat-shocked *hs-rsh(161)* (red line, n=3) 6-minute open-loop continuous flights with two distinct visual objects.



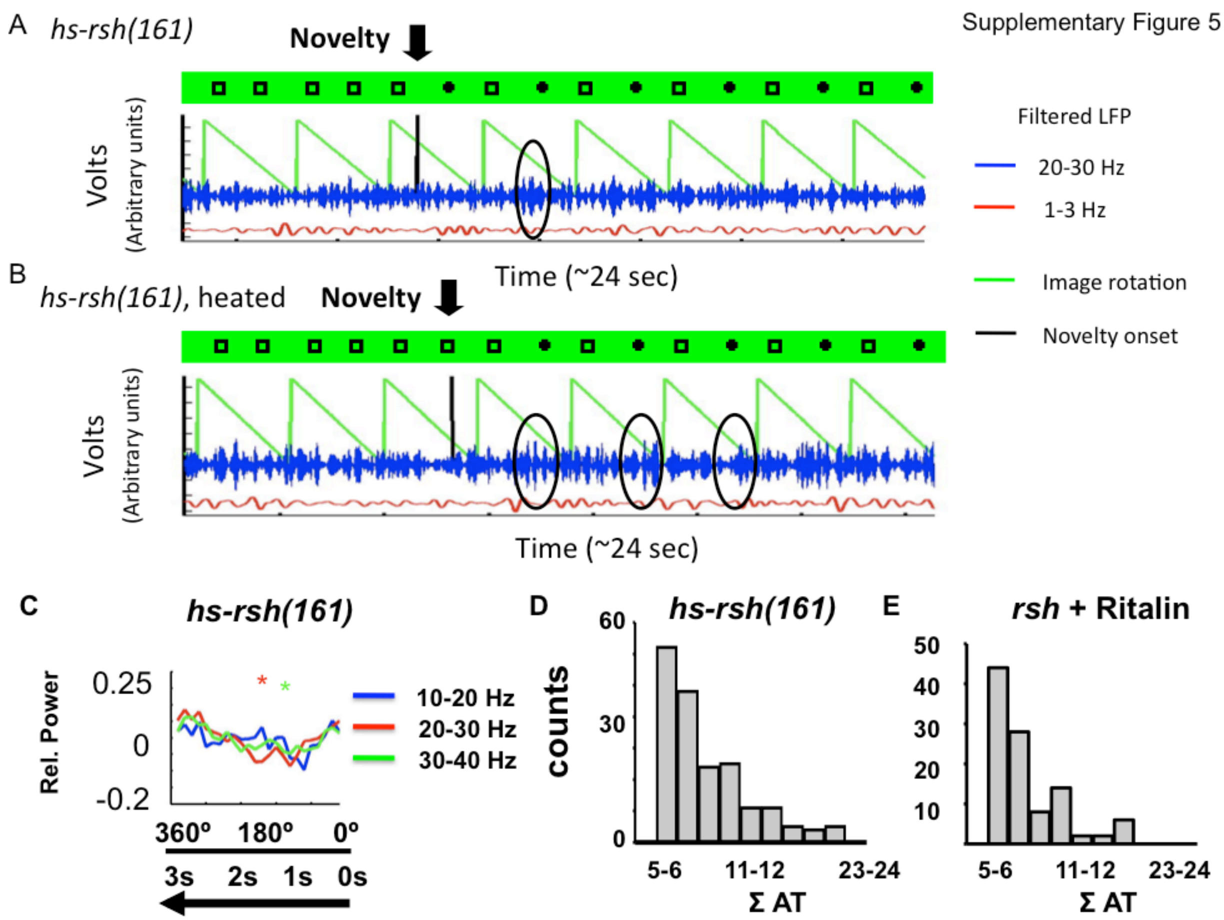
Supplemental Figure 3

Supplemental Figure 4. Brain activity versus thorax activity. **A** 6s raw trace of thoracic potentials (blue) and brain LFP activity (red) in a *radish* mutant. The fly transitions to flight in the middle of the trace. **B** Z-scored spectral analysis of brain and thorax activity in a *radish* mutant, for 1-10 Hz frequencies.

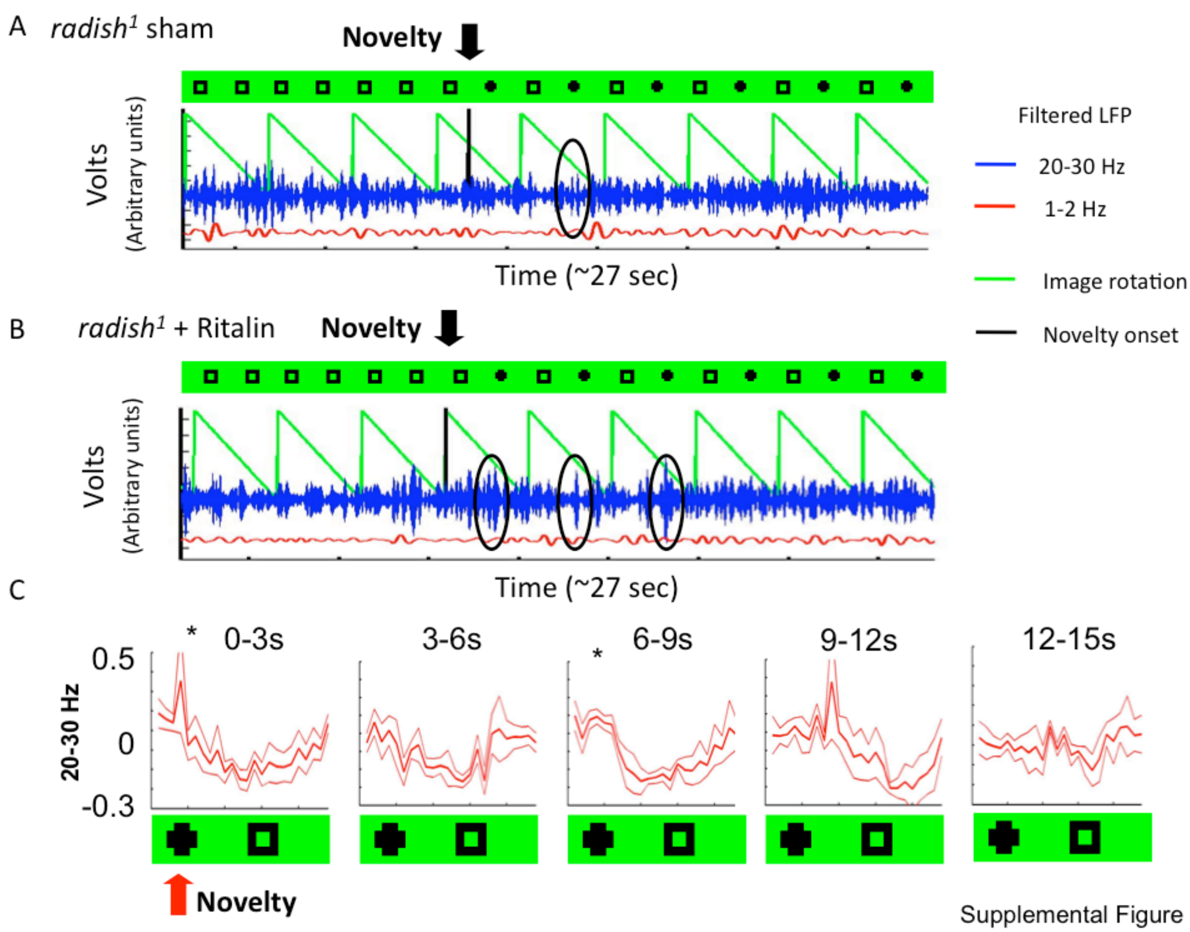


Supplemental Figure 4

Supplemental Figure 5. Brain responses to novelty in *hs-rsh(161)*. **A** Filtered LFP traces for a non heat-shocked *hs-rsh(161)* fly. **B** Filtered LFP traces for a (different) heat-shocked *hs-rsh(161)* fly. **C** Average novelty response for three frequency domains for 4 heat-shocked *hs-rsh(161)* flies. * = significantly different responses ($P < 0.05$, by *t*-test) between competing objects, color coded by frequency domain. **D** Distribution of summed alternation tempos (Σ AT, see Methods) for 4 heat-shocked *hs-rsh(161)* flies. **E** Distribution of summed alternation tempos (Σ AT, see Methods) for 5 *radish* mutants treated to 0.5 mg/ml Ritalin.



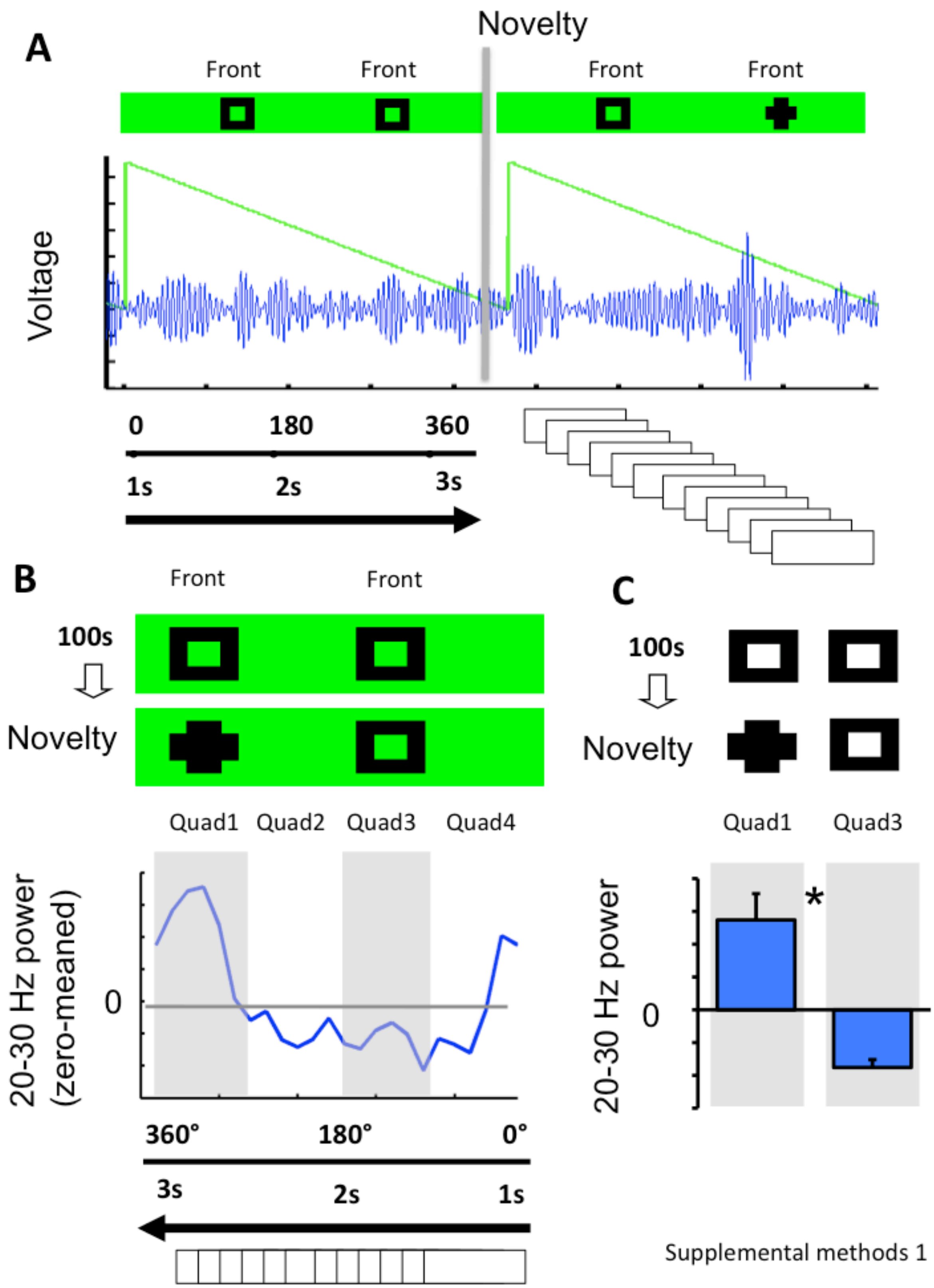
Supplemental Figure 6. Ritalin treatment of *radish* mutants. **A** Filtered LFP traces for a *radish* mutant. **B** Filtered LFP traces for the same fly after Ritalin feeding. **C.** Average 20-30 Hz novelty response partitioned into successive 3 s epochs for *radish* mutants fed on Ritalin (* = significantly different responses ($P < 0.05$, by t-test) between competing objects).



Supplemental Figure 6

Supplemental Methods 1: 20-30 Hz response calculations.

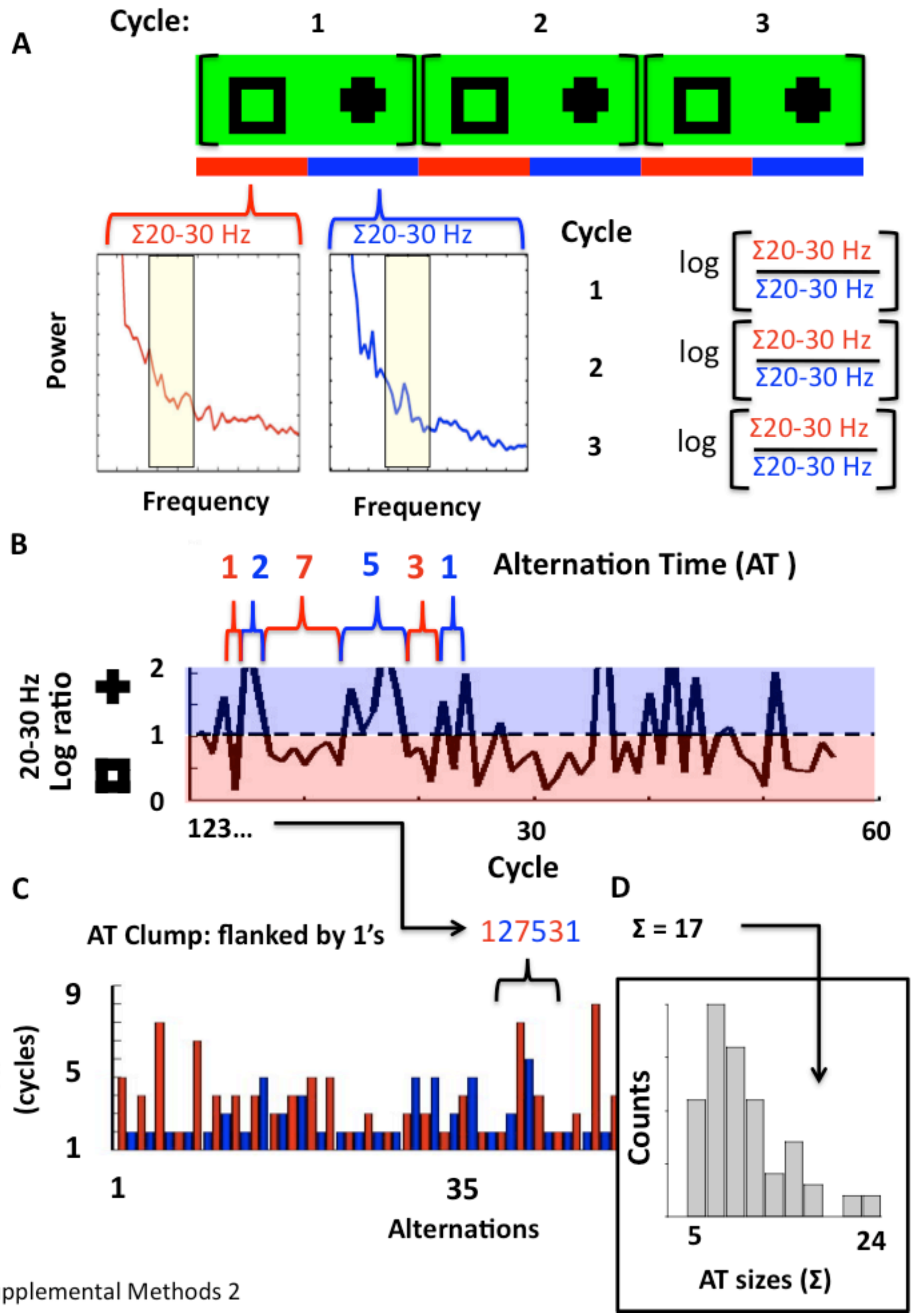
A Three signals are recorded during an experiment: the position of the rotating visual panorama (green line), brain local field potentials (a 20-30 Hz bandpass-filtered trace is shown, blue line), and a trigger indicating a change in scene (gray bar). Each object (a square or a cross), sweeps in front of the fly at a specific time of the panorama rotation sequence, once every 3 seconds. The objects are 180° apart. 20-30 Hz power is calculated (by Fast Fourier Transform, FFT, in Matlab) for 24 overlapping windows covering the entire panorama rotation sequence (12 overlapping rectangles are shown). **B** Average 20-30 Hz activity from the 24 FFT calculation are plotted onto the 3s rotation sequence, with image flow moving from left to right (inverted from A). For novelty experiments (100s training to squares followed by one of the squares changing to a cross), five such graphs from five separate novelty transitions are averaged to display a novelty response per fly. These data are then zero-meaned and normalized in order to produce average responses (± s.e.m.) among flies within a strain (as in Figure 6). **C** To determine whether a strain is producing a significant response, 20-30 Hz activity is contrasted for either object (coinciding with when that object sweeps in the quadrant in front of the fly). Thus, the average 20-30 Hz activity (from B) for the quadrant representing the novel object (Quad1) is contrasted (by *t*-test) with the average 20-30 Hz activity representing the competing object (Quad3). A significant difference ($P < 0.05$) indicates the 20-30 Hz selection / suppression effects characteristic of a response to visual salience effects such as novelty.



Supplemental methods 1

Supplemental Methods 2: Attention Span Calculations

A Summed 20-30 Hz power is calculated for the time (1.5s) each object is in front of the fly. Thus, there are two numbers calculated for every full rotation of the panorama, (consisting of two distinct objects, a square and a cross, sweeping across in succession). A log ratio of these numbers is calculated for every rotation cycle, yielding a ratio for every successive cycle (with a total of around 130 cycles for experiment per fly. **B** The ratios are plotted in succession for the experiment (black graph). When 20-30 Hz activity was greater in response to the cross, the graph is in the blue zone; when activity was greater in response to the square, the graph is in the red zone. From this data, an “Alternation Tempo” (AT) series is calculated (red or blue numbers, corresponding to the square and the cross, respectively). These are the durations (in cycles) that the ratio is biased to one or the other object (above or below a ratio of 1) before alternating. AT durations are calculated for the entire experiment (6 are shown in b). These are then plotted as histograms in **C** The AT sequence “127531”, is indicated as a clump of alternating red and blue histograms. A clump is defined as a group of contiguous AT values where each is larger than 1, but flanked on both sides by 1’s (as in the sample clump). Clump size (Σ) was calculated by summing the AT values within a clump. To quantify this behavior of the 20-30 Hz brain activity in response to competing images, we tallied clump sizes across experiments for multiple flies within a treatment or genotype. A histogram of clump sizes was then plotted, as shown in **D** Only sizes 5 or above were considered. This simple set of calculations is aimed at determining whether there are contiguous epochs of time (visualized as Alternation Tempo (AT) clumps) when the fly’s 20-30 Hz brain activity is strongly biased to one or the other object. To test whether this potential bias is significant, we shuffled (by permutations) the original series of 20-30 Hz ratios (the black plot in b.) and then recalculated AT durations for the shuffled data. Then, clump sizes were recalculated according to the same criteria (flanked by 1's, $\Sigma = 5$ or larger) and size distributions graphed as histograms. To test whether real data (or mutant data) were different from shuffled data, we performed a Kolmogorov-Smirnov test for distributions, with significance set at $P < 0.05$. Distributions that were significantly different from the shuffled set show that 20-30 Hz activity is successively biased (in time) for one or the other object. We suggest that this is a form of working memory relevant to selective attention.



Supplemental Methods 2