Figure 5 Supplement 3

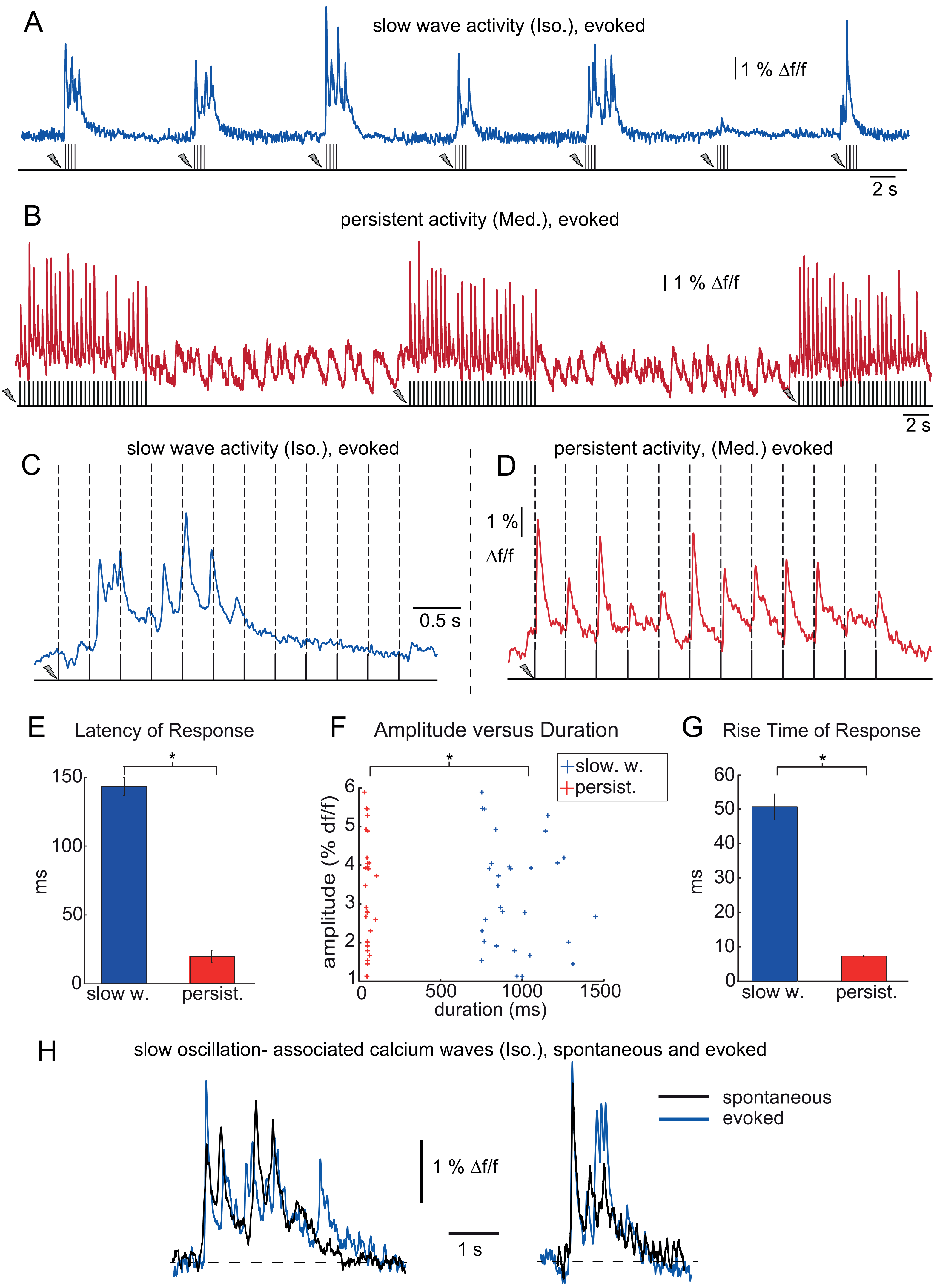


**Figure 5 Supplement 3. Neuronal response patterns upon sensory stimulation for slow wave and persistent activity.** (A) Calcium trace recorded during slow wave activity (blue). Electric forepaw stimulation at 9 Hz for 1 s every 10 s. Calcium waves were evoked in an all-or-none manner. The last pulse train started during the occurrence of a spontaneous wave. (B) Calcium trace recorded during persistent activity (red). Electric forepaw stimulation at 3 Hz for 10 s every 30 s resulted in short-latency calcium transients upon each pulse. (C) Individual sensory evoked slow calcium wave, note that wave is locked to the onset of the first stimulus with a latency of 140 ms (stimulation paradigm: 3 Hz, 4 s). (D) Sensory-evoked calcium responses during persistent activity (stimulation paradigm: 3 Hz, 4 s). Each pulse evoked an individual response. (E) Latencies of evoked calcium waves during slow wave activity (blue) vs. responses during persistent activity (red). Latencies differ significantly by more than 100 ms. (F) Scatter plot displaying mean response amplitudes versus durations measured at 50 % of maximum peak in the two conditions. Duration of responses (slow wave vs. persistent response) differs significantly by more than 900 ms. (G) Mean rise times of responses in the the two conditions differ significantly. Asterisks indicate significant difference (rank sum test, $p < 0.0001$, 30 events, 3 animals). (H) Slow oscillation-associated calcium waves evoked by sensory stimulation are indistinguishable from spontaneously occurring waves.

For slow wave activity, we observed electric forepaw stimulation to reliably evoke slow waves locked to stimulus onset (A) with a mean latency of 143 ms ± 7 ms (30 events, 3 animals, E), in reasonable agreement with studies in mice using visual or auditory stimulation (Grienberger et al., 2012; Stroh et al., 2013). Regarding signal characteristics, stimulus-evoked calcium waves were not distinguishable from spontaneous waves (H), again in agreement with previous studies (Stroh et al., 2013). Stimulating with multiple pulses with a frequency of 3 Hz and pulse train duration of 4 s led to calcium waves with latencies similar to those waves induced by single pulses (C). Subsequent pulses within a given pulse train did not evoke additional waves, suggesting refractory behavior of those waves, as previously shown in mice. Waves followed all-or-none characteristics (A), with a probability of induction of 72 % ± 8 % (5 animals, 12 traces). In the persistent activity condition, we detected neuronal calcium responses upon sensory stimuli with a significantly shorter latency of 20 ms ± 4 ms and a significantly shorter duration of 55 ms ± 3 ms, in agreement with previous studies (Schmid et al., 2016) versus 959 ms ± 36 ms in slow wave state (30 events, 3 animals, E, F). The amplitudes of persistent activity responses slow waves did not differ significantly (F), but varied between individual animals due to different staining efficiencies. It has to be noted that amplitudes of calcium transients can only be meaningfully compared within one experiment, as the fluorescence amplitudes depend on many local parameters, such as the concentration of the calcium indicator in the region and the position of the optic fiber. Responses during persistent activity exhibited significantly shorter rise times compared to slow waves (G), suggesting a different mechanism of activity initiation.