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Toward the development of a sensitive, pre-clinical screen for neurological diseases from spontaneous neural coordination in juvenile and young–adult C57BK6 mice

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ABSTRACT

Spontaneous Frequency Bursts (SFBs) are a newly discovered form of long-distance neural coordination. They have several distinctive properties, including near-simultaneity of occurrence (±25–50 ms) across distant brain regions and high within- and across-site coherence in multiple low and high frequency bands, presumably requiring high axonal, dendritic and vascular integrity. We examined whether SFBs occurred in young and young–adult C57BK6 mice with properties similar to those seen in rats. We found that across the entorhinal and piriform cortices, SFBs occur robustly in young and young–adult mice under light anesthesia, and that their rate of occurrence dropped sharply as anesthetic levels increased, as in rats. Moreover, murine SFBs showed high cross-site coherence in multiple frequency bands, including those that require exquisite action potential timing to be maintained across long distances. We discuss our findings in light of SFBs potential as a pre-clinical biomarker for diseases affecting action potential firing and local field potential coherence, especially in high frequency ranges (20–30 Hz and beyond).

Subthreshold neuronal oscillations, particularly in specific frequency bands such as the delta, theta and gamma ranges (1–4 Hz, 5–11 Hz, and 35–90 Hz, respectively), are increasingly being recognized as playing an important role in neural coding as well as in the maintenance of cross-brain coordination of neural activity [7,17,19,20,24,25,30,31,42,43,45]. Our laboratory recently discovered a novel form of cross-area neural coordination in rats, with distinctive frequency and coherence profiles, which we termed Spontaneous Frequency Bursts (SFBs; [16]). In spectrograms and continuous wavelet decompositions of local field potential recordings, SFBs were observed nearly simultaneously across the anterior piriform, posterior piriform, entorhinal and motor cortices, and occurred at the highest rate during light anesthesia and while awakening from normal sleep. SFBs' distinctive characteristics included a fixed duration of ∼250 ms, an appearance as broadband and nearly continuous high energy from 1 Hz to 40+ Hz, and coherence across recording sites that were particularly high in portions of the beta and high-gamma frequency ranges (20–30 Hz and 70–90 Hz).

Sustained action potential firing, membrane oscillations and coherence are particularly hard to maintain in high frequency bands [12,36,47]. Because of this, and because deficits in sustained action potential firing and other neurophysiological properties that may be critical to SFB generation are seen in several neurodegenerative disorders [5,6,22–24,27,33,40,41], SFBs may serve as an especially sensitive, early biomarker of one or more of these diseases in humans or animal models. As a first step toward examining this possibility, we tested for the existence and relative properties of SFBs in normal, young vs. young–adult C57BK6 mice (a commonly used background strain for disease mutants) during light as well as deep anesthesia. We examined their rate of occurrence in these two states in mice of each age group, as well as SFBs' average duration, peak frequencies up to 100 Hz, power spectral densities, and cross-site coherence profiles, while also taking steps to ensure that SFBs did not result artifactually from inappropriate LFP time series characteristics or equipment problems.

All procedures were approved in advance by the Animal Care and Use Committee at the University of Florida. We performed our experiments on two groups of male C57BK6 mice, 5 aged 5 months and 5 aged 2–2.5 months. We studied SFBs in these age ranges to examine age groups that are typically pre-histopathological and cognitively normal in strain-, age- and sex-matched disease animal models. Each mouse underwent acute, simultaneous implantation of 8 wire electrode bundles (stainless steel, teflon-coated, impedance ∼300 kΩ; NB Labs, Denison, TX) into the anterior piriform (aPIR) and entorhinal (ENT) cortices. Five-month

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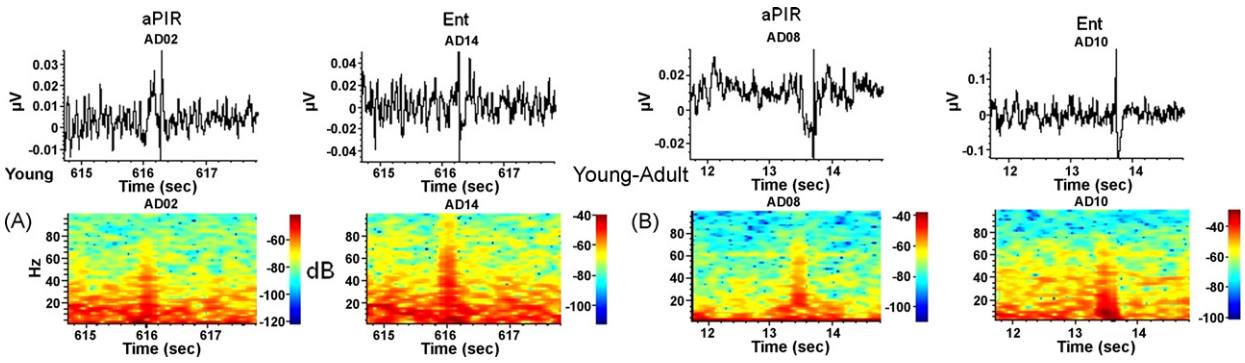

Fig. 1. Typical examples of LFP time series and their respective spectrograms across the anterior piriform (aPIR) cortex and entorhinal (ENT) cortex for (A) young mice and (B) young–adult mice. The analyzed data included only portions of each animal's time series containing SFBs. In the time series, note the absence of square waves, which if present could generate artifactual "SFBs." In the spectrograms, note the near-simultaneous occurrence of the SFBs across sites for each age group.

old mice received stereotaxically performed craniotomies (Kopf Instruments, Tujunga, CA) for multi-electrode implantations at the following locations relative to bregma: aPIR: 1.34 mm anterior, 2.5 mm lateral; ENT: 2.92 mm posterior, 3.5 mm lateral. Ventral coordinates were determined from a standard mouse brain atlas [32], post-mortem histological analyses, and the determination of electrophysiological hallmarks of correct implantation, yielding ventral coordinates of 3.65 mm and 4.10 mm for young–adult mice. In the young mice, we determined the coordinates for each recording site using a similar combination of techniques, yielding the following anterior, lateral, and approximate ventral, coordinates for young animals: 1.34 mm anterior, 2.5 mm lateral, and 3.50 mm ventral (aPIR); 2.92 mm anterior, 3.50 mm lateral and 4.0 mm ventral (ENT). Grounding craniotomies were made roughly 2 mm from electrode implantation sites, taking care to avoid ventricles. Anesthesia was induced with a 95% oxygen and 5% isoflurane mixture for 4 min and then stabilized to 0.50–1.5% isoflurane for the duration of the surgery. Surgeries typically lasted 3 h.

Animals were brought to a stable state of light anesthesia by slowly decreasing Isoflurane from ~1.0% to 0.50–0.75% as the electrodes were lowered at 100 μM/min to their ventral locations. After the correct electrode depth was reached, we ensured that the animals were in our state of light anesthesia, carefully defined as having the presence of all reflexes but the corneal; we defined deep anesthesia as the point at which hindpaw reflexes were barely absent with the breathing rate remaining >50% of that in the Light state. For each state, once determined to be stable, and after having determined via 1 min sample recordings that there was minimal line noise (<3 dB), we made a 10 min recording. Local field potential recordings were low-pass filtered at ~140 Hz and sampled at 1 kHz. Following adequate recordings, a low current was passed bipolarly through two wires at each implant site, the animals were deeply anesthetized and perfused with a ferri-/ferro-cyanide solution, and histology was performed to verify correct electrode placement.

SFBs were first visualized with spectrograms (NeuroExplorer 4.0, Dallas, TX) made from 2 Hz to 100 Hz, using a window size of 512 m and a sliding window distance of 25 m. They were compared to their corresponding LFP time series to screen out SFBs that may have resulted artifactually from square waves, having ruled out other sources of artifactual generation in our previous work [16]. For SFBs validated in this manner, SFB start time, end time, and peak frequency were coded in SPSS 15.0 (SPSS Inc., Chicago, IL) from 1 Hz to 100 Hz using a variety of window sizes and sliding distances. With NeuroExplorer we next computed and plotted, for each animal and recording site, the power spectral densities from 1 Hz to 100 Hz across all the start-to-end SFB intervals (256 frequency values) to ensure that energies in each potentially coherent frequency range also existed with adequate power. Finally, we performed coherence analyses using similar parameters.

With SPSS we first calculated the means and standard deviations for the rate of cross-site occurrence, peak frequencies and duration for young and young–adult animals in the Light and Deep anesthetic states. We also performed three two-way ANOVAs to test for main effects and interactions involving age (young vs. young–adult) and anesthetic state (Light vs. Deep), on SFB occurrence rates, peak frequencies and durations.

With NeuroExplorer we calculated SFBs' power spectral densities and coherence plots, only on LFP time series segments containing SFBs. Using a relatively common method for testing whether coherence in specific frequency ranges is significant (e.g. [35]), we evaluated whether coherence in the anticipated portions (given the rat results) of the theta, beta, low-gamma and high-gamma frequency bands during SFBs differed from coherence during 250 ms non-SFB portions of the young and young–adult animals' time series. For each animal in a given age group, we derived the peak coherence coefficients in these ranges for concatenated SFB vs. concatenated non-SFB time series segments (as with rats, only for Light recordings; the Deep recordings contained too few SFBs to yield good coherence calculations). For each age range, we tested the overall difference between SFB and non-SFB periods using a multivariate analysis of variance (MANOVA), with the four frequency ranges as dependent variables and SFBs vs. non-SFBs as the independent variable. As planned comparisons for each age group, we tested the results for each frequency range with one-tailed, paired *t*-tests.

We found that overall, SFBs in young and young–adult mice were similar, and that they shared qualitatively similar properties to those seen in young–adult rats. As shown in Fig. 1, both young–adult and young mice displayed SFBs that lasted ~250 ms (durations: $\text{mean}_{\text{young}} = 230.4 \pm 28.05$ ms, $N = 512$; $\text{mean}_{\text{old}} = 286.8 \pm 45.2$ ms, $N = 205$) and they exhibited similarly high and broadband energy from ~2 Hz to 50 Hz (peak frequencies: $\text{mean}_{\text{young}} = 48.20 \pm 24.11$ Hz, $N = 432$; $\text{mean}_{\text{old}} = 46.55 \pm 14.67$ Hz, $N = 199$). Similarly, young and young–adult mice expressed roughly equal numbers of SFBs/min ($\text{mean}_{\text{young}} = 3.650 \pm 5.426$, $N_{\text{minutes}} = 80$; $\text{mean}_{\text{old}} = 3.454 \pm 5.651$, $N_{\text{minutes}} = 55$).

We analyzed whether SFB occurrence rate, duration or peak frequency as a function of age group or anesthetic depth using two-way ANOVAs, testing for main effects and interactions. We found that the number of SFBs/min did not differ between young and young–adult animals ($F(1,131) = 0.0408$, $p = .8400$), but the rate did vary across the Light and Deep states, with more SFBs occurring during the Light state ($F(1,131) = 6.338$, $p = .0130$). Moreover, there was a marginal interaction among age group and anesthetic depth, with young animals having the highest rate of SFB occur-