SUPPLEMENTARY NOTE

1. Pharmacological profile of the Ca^{2+} waves

   The strong sensitivity to anesthetics precluded two-photon imaging-based analysis of the Ca^{2+} waves *in vivo*. Therefore, the mechanisms underlying the Ca^{2+} waves were analyzed in experiments performed in slices. We found that the Ca^{2+} waves recorded in slices were accompanied by 0.5- to 2.6-s-long bursts of 6-20 action potentials that were paralleled by changes in field potential (mean amplitude 34 ± 4 µV, n=28). The waves were blocked by TTX and by a mixture of the ionotropic glutamate receptor antagonists NBQX and APV. The frequency of Ca^{2+} waves was only slightly reduced by NBQX, but blocked significantly by APV and the GABA\textsubscript{A} receptor blocker bicuculline (the respective reduction in wave frequency: 81.1 ± 9.1% and 78.4 ± 4.55 %), suggesting a dominant role of NMDA and GABA\textsubscript{A} receptors for their generation in mice. As in the rat cortex\textsuperscript{1}, the Ca^{2+} waves propagated along the longitudinal axes of the cortical slice in both the posterior-to-anterior (58% of the waves) and anterior-to-posterior directions (42% of waves, n=6 mice). The average velocity of propagation was 7.0±1.5 mm/s in the posterior-to-anterior and 7.1±1.0 mm/s in the anterior-to-posterior direction. These values were higher than those found in rat cortical slices (~2 mm/s\textsuperscript{1}).

2. Ca^{2+} waves and spindle oscillations in the immature cortex

   The results revealed a marked similarity of the Ca^{2+} waves detected *in vivo* with those recorded in cortical slices. These Ca^{2+} waves seem to reflect an activity that is distinct from the electrically recorded sharp waves and/or spindle oscillations in anesthetized rats\textsuperscript{2-4}. More specifically, (i) the sharp waves/spindle oscillations are accompanied by spontaneous animal movements\textsuperscript{3,4}, whereas the Ca^{2+} waves are
not, (ii) sharp waves are present in urethane-anesthetized animals\(^2\), whereas the Ca\(^{2+}\) waves both in vivo and ENOs in vitro are blocked by urethane, (iii) the majority of cortical spindle-bursts represent sensory feedback signals, which result from spinal-cord generated muscle twitches\(^4\) and thus should be absent in in vitro preparations. Finally, the neonatal spindle-bursts are present during a wide range of behavioral states\(^4\), whereas cortical Ca\(^{2+}\) waves occur during sleep-like resting periods.

3. Evidence for Ca\(^{2+}\) waves in preterm human babies

Distinctive features of the Ca\(^{2+}\) waves in neonates include their low frequency (0.02 - 0.2 Hz), their marked sensitivity to anesthetics, their intrinsic cortical origin, and their occurrence during the developmental stage, in which GABA has a depolarizing action and produces Ca\(^{2+}\) signals in neurons\(^1,5\). Remarkably, recent direct current EEG recordings in quietly sleeping preterm human babies revealed a slow-wave activity with similar frequency and duration\(^6\). This activity was prominent in the temporo-parietal cortex and was restricted, as in rodents\(^1,7\), to a distinct period of early development, in which GABA has a depolarizing action.

References