Synchronized Spikes of Thalamocortical Axonal Terminals and Cortical Neurons Are Detectable Outside the Pig Brain With MEG

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INTRODUCTION

High-frequency oscillations (HFOs) have been observed in human somatic-evoked potentials (SEPs) (Yamada et al. 1988) and somatic-evoked magnetic fields (SEFs) (Curio et al. 1994; Hashimoto et al. 1996). They are now known to be generated in the primary somatosensory cortex (SI) with a peak in the amplitude spectrum around 600 Hz (Hashimoto 2000). This so-called “600-Hz” signal has attracted interests because some have suggested that it may reflect synchronized population spikes of the thalamocortical afferents or cortical neurons (Curio 2000; Gobbelé et al. 1998). Although this possibility is important for future applications of MEG and EEG, the origin of the signal is still unknown and thus it is unclear whether HFOs provide new information complementing conventionally recorded slow signals below 100 Hz.

We determined the origin of the HFO in the SI of an in vivo animal model (piglet), since the origin can be determined directly by comparing the SEF outside the brain with simultaneously measured intracortical SEPs at different depths before and after cortical injection of synaptic transmission blockers. The pig is gyrencephalic with a large, convoluted brain, so that our results should be relevant for interpreting human magneto- and electroencephalographic (MEG and EEG) signals.

METHODS

Animals were prepared as previously described (Okada et al. 1999a,b) using an approved protocol. The piglet (3–5 wk old, 7–11 kg) was anesthetized intravenously with ketamine (4 mg · kg⁻¹ · h⁻¹) and xylazine (1 mg · kg⁻¹ · h⁻¹), artificially ventilated, and secured in a headholder. Additional anesthetics were given as needed to maintain a surgical level of anesthesia. The scalp and the skull overlying SI were removed to expose the projection area of the snout. The snout, corresponding to the hand in humans, is projected contralaterally onto the large rostrum area of SI (Fig. 1A) (Craner and Ray 1991). After the surgery, the animal was placed in a magnetically shielded room. Anesthesia was reduced (ketamine, 1 mg · kg⁻¹ · h⁻¹; xylazine, 0.25 mg · kg⁻¹ · h⁻¹) to obtain a light anesthetic level as monitored by MEG. Tubocurarine chloride was given intravenously (0.3 mg · kg⁻¹ · h⁻¹) to prevent reflexive movements. The electrocardiogram was monitored continuously to ascertain sufficient anesthetic level. A sulcal area in SI (Fig. 1A, inset, ×) was activated by stimulating the snout with a bipolar electrode (5 mA, 50 μs, 1/s). The projection area was determined by localizing the generator of the initial cortical component of the SEF after an extensive SEF mapping at the beginning of each experiment. Measurements were obtained with a high-resolution, four-channel MEG system whose sensing coils were 2 mm above the exposed cortex. Once the field pattern was determined, the four coils were centered over the projection area (Fig. 1A). In study 1, the presence of the HFO in the SEF was determined by recording the conventional wideband SEF (1–3,000 Hz) simultaneously with a narrowband SEF (417–2,083 Hz filtered with an FIR digital filter on-line). In study 2, two components of the HFOs were distinguished with an antagonist of excitatory glutamatergic neurotransmission [kynurenic acid (Kyna) 20 mM dissolved in physiological saline] injected into the projection area of the snout in SI at a rate of 0.23 μl/min for 30–60 min. Effects of Kyna on the SEF and intracortical SEPs were compared by simultaneously measuring the signals with the MEG sensor above the cortex and a linear array of 16 electrodes (150 μm apart, 2 MΩ at 1 kHz) in the cortex. Because MEG is...
sensitive to signals from cortical sulci, the array was inserted horizontally into the sulcal wall of SI (Fig. 1A) for laminar potential recordings. Both SEFs and SEPs were filtered with same filters. In study 3, laminar origins of the two components were determined from depth profiles of the wide- and narrowband SEPs with and without Kyna. Recording locations in the cortex were determined from slices stained with Klüver-Barrera method.

Study 1 (18 piglets) confirmed the presence of HFOs with an amplitude peak around 600 Hz in the SEF and showed that they have properties similar to the “600-Hz” signal in humans. The wideband SEF (Fig. 1B) consisted of an initial cortical component corresponding to N20m of human SEF. The direction of magnetic field of the porcine N20m indicated that its underlying current was directed from deep toward shallow layers of the cortex as in humans. Digital filtering of this SEF (labeled “high-frequency SEF”) revealed HFOs starting at the onset of the porcine N20m. The HFOs at SQ2 and SQ4 were opposite in direction, indicating they were produced close to the generator of “N20m” as in humans. The difference of these two waves, “SEF diff wave,” is a first-order estimate of the cortical current (Cohen et al. 1980). The square of the difference wave, “SEF power,” clearly showed HFOs. The amplitude spectrum (Fig. 1C) demonstrated that the wideband SEF consisted of a dominant low-frequency signal below 200 Hz with a peak frequency of 70 Hz, a trough around 480 Hz, and a clear peak near 600 Hz (580 Hz here). This spectrum is very similar to that of human SEFs (Curio et al. 1994; Hashimoto et al. 1996). Although not shown, the HFOs were similar to human HFOs in other respects. For example, the signal became weaker as anesthetic level was increased and as depth of sleep monitored by spontaneous MEG increased (Hashimoto et al. 1996; Yamada et al. 1988).

Study 2 (12 piglets) tested a hypothesis that the HFO in the SEF is produced by thalamocortical axons presynaptic to the first cortical synapses and postsynaptic cortical neurons. Figure 2A shows simultaneously measured narrowband SEF and intracortical SEP from one animal before injecting Kyna. The SEP difference waveform between SQ2 and SQ4 was highly correlated to the intracortical SEPs, here shown for layer VI. During this baseline condition, the SEF power exhibited two components, one centered around 8 ms and another between 10 and 15 ms poststimulus. They were also seen in the group average shown on the right where the average SEF power is at the top and the superimposed SEF powers of all 12 animals at the bottom. The amplitudes of components 1 and 2 during time windows b and c were 0.012 ± 0.003 and 0.049 ± 0.013 (SE) pT², respectively, compared with the prestimulus amplitude of 0.0035 ± 0.0007 pT² during time window a. Their amplitudes compared with the prestimulus level were statistically significant at $P < 0.01$ (t [df = 11] = 2.74 and 3.37 for components 1 and 2, respectively). During Kyna injection (Fig. 2B), component 1 was hardly affected by Kyna, but component 2 was largely abolished. Component 2 partially recovered during washout (Fig. 2C). The superimposed traces of SEF and SEP powers (Fig. 2D) clearly show the selective effect of Kyna on component 2. These results suggest that components 1 and 2 were pre- and postsynaptic, respectively, to the first glutamatergic synapse in the cortex.

Study 3 (5 piglets) revealed the laminar origins of these two components. As shown in Fig. 3A, the laminar profile in the baseline condition contained HFOs in all cortical layers. During Kyna injection, the initial signals were found in layers IV–VI, but the later HFOs were largely abolished as in study 2. During washout (4 h after stopping Kyna injection), the HFOs were again found in all layers. The HFOs were first seen in layer IV and the bottom part of layer III and then after some
delay in the deeper and superficial layers. The wideband SEPs were also suppressed by Kyna but recovered during the washout.

The laminar origins of the initial signals are shown in Fig. 3B, which compares the laminar profiles before and during the administration of Kyna at four time points illustrated at the top. At 6.4 ms, the SEP was negative in the deep layers without a polarity reversal. At 7.2 ms, the potential was negative in the bottom of layer III and in layer IV, indicating the arrival of thalamocortical volley. A similar pattern was obtained at 7.8 ms which corresponds to the latency of the Kyna-insensitive component shown in Fig. 2. After a delay of about 1.1 ms, indicating a monosynaptic delay, the potentials in layers II/III increased abruptly, reaching a peak at 9.4 ms. These data indicate that the Kyna-insensitive component was produced in layer IV by specific thalamocortical axonal terminals and the Kyna-sensitive component was initially monosynaptically produced and later in several layers.

The preceding effects of Kyna may be questioned for two reasons. First, the suppression of component 2 may be due to the pH change during the injection of the Ringer plus Kyna. The pH of the Ringer with 20 mM Kyna was 6.5 rather than the physiological value of 7.4 for the intact cortex. Second, the suppression of component 2 in the presence of Kyna may be due to some nonspecific effect of the intracortical injection e.g., the volume effect of the Ringer solution used as the carrier, rather than to the specific effect of Kyna. We thus carried out a control study (3 animals) to test for these confounding factors. The procedure was same as study 3 except that the SEPs were measured during baseline, injection of Ringer with its pH adjusted to 6.5 with HCl to test for the volume and pH effects, Ringer washout, injection of Ringer plus Kyna with pH of 6.5 to check for the Kyna specific effect, and final washout. The results were the same as in study 3. Figure 3C, left, compares the intracortical SEPs obtained from one animal in three layers (rather than at all the recorded locations due to space limitation) before and after injecting the Ringer alone (conditions 1 and 2). There was virtually no difference. Figure 3C, right, shows the waveforms during the Ringer washout and injection of Ringer plus Kyna (conditions 3 and 4). Component 1 was intact, but component 2 was greatly suppressed in condition 4. We also carried out another control study (3 animals) in which the pH of the Ringer in condition 2 was 7.4 and obtained the same results. Thus the effects of Kyna observed in studies 2 and 3 were specifically due to the synaptic transmission blocking by Kyna.

Figure 3 indicates that the frequency content of the presynaptic component may be higher than for the postsynaptic component. Figure 3D confirms this visual impression. The Fourier spectrum of the intracortical SEP in layer IV (marked by * in Fig. 3C) had three peaks (567, 1,134, and 1,418 Hz here) in condition 3 as in other layers. Kyna injection in condition 4 greatly suppressed the 567 Hz component, peaking here at 709 Hz, but it had smaller effects on the 1,134 and 1,418 Hz components (here 1,063 and 1,489 Hz) with the 1,134-Hz component being affected least.
DISCUSSION

Our results indicate that MEG is capable of detecting the HFOs outside the brain that are produced by the presynaptic thalamocortical axonal terminals, monosynaptically activated postsynaptic cortical neurons and cortical neurons subsequently activated in the superficial and deeper layers of SI. Although the signals were measured above the exposed cortex, we can assume that similar signals may be detected above an intact scalp since MEG is known to be transparent to the scalp and skull (Okada et al. 1999a,b).

The presynaptic component most likely reflects a dominance in the high frequency range of the synchronize population spikes generated by the thalamocortical terminals. The relationship between the postsynaptic HFOs and the underlying spike activity is, however, still not well established. The postsynaptic HFOs may be due to inhibitory neurons (Hashimoto et al. 1996), but there is still no evidence demonstrating that inhibitory neurons are capable of producing MEG signals. Alternatively, they may be due to excitatory neurons whose activities are modulated by inhibitory neurons (Jones et al. 2000; Kandel and Buzsáki 1997).

It is now known that fast spiking neurons, believed to be inhibitory in the rat SI, are capable of firing at 400–600 Hz (Jones et al. 2000; Kandel and Buzsáki 1997).

The pre- and postsynaptic components were distinct in latency in the intact cortex without Kyna. They were also distinguished by frequency in the intracortical SEP. Klostermann et al. (1999) noted differences in the frequency content of the early and later components of the HFOs in the human SEP (700 and 494 Hz, respectively). Thus it may be possible to determine characteristics of the presynaptic activity, reflecting properties of the afferent pathway to the cortex, and the postsynaptic activity, reflecting cortical properties.

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REFERENCES


