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Interaction of slow cortical rhythm with somatosensory information processing in urethane-anesthetized rats

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Abstract

Slow cortical rhythm (SCR) is a rhythmic alteration of active (hypopolarized), and silent (hyperpolarized) epochs in cortical cells. SCR was found to influence sensory information processing in various models, but these studies yielded inconsistent results. We examined sensory processing in anesthetized rats during SCR by recording multiple unit activity (MUA) and evoked field potentials (eFPs). Evoked field potentials as well as spontaneous FPs changes around spontaneous activations were analyzed by subsequent current source density (CSD) analysis.

MUA responses and eFPs were recorded from the hindlimb area (HL) of the somatosensory cortex (SI) to electrical stimuli of the tibial nerve during active and silent states, respectively. Stimulus-associated MUA above the ongoing background activity did not differ significantly in active vs. silent states. Short latency (< 50 ms) eFP responses consisted of a sequence of deep-negative and deep-positive waves. Parameters of the first negative deflection were similar in both states. Stimulation in the silent state occasionally induced 500-700 ms long spindles in the alpha range (10-16 Hz). Spindles were never observed in responses to active state stimulation.

CSD analysis showed moderately different cortical sink-source patterns when the stimulus was applied during active vs. silent state. Sinks first appeared in layer IV, V and VI, corresponding sources were in layer I/II, V and VI. Stronger activation appeared in the infragranular layers in the case of active state. CSD of spontaneous FPs revealed some sequential activation pattern in the cortex when strongest and earlier sink appeared in layer III during active states.

Keywords

current source density analysis; evoked field potential; hindlimb area, rat; somatosensory cortex; slow cortical rhythm

1. Introduction

In both anesthesia and natural sleep, a low-frequency (< 1 Hz) rhythmic activity, slow cortical rhythm (SCR) is present in the cortical electroencephalogram (Steriade *et al.*, 1993a; Steriade

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et al, 1993b). A similar pattern has been reported in cortical slabs (Timofeev *et al*, 2000), and in neocortical slices (Sanchez-Vives & McCormick, 2000). Slow cortical rhythm can have different manifestations depending on the type and depth of anesthesia and on the depth of natural sleep, but in deep urethane anesthesia it is characterized by the alternation of almost isoelectrical EEG periods with short epochs of activity consisting of waves at different frequencies (Grahn & Heller, 1989; Dtri *et al*, 1997). Recording with transcortical electrodes revealed that the active epochs start with a sudden, deep-negative potential shift (Grahn & Heller, 1989; Dtri *et al*, 1997). This shift is caused by a strong depolarization in pyramidal as well as in non-pyramidal neurons (Metherate & Ashe, 1993). Hyperpolarization and virtually no synaptic activity is seen in pyramidal cells during the isoelectrical period (silent state), while strong synaptic activity and action potentials ride the depolarizing wave in the active state (Metherate & Ashe, 1993). Active (depolarized) states were first referred as UP states, while silent (hyperpolarized) epochs as DOWN states in the basal ganglia (Wilson & Kawaguchi, 1996). Strong correlation was found between SCR states and membrane potential fluctuations of striatal medium spiny neurons (Kasanetz *et al*, 2006), hippocampal interneurons (Hahn *et al*, 2006) and cortical neurons (Steriade *et al*, 1993a; Haider *et al*, 2006; Hasenstaub *et al*, 2007; Haider *et al*, 2007). SCR was declared to be of cortical origin as thalamic ablations did not abolish cortical active and silent states (Steriade *et al*, 1993b) and cortical ablations prevented active states from occurring in the striatum and thalamus (Nita *et al*, 2003; Steriade *et al*, 1993b). The SCR is synchronized over the cortical mantle (Volgushev *et al*, 2006), and also reaches subcortical targets, including the striatum (Wilson & Kawaguchi, 1996), basal forebrain (Szentgyorgyi *et al*, 2006), subthalamic nucleus-globus pallidus (Magill *et al*, 2000), hippocampus (Wolansky *et al*, 2006), thalamus (Steriade *et al*, 1994), and pedunculo pontine tegmental nucleus (PPT) (Balatoni & Detari, 2003).

Interaction of active and silent states with cortical information processing has been examined in several studies. N20 component of the somatosensory evoked potentials was found to be larger when stimuli were applied during the depolarized state compared to responses evoked during the hyperpolarized state in human experiments (Massimini *et al*, 2003). Somatosensory stimulation evoked responses with larger amplitude when stimuli were applied during the depolarized state in cats (Rosanova & Timofeev, 2005). UP and DOWN states were found to modulate sensory-evoked postsynaptic potentials and action potentials in the rat barrel cortex. In these experiments, stimuli were more effective in the DOWN state (Sachdev *et al*, 2004; Petersen *et al*, 2003). In a recent study, however, more complex interactions were found between SCR and evoked responses in the barrel cortex (Hasenstaub *et al*, 2007). UP and DOWN states alter processing of not only somatosensory, but visual stimuli as well in cats (Arieli *et al*, 1996; Azouz & Gray, 1999; Anderson *et al*, 2000; Haider *et al*, 2007). However, relatively few data are available from rats from cortical areas other than the barrel cortex. Therefore, we focused on the hindlimb area (HL) of the primary somatosensory cortex (SI), which is less investigated compared to the barrel/vibrissae system. The aim of the present experiments was to examine the influence of SCR states to information processing in urethane-anaesthetized rats by applying electrical stimuli to the tibial nerve and recording neuronal and field responses in the HL area of the SI. In addition to the evoked field potentials (FPs), we also analyzed spontaneous FP data recorded from this area during transitions between active and silent states.

2. Results

2.1. Unit activity

Spontaneous MUA in the HL area of the SI showed strong correlation with the SCR. High frequency spike activity was seen during active states, while cells virtually ceased firing during silent states (Fig. 1, top row). This difference is clearly seen in the 50 ms long baseline part of

PSTHs (Fig. 1, bottom row) created for stimuli applied during active and silent states, respectively. Number of spikes during the 50 ms prestimulus period was significantly ($p < 0.05$; Student's paired t-test) higher in the active state (1.987 ± 0.786 spikes) than in the silent state (0.573 ± 0.418 spikes). Somatosensory stimuli induced activation of unit activity in both states of the SCR (Fig. 1, bottom row). Unit responses lasted for about 12 ms in both active and silent states and showed two characteristic peaks at about 9 and 20 ms. The amplitude of the first peak was larger by 59 % during active states compared to silent states. Similarly, the second peak was also larger by 16 % in active states. However, due to the different baseline levels, only 1.22 ± 0.87 spikes were seen above background firing in active states while 1.58 ± 1.06 spikes were evoked in silent states. The difference was not significant (Student's paired t-test). After termination of the response, MUA returned to the baseline level in the case of active state stimuli, while a long, moderate activation was seen in silent states.

2.2. Evoked FPs

Tibial nerve stimuli elicited clear evoked potentials during both phases of SCR. Short latency responses (up to 50 ms) consisted of a sequence of negative-positive waves (active state: 1-4, silent state: 1-2; see a representative example on Fig. 2, left). The onset latency of the first deep-negative wave was virtually the same in active and silent states (9.83 ± 1.48 ms and 9.88 ± 1.46 ms, respectively). Similarly, no difference was seen in the peak latency (15.78 ± 4.04 ms and 15.78 ± 4.02 ms, respectively). Peak amplitude was slightly larger in silent state compared to active state in all individual animals ($n=6$) and in the grand average (315.03 ± 118.50 μ V and 278.58 ± 112.95 μ V, respectively), but the difference was not statistically significant (Student's paired t-test). In all but one case (5 out of 6 rats), a second depth-negative wave appeared in active state responses (latency: 25.82 ± 2.79 ms). In contrast, this second wave appeared only in half of the animals in response to silent state stimulation (3 out of 6 rats) with a latency of 25.80 ± 2.01 ms.

Longer latency components of the response showed a stronger dependency on the SCR. In silent states, short latency responses were occasionally followed by spindles in the alpha range (10-16 Hz). Spindles started at 100-120 ms after the stimulus and lasted for 500-700 ms. In all, but one animal, these patterns were eliminated by the averaging procedure (Fig. 3), thus their phase was not stimulus-locked. Spindles were never observed in responses to stimuli given in the active state.

2.3. Spontaneous FPs

Spontaneous FPs recorded from the HL area of the SI showed low frequency (<1 Hz) oscillation between large EEG shifts with superposed high-frequency activity (active states) and intermittent silent states (Figure 4, left). The shifts showed characteristic polarity as well as amplitude distribution. Positive shifts appeared in the supragranular layers and in the granular layer while negative shifts were recorded in the infragranular ones (Figure 4, left). To facilitate comparison of shift amplitudes, ratios of maximal values (measured between the dotted lines) to the value recorded on the cortical surface are also indicated (Figure 4, left). The largest shift was present in layer VI at a depth of 1500 μ m with an amplitude almost twice compared to the surface value. The smallest amplitude shift appeared in the middle of the cortex at about 1050 μ m with an amplitude about one fifth (0.206) compared to the reference.

2.4. CSD analysis

2.4.1. Spontaneous activity—Cortical CSD profiles showed a complex series of sink-source patterns during spontaneous SCR epochs. For the sake of clarity, sinks were marked by Roman numbers (active state: I.-III.; silent state: IV.-VI.) while sources by capital letters (active state: A-C; silent state: D-E). During active states, a characteristic sink appeared in layer III (sink I.; see Figure 4, middle column, between 400-600 ms). Sink I clearly showed two fused

parts which reflected the two large waves that appeared in the spontaneous FPs during active states in the upper layers (Figure 4, left). Compared to sink I, which was strong and had a long duration (> 200 ms), a smaller, shorter and slightly longer-latency sink was present in the deeper part of the layer V, centered at around 1200 μ m (sink II). A third sink also appeared (sink III) at the border of layer VI and the white matter. It had the longest latency among the three active state sinks. Corresponding sources appeared in layer I/II (source A) and in layer VI (source B and source C). Source B appeared in the upper part of the layer (at ≈ 1500 μ m). Source C was generated in the deeper part (at ≈ 1800 μ m) and it was shorter and weaker compared to source B. Among the sources that appeared during active states, source C showed the shortest latency. When the activate state ended and the silent one began (see Figure 4, middle, from about 700 ms), the previous sink-source configuration reversed. In the locations where sinks were found during active state, sources showed up in silent state and vice versa (for example, see the source A $\rightarrow$ sink IV or the sink I $\rightarrow$ source D transitions). This phenomenon resulted in a “chessboard-like” pattern. Sink III represented an exception to this rule, as this was not followed by a source during silent state.

2.4.2. Evoked responses—Similar to the spontaneous activity, multiple series of sink-source patterns were found after somatosensory stimulation in both states of the SCR (Figure 2, right). In this case, sinks were marked by Arabic numbers (1-3) while sources by letters (a-c). Data are expressed as mean $\pm$ SD ($n=4$ in most cases). Moderate differences were only seen in the sink-source patterns following stimulation in active vs. silent state of SCR.

Hindlimb stimulation applied during active as well as during silent states evoked a strong sink in layer IV (sink 1; Figure 2, right) with two peaks separated by 10 ms. The first peak was more pronounced in the silent state than the second one, while they were similar in strength in the active state. Onset latency of sink 1 was very similar in both cases (10.18 ± 1.48 ms in active and 10.33 ± 1.58 ms in silent state). This sink was also present in layer III in the active state, while it was transferred to layer III with a delay of about 15 ms in the silent state. Sink 1 persisted for 153.40 ± 67.01 ms in active state and for 168.75 ± 99.45 ms in silent state, and was associated with two strong sources. Source B in the infragranular layer (layer V-VI) clearly corresponded to the first part of sink 1 in layer IV showing two distinct peaks. It had a significantly longer duration in the active state than in the silent state (123.85 ± 5.62 ms and 24.35 ± 6.14 ms, respectively; $p < 0.05$; Student's paired t-test). Source A in the supragranular layer (layer I-II) was locked more to the part of sink 1 in layer III. It appeared simultaneously with source B in the active state, but was delayed in the silent state clearly following the spread of sink 1 to layer III. An additional source (source C) appeared in layer VI between the two peaks of source B with a similar latency in both states (17.73 ± 5.98 ms in active, 20.48 ± 5.23 ms in silent, respectively). It was more pronounced in the silent than in the active state. A short latency sink (sink 2) also appeared in layer VI. Its timing and intensity reflected the characteristics of sink 1 in layer IV. Two peaks could be distinguished in the active state in parallel with the peaks of sink 1, while only one, weak peak was seen in the silent state during the first stronger peak of sink 1 in this state (see Figure 2).

In the active state, another sink was observed in some rats that depended even more on the ongoing cortical activity. It appeared in layer V in 3 out of the 4 rats in case of active state stimuli, but it was present only in one rat after silent state stimulation. Sink 3 was delayed compared to sink 1 and sink 2 with an onset latency of 31.7 ± 9.85 ms ($n=3$) in the active state. In the single case when it was present after silent state stimuli, it had an onset latency of 23.3 ms (data not shown).

Sink-source configuration during spindles in the alpha range evoked by stimuli applied in the silent state was also examined. Spindles were associated with two series of sinks in layer II/

III and in layer V, respectively (Figure 5). Corresponding sources appeared in layer IV. All three series of sinks and sources were in close temporal association with each other.

2.5. Histology

Histology confirmed that recording sites were located in five cases in the lateral, in one case in the medial part of the HL area of the SI between -1.30 and -1.40 mm from Bregma (Fig. 6). In control recordings from adjacent cortical regions outside the coordinates of the HL area, no responses (MUA or eFPs) were observed (data not shown). Laminar analysis showed that in both active and silent states the largest amplitude responses were recorded from layer V (4/6 animals) or at the border of layer V and VI (2/6 animals) at an average depth of $916 \mu\text{m} \pm 285 \mu\text{m}$ from the cortical surface. The average thickness of the cortex at the HL area was $1874 \pm 184 \mu\text{m}$.

3. Discussion

We examined the influence of SCR on somatosensory information processing by first recording multiple unit activity then evoked and spontaneous FPs with subsequent CSD analysis. Our results show that tibial nerve stimulation in urethane-anaesthetized rats is able to evoke responses during both states of the SCR. Stimulus-associated MUA above the ongoing background activity in SI did not differ significantly in active vs. silent states (Fig. 1). Amplitude of the short-latency eFPs tended to be larger during silent states, while peak latencies were the same during both states (see Fig. 2, left). CSD analysis of the eFPs indicated stronger activation in the infragranular layers following stimuli applied during active cortical states compared to silent ones (Fig. 2, right).

In contrast to the original concept of Steriade (Steriade *et al*, 1993c;Timofeev *et al*, 1996), recent data have shown that sensory information can reach the cortex in both phases of the SCR. However, it is still not clear, how SCR influences (thalamo)cortical information processing. Experiments addressing the cortical effects of various sensory inputs during different (thalamo)cortical states yielded contradicting results in different species as well as in different studies using the same animal model. Neuronal responses evoked by whisker stimulation in the rat barrel cortex were larger during DOWN states compared to UP states (Petersen *et al*, 2003;Sachdev *et al*, 2004). However, in a recent study, these results were only verified for the presentation of short and simple stimuli. When a prolonged and variable (more natural) whisker stimulation pattern was used, increased responsiveness was found during UP states (Hasenstaub *et al*, 2007). Central (in this case, thalamic) stimulation was also more effective at evoking spikes in the DOWN state, than in the UP state in rats (Sachdev *et al*, 2004). We used single, short somatosensory stimuli and found similar responses after active as well as silent state stimulation. Responses tended to be larger to silent state stimulation but the difference between active vs. silent state responses was not significant. Thus, our results were in agreement with the results of previous studies in the barrel/vibrissae system, though we did not test the effect of the long and variable stimuli. In contrast, visual stimuli evoked more spikes and larger depolarization in the cat visual cortex, when presented in the depolarized state (Arieli *et al*, 1996;Azouz & Gray, 1999;Anderson *et al*, 2000;Haider *et al*, 2007). In ferret slices *in vitro*, more action potentials were evoked during the UP state than in the DOWN state in response to the stimulation of the white matter (Shu *et al*, 2003). The contradictory data from cats and rats raises the possibility of species differences as it was suggested recently (Haider *et al*, 2007).

Thus, evoked responses can be larger during silent or DOWN states compared to the active or UP ones in case of single stimuli. Several factors can contribute to this phenomenon. Some factors can play a facilitating role in this issue (i.e. can increase the amplitude of the response), but others can play a preventing role. When we study this question, we may see the overall

summary of all these pro and con factors. Membrane resistance differences during UP and DOWN states can be pro factors. In UP states, neurons have a higher background activity, but their membrane resistance is about one fifth of that measured during DOWN states, at least in anaesthetized cats (Destexhe & Pare, 1999) and rats (Cowan & Wilson, 1994). Because of the higher resistance, synaptic currents can induce larger membrane potential changes during DOWN states than in UP states leading to increased amplitude of eFPs. Spike threshold seems to be also higher during UP states (Hasenstaub *et al*, 2007). Activity changes of inhibitory neurons may also be important in influencing the amplitude of the eFP. Peripheral stimulation was found to activate both excitatory and inhibitory neurons in layers II-V of the cortex (Armstrong-James *et al*, 1992; Armstrong-James & Fox, 1987). Increased baseline activity levels of cortical neurons mean that inhibitory circuits are also more active during UP states than in DOWN states (Hasenstaub *et al*, 2005). Thus, external stimuli arriving during active states may induce more activation in inhibitory neurons compared to silent states. In other words, balance between excitation and inhibition in the cortex may be shifted toward excitation in response to a stimulus arriving during silent state leading to increased responsiveness during this cortical state. Thalamic mechanisms may also play an important role, as during DOWN states, a possible increase in bursting of thalamic relay neurons was suggested (Timofeev *et al*, 1996; Rosanova & Timofeev, 2005).

Our CSD data are generally in agreement with previous studies applying central or peripheral stimulation and describing CSD profiles in the SI (Castro-Alamancos & Connors, 1996; Kandel & Buzsaki, 1997; Jellema *et al*, 2004). However, there are two basic differences between our experiments and the other reports. Firstly, we analyzed separately the effects of stimuli given in different phases of slow cortical rhythms. Secondly, CSD analysis in previous studies was performed only for the first 30 ms (Castro-Alamancos & Connors, 1996; Jellema *et al*, 2004) or 50 ms of the post-stimulation period (Kandel & Buzsaki, 1997). We found sinks and sources bearing longer durations than 30 or 50 ms thus extended the analysis to longer periods after stimuli. Thus, our CSD data give information for the first time about short- and midlatency sink and source patterns generated by cortical circuits in the HL area of the SI in response to peripheral stimuli applied during different phases of the SCR in anesthetized rats.

Specific TC afferents terminate mainly in layer IV and lower part of the layer III in the SI (Jensen & Killackey, 1987; Herkenham, 1980). A less dense projection formed by collaterals of incoming thalamic fibers also terminate in layer VI (Zhang & Deschenes, 1998). The thalamic afferents establish synapses on dendrites of about all types of neurons at their depth of termination, but mainly on pyramidal and stellate (granule) cells. Stellate cells have spherical dendrite arborization, thus generate closed-field potential changes and contribute minimally to CSD. However, axons of these cells are important in the spread of activity within the cortex as their axons together with axon-collaterals of pyramidal neurons ascend and descend to form synapses on the apical dendrites of layer II, III and V pyramidal cells (Armstrong-James *et al*, 1992).

In agreement with the anatomical description of this circuitry, the earliest sinks (sink 1 and 2) in our experiments appeared in layers IV and VI reflecting the activity arriving through primary thalamic afferents (Figure 2). Shortly after its initialization, sink 1 shifted toward layer III, as it was already described in earlier studies (Castro-Alamancos & Connors, 1996; Kandel & Buzsaki, 1997). In these publications, the upward shift was suggested to be induced directly by thalamic afferents terminating on the basal dendrites of layer III pyramidal cells and indirectly by axon collaterals of granule cells converging on the same neurons. In our experiments, a sink in layer III together with a corresponding source in layer I was already present before the stimulus arrived in the active state (Figure 2). Currents induced by the stimulus were summed with these preexisting currents leading to the early appearance of the upper component of sink 1 in layer III together with its source in layer I. Neither the generation

of the active state, nor its spread to the whole cortical mantle and to subcortical structures is well understood. However, it seems from our data, that pyramidal cells in layer III have an important role in these processes. Our CSD data of the spontaneous activity support this hypothesis as a strong sink appeared in layer III during active states (sink I, see Figure 4, middle), which preceded the sink in layer V (sink II).

In an earlier study, the median nerve was stimulated electrically and EPs were recorded in the forelimb area of the SI in ketamine anaesthetized rats (Jellema *et al*, 2004). The authors found a short-latency sink-source configuration in layer Vb, and suggested that it reflected the population spike of layer Vb pyramidal cells. In addition, the authors described a superficial sink in layer I-II. These sinks were not observed in our experiments and were not detected by other authors (Castro-Alamancos & Connors, 1996; Kandel & Buzsaki, 1997). We found a characteristic sink in layer V (sink c), but it was much more delayed and appeared mostly after active state stimulation (see Figure 2). These differences in sink-source patterns might be attributable to different technical details (anesthesia, spatial resolution of the CSD analyses, recording sites in the SI).

Infragranular layers (layer V and VI) appeared to be more active during active state compared to the silent one (Figure 2) in case of somatosensory stimulation. Sink 2 was larger both in amplitude and duration as well as source b in case of active state stimuli. Sink 3 was absent in silent state with only one exception. These differences may reflect the different cortical information processing mechanisms. It can be hypothesized that activity of thalamic afferents terminating in layer VI may be lower in silent state compared to the active one. Absence of sink 3 in silent state (Figure 2) may show the presence of a cell population in layer V which is activated through local circuits when the stimulus arrives in active state, but not in the silent state. In general, active states may allow for all sort of polysynaptic mechanisms that could account for late events as seen in case of source b and sink 3 (Figure 2, active).

We also examined the cortical current generators of spontaneous FPs in the HL area of the SI with CSD analysis. As shown on Figure 4, polysynaptic activation during active states resulted in a strong sink in layer III (sink I.) and a weaker and longer-latency sink in layer V (sink II.). No sink was found in layer IV and only a weak sink appeared at the ventral border of layer VI (sink III.). This is in agreement with the idea that thalamic input is not involved in the generation of active states (Steriade *et al*, 1993b; Nita *et al*, 2003) as the above mentioned layers receive most of the thalamic input (Jensen & Killackey, 1987; Herkenham, 1980; Zhang & Deschenes, 1998). SCR was suggested to originate from layer V based on *in vitro* data (Sanchez-Vives & McCormick, 2000) and to be - at least, partly - a travelling wave (Sanchez-Vives & McCormick, 2000; Massimini *et al*, 2004; Luczak *et al*, 2007). Our CSD data may reveal some sequential activation in cortical circuits as activation in layer III (sink I.) appeared earlier compared to layer V (sink II.) during active states (Figure 4, middle).

Long-latency evoked potential components are more difficult to interpret than short-latency ones because of the possible recruitment of various neuronal circuits in time. Characteristic long-latency components of evoked responses in our experiments were spindles in the alpha range (10-16 Hz). Spindles were only observed when stimuli were given in silent state (Fig. 3). It was suggested previously (Steriade *et al*, 1993b) that sensory stimuli can only induce spindles in silent states due to the deinactivation of the low threshold Ca-spike mechanism in thalamic reticular and relay neurons (Destexhe *et al*, 1999). Any excitatory input reaching the cells in this state would result in fast Na-spike bursts riding the slow Ca-spike (Steriade *et al*, 1993c; Timofeev *et al*, 1996). These bursts in turn would induce a short spindle sequence by interacting with the reticular nucleus. In contrast, our experiments showed that short latency responses to peripheral stimuli were similar in active and silent states, though they were often followed by short spindle sequences in silent states. Our CSD data indicated that evoked

spindles were represented by a series of sinks in layers II/III and V, while corresponding sources were located in layer IV (Figure 5). The frequency of these spindle waves fell in the alpha (10-16Hz) range. With TC spindles, sinks in layer IV would be expected, induced by the incoming spike volleys of thalamic relay neurons. It was previously suggested that, in contrast to spindles, alpha waves are generated in the cortex itself (Steriade *et al*, 1990). As sinks during the evoked spindles in our experiments were found in layer V (and not in layer IV), it might be possible that these waves were generated by the cortex in response to the stimuli. In our experiments, spindles in the alpha range appeared in layer IV and mostly in layer V. However, spontaneous spindles were found to peak in the granular layer (layer IV), to appear with smaller amplitude in the supragranular layers, and missing in the infragranular layers in unanesthetized animals (Kandel & Buzsaki, 1997).

In summary, our results give some insight into the neuronal processes underlying sensory information processing during the different states of the SCR. While these states reflect strong differences at the neuronal level, surprisingly small differences were found between responses to stimuli given during the active and silent state, respectively. Future studies using naturally sleeping animals may provide a better understanding of the generation and spread of slow cortical rhythm and might help to elucidate the functional significance of this characteristic rhythm in the sensory information processing.

4. Experimental procedure

4.1. Surgical and recording procedures

Male Sprague-Dawley rats (n= 6), weighing between 320 and 410 g were anesthetized with urethane (1.2 g/kg, i.p.) and fixed in a stereotaxic frame (David Kopf) with the top of the skull set horizontal to comply with the atlas of Paxinos and Watson (Paxinos G. & Watson, 1998). A 4 × 3 mm craniotomy centered 1.5 mm posterior to Bregma and 1.5 mm lateral to the midline was made to expose the HL representation area in the right hemisphere. The dura mater was left intact during the whole experiment. Rectal temperature was maintained at 37 °C with a heating pad attached to a thermostatic instrument. Supplementary doses of urethane were given if needed. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and with the guidelines of the local Animal Care and Use Committee.

4.2. Electrophysiological recordings

MUA was recorded from the SI with extracellular metal microelectrodes (tip resistance 2 MOhm; A-M-Systems, Sequim, WA, USA). A stainless steel screw placed above the cerebellum served as reference. The microelectrode was slowly moved by a hydraulic microdrive (Narishige, Tokyo, Japan) to a depth of 1-1.2 mm below the dura. The signal was filtered, amplified (300 Hz-5 kHz, gain: 10000x; A-M Systems) and displayed by an oscilloscope. EEG was simultaneously monitored from the contralateral HL area of the SI (A: Br -1.3 mm, L 2.2 mm) with a bipolar transcortical electrode and conditioned with the same amplifier (filter: 0.1 Hz-500 Hz, gain: 1000x).

The exposed part of the SI was systematically explored to locate the area where neurons responded to light mechanical stimulation of the left hindpaw. When responsive units were found, two stimulating needle electrodes were inserted into the HL close to the ankle to enable electrical stimulation of the tibial nerve, and unit responses were recorded to electrical stimulation of the nerve. The threshold stimulus voltage was defined as the lowest intensity that elicited minimal movement in the hindpaw ($6.92 \text{ V} \pm 3.67 \text{ V}$). During the whole experiment, stimulus strength twice the threshold was used. Duration of the stimuli was 90 μ s in all cases.

Transitions to hypopolarized, active states were clearly indicated by an increase in MUA, and by a sharp, deep-negative shift with superposed high-frequency activity in the EEG recorded from the contralateral hemisphere. These changes appear almost simultaneously, though there may be a minimal delay on FP level compared to the level of units (Kasanetz *et al*, 2006). Selective stimulation during active and silent states was achieved by feeding the EEG signal into a comparator. When deep negativity reached a pre-set level during the transition to the active state, the comparator triggered a stimulator (Master8, AMPI, Jerusalem, Israel). Selection of a short stimulus delay (50 ms) ensured that the stimulus arrived during the fully developed active state. A delay of 600 ms from the onset of the deep negative EEG shift (see Figure 1, right) resulted in stimuli that arrived in the silent state, when cortical neurons had recovered from the previous depolarization caused by the active state. A total of 50-50 stimuli were applied during active and silent cortical states, respectively. Unit responses were recorded in 2000 ms long blocks (1000 ms pre-stimulus, 1000 ms post-stimulus periods).

When this part of the recording session was completed, the metal microelectrode was pulled back from the brain. A small drop of Fast Green dye (Sigma) was placed on the tip of the microelectrode and the electrode was reinserted into the superficial layers of the cortex to mark the exact location of the HL area. Then the microelectrode was replaced by a 16-channel vertical electrode array (Elektroencefalograf Ltd., Budapest, Hungary) to record spontaneous and evoked FPs from different cortical layers at the same recording site. Recording contacts of the array were made of 40- μ m platinum-iridium wires, with an interelectrode distance of 150 μ m. Impedance of the recording sites were taken as 1 M Ω at 100 Hz (Ulbert *et al*, 2001). At the tip of the array, an additional 50- μ m stainless steel wire was built into the array to enable marking of recording sites by depositing iron ions for a subsequent Prussian blue reaction.

Following insertion of the electrode array, tibial nerve stimuli were applied during active and silent states (50-50 stimuli, respectively) and eFPs were recorded (length 2000 ms; 1000 ms pre-stimulus - 1000 ms post-stimulus). EEG signals were fed to a 16-channel differential amplifier (Supertech Ltd., Pecs, Hungary), conditioned (filter: 0.1 Hz-1000 Hz, gain: 5000x) then digitalized at 3 kHz with 16 bit resolution (Labview; National Instruments, Austin, TX, USA). All data were stored on disk for off-line analysis.

4.3. Data analysis

Analysis was carried out off-line using custom designed software. The program enabled visual inspection of the recorded signals, digital filtering, spectral analysis of the EEG signal, averaging of single evoked responses and calculation of firing rates from selected epochs of neuronal activity.

To analyze MUA, spikes were separated from noise by setting a threshold level. Artifacts were eliminated by spike shape information (Detari *et al*, 1997). Peri-stimulus time histograms (PSTHs) were calculated to examine the changes of neuronal activity before and after stimulation (bin width: 1 ms).

Evoked FPs were recorded separately in active and silent states, and averaged using a custom-written Matlab (MathWorks, Inc., Natick, MA, USA) script. In the first step, stimulus timing was visually examined and erroneously timed stimuli (e.g. when stimulus arrived in active state instead of silent state and vice versa) were excluded from averaging. EFPs recorded during active states were riding on the deep negative EEG shifts characterizing active states. To facilitate analysis of the evoked potentials, negative shifts recorded without stimulation were averaged and subtracted from the averaged curves recorded during stimulation. From the 16 signals recorded by the electrode array, the channel on which the first negative wave appeared with the shortest latency and largest amplitude was selected for eFP analysis. Amplitude and latency differences seen in active vs. silent state responses as well as parameters of individual

sinks and sources were compared statistically by paired, two-tailed Student's t-tests. Statistical tests were performed using InStat (GraphPad, San Diego, CA, USA). Statistical significance was accepted at the $p < 0.05$ level.

One-dimensional current-source density (CSD) analysis was performed using the averaged spontaneous and evoked FP data of the 16-pole recording array to accurately locate the synaptic currents inducing the local extracellular potential changes. CSD plots were calculated by approximating the second spatial derivative of voltage using a custom-written Matlab script. CSD calculation used the following formula (Mitzdorf, 1985):

$$CSD(h,t) = \frac{\Phi(h - n\Delta h, t) - 2\Phi(h, t) + \Phi(h + n\Delta h, t)}{\Delta h^2}$$

where CSD(h,t) is the CSD at a fixed time t and depth h, $\Phi(h,t)$ is the averaged eFP at time t and depth h. In our experiments, Δh was 150 μm , while three points Hamming spatial filter was applied ($n=3$). To eliminate background noise, threshold levels were defined using maximum values in a given data set. In most cases, 20 % of the maximum value was used.

4.4 Histology

At the end of the experiment, a 50 μA positive direct current was passed through the stainless steel electrode at the tip of the electrode array for 60 s. The animals were perfused transcardially with 150 ml of 0.9% saline followed by 400 ml of fixative containing 4% of paraformaldehyde and 2 % of potassium ferrocyanide to produce a Prussian blue reaction with the iron ions deposited from the electrode. Brains were removed and postfixed overnight at 4 °C in the same fixative. Coronal sections (50 μm) were cut with a vibroslicer, mounted and stained in gallocyanine solution overnight. After dehydration, slices were coverslipped with Canada balsam. Bright-field light-microscopy was used to locate recording sites, which were marked on the appropriate plates of the stereotaxic atlas of Paxinos and Watson (Paxinos G. & Watson, 1998).

The thickness of the cortex at the recording site and the distance of the iron deposit from the cortical surface were measured on photographs taken by an Olympus BFX51 microscope equipped with an Fview-2 CCD camera. Photographs were analyzed using an image-analysis program (Analysis; Olympus, Japan). Distances were measured in parallel with the apical dendrites of pyramidal cells. Borders of cortical layers were determined by inspecting the microphotographs and comparing them with data available in the literature (Skoglund *et al*, 1996). In this way, position of recording points of the electrode array in different cortical layers was determined. Sub-layers Va and Vb were not distinguished within layer V.

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Abbreviations

CSD, current source density; EEG, electroencephalogram; eFP, evoked field potential; FP, field potential; HL, hindlimb; MUA, multiple unit activity; SCR, slow cortical rhythm; SI, primary somatosensory cortex; TC, thalamocortical.

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Figure 1. Multi-unit responses to tibial nerve stimulation during different phases of the SCR in the hindlimb area of somatosensory cortex. Top: original multiple unit and EEG recordings from a representative rat. Bottom: grand average peri-stimulus time histograms (PSTHs) of multiple unit responses during active and silent states, respectively, from five individual rats. Bin width for PSTHs was 1 ms.

Figure 2. Averaged eFPs (n=50; left) from the HL area of the SI and their corresponding CSD profiles (right) in case of active and silent state stimulation, respectively. Data presented here are derived from a representative rat. In the middle, cortical layers are marked with numbers while lines indicate the borders of the layers. Stimulus arrived at 0 ms. Sinks are marked by numbers (1-3) while sources marked by letters (a-c).

Figure 3.

Occurrence of spindles in response to stimulation during the silent state. Spindles were never observed in responses to stimuli given in active state. Panel A, left: representative single responses recorded in active and silent states, respectively. Panel A, right: power spectra calculated for epochs marked by vertical broken lines. Panel B shows responses and power spectra from another rat. In this rat, curves were high pass filtered at 3 Hz. In case of silent state responses, peaks belonging to the alpha range (14.65 Hz and 11.72 Hz, respectively) are seen in the power spectra in both rats. These peaks are missing in the power spectra of the active state responses.

Figure 4.

Spontaneous FP activity in the HL area of the SI. Data presented here are derived from a representative rat. Left: averaged spontaneous FPs (n=50 epochs). Middle: CSD profile of the spontaneous activity. Cortical layers are marked with numbers while lines indicate the borders of the layers. Sinks are marked by Roman numbers (I.-VI.) while sources marked by capital letters (A-E). On the left and middle panels, vertical dotted lines indicate the approximate borders of an active state between 350 and 700 ms. Between 700 and 1000 ms, a part of a silent state is seen. Right: relative amplitudes of the EEG shifts seen during active states. The graph also shows the polarity of the shifts. Positive shifts appeared in the supragranular layers and in the granular layer while negative shifts were present in the infragranular ones. For comparison, shift amplitude on the cortical surface was given as a reference (with a value of 1).

Figure 5.

CSD profile of spindles evoked by stimuli applied in silent state of the SCR in one rat. Spindles are represented by two series of current sinks in layer II/III and in layer V with an onset latency about 100 ms. Corresponding sources are located in layer IV. All three series of sinks and sources were closely associated with each other. Left: averaged eFPs ($n=50$). Right: CSD profile. In the middle, cortical layers are marked with numbers while lines indicate the borders of the layers. Stimulus arrived at 0 ms. Averaged eFPs were notch filtered between 48 and 52 Hz.

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