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Cortical evoked responses to magnetic stimulation of macaque's abdominal wall in sleep-wake cycle

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EEG and eye movements (magnetic search coil method) were recorded in sleep and wakefulness in a monkey (*Macaca fascicularis*) while the animal was sitting in a primate chair. Single pulse magnetic stimulation was applied to the monkey's abdominal wall using a circular coil and a Magstim 200 stimulator. Magnetic stimuli did not wake the sleeping animal, and being applied during slow wave sleep evoked clear responses in EEG with a latency of 80–100 ms. These responses disappeared during wakefulness and rapid eye movement sleep. Control experiments confirmed that these responses were not caused by the acoustic clicks produced by the magnetic coil. Results of this study further confirm that during sleep, signals from visceral organs reach the cortical areas which in wakefulness process exteroceptive sensory information. This observation indicates that magnetic stimulation may be a useful tool for researching neural connectivity reorganization within the sleep-wake cycle.

Key words: Cortical evoked responses, magnetic stimulation, visceral stimulation, monkey, sleep

It was shown in cats that evoked responses to electrical stimulation of intestinal mucosa or directly of splanchnic nerve in cortical and subcortical projections of this nerve, increased during sleep (Kukorelli and Juhasz 1983). Later it was found that electrical stimulation applied during slow wave sleep (SW sleep) to the area of the stomach and small intestine in cats (Pigarev, 1994) evoked responses of neurons in visual cortical areas which in wakefulness process signals from the retina. These visceral responses disappeared in wakefulness. Recently it was shown that also in monkeys during slow wave sleep intraperitoneal electrical stimulation evoked responses in the occipital cortical area in monkeys (Pigarev et al. 2006a). This suggested that the same cortical areas which process signals from exteroreceptors in sleep can be involved in analysis of incoming signals from interoreceptors – a conclusion that opens a new and stimulating view of the possible function of sleep and neuronal control of visceral functions. However, the research of viscero-cortical interaction in sleep-wake cycle has several inherent technical problems. Firstly, in order to have naturally sleeping animals, experiments should be performed under chronic conditions. Similarly, most studies of visceral systems were performed in acute preparations, and known methods for controlled stimulation of visceral receptors in chronic experiments, as for example in the work (Kukorelli and Juhasz 1983) were connected with intraperitoneal operations. Direct intraperitoneal stimulation is not acceptable for studies of humans.

During the last years magnetic stimulation began to be widely used in physiological and medical practice (for review see Rothwell et al. 1991, Rossini and Rossi 1998). Being very similar in nature to electric stimulation (most likely that magnetic pulse induces the current which excites the tissue) magnetic stimulation perceptually is “softer”. By our own observations magnetic pulses applied by the coil located on the skin overlying the abdomen do not produce a painful sensation, and are felt as short and mild mechanical knocks.

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Another advantage of magnetic stimulation was that it can excite tissue located up to 3–4 cm from the surface of the stimulating coil (Marg and Rudiak 1994). This allows the use of magnetic stimulation even for transcranial activation of certain brain areas. Thus it can be expected that a magnetic pulse applied at the surface of the abdomen would reach visceral receptors or excite neurons of visceral plexuses and ganglia located within a 3–4 cm depth. A magnetic pulse might also evoke contraction of intestinal or stomach muscles, thus producing "secondary" excitation of visceral receptors synchronized by the pulse.

The aim of this study was to assess magnetic stimulation as a potential tool for the research of the reorganization of neuronal connectivity in the sleep-wake cycle. We would like to find answers to the following questions: (1) whether magnetic stimulation of the abdomen in monkeys can be applied during sleep without waking the animal; (2) whether magnetic stimulation of the abdomen will evoke cortical responses in non-somatosensory cortical areas during sleep, and whether these responses could be understood to be responses to visceral stimulation; (3) whether cortical evoked responses to magnetic stimulation depends on the phase of the sleep-wake cycle.

The monkey (*Macaca fascicularis*) was trained to sit and later to sleep in a primate chair. Recording of eye movements and EEG were performed in chronic experiments where the head of the animal was painlessly restrained by a frame surrounding the head (Pigarev et al. 1997). The magnetic search coil for eye movement recordings, and the frame for head fastening were implanted under pentobarbital anaesthesia (Nembutal®, Sanofi, 7 mg/kg/h i.v.) under aseptic conditions. For five days after surgery the animal received antibiotic treatment (clindamycin, Sobelin® Solubile 300, Upjohn, 30 mg/kg three times a day).

Surgery and daily treatment of the animal were performed in accordance with the Ethical Principles for the maintenance and use of animals in neuroscience research (Ethical Principles for Maintenance and Use of Animals in Neuroscience Research, 1987), and NIH guidelines for the care and use of animals.

EEG was recorded by means of an electrode that was implanted, during the same operation, into the skull above the dura at the occipital pole (above visual area V1). Reference electrodes were distributed over several points on the frontal part of the skull. Recordings were made using a band pass filter of 0.5–40 Hz. Positivity of the "occipital" electrode is shown in the figures as upward inclination.

For magnetic stimulation a circular coil (11 cm diameter) was fixed in the primate chair so that it touched the abdominal surface of the monkey's body when the animal was sitting in a normal relaxed pose. The upper edge of the coil was located at the lower part of the chest. The relative positions of the body and the coil were monitored with an infrared video camera. Single magnetic pulses with maximal intensity of 1 T were generated by a Magstim 200 stimulator (The Magstim Company, Whitland, Dyfed, UK). Pulses were applied with intervals of at least one minute.

Time of magnetic stimulation, vertical and horizontal components of eye movements and EEG were recorded on tape and were analyzed off-line. Applied magnetic stimuli were used as triggers for EEG averaging.

Magnetic stimuli, applied both in wakefulness and in sleep, evoked stable inclinations of EEG with constant amplitude, and extremely short latency (5–6 ms) corresponding to the expected delay of the used filter. When we changed the polarity of magnetic pulse the polarity of this short latency component also changed. Thus, we considered it to be the result of magnetic stimulation. It was found that in wakefulness no other responses were seen in synchrony to the magnetic pulse. All responses to magnetic stimuli in wakefulness were averaged (solid line in Fig. 1A), and smoothed (thin dashed line in Fig. 1A). The curve obtained this way (Fig. 1B) was regarded as the pure stimulation result. In further analysis, the amplitude of the result (Am in Fig. 1B) was standardised to the amplitude of the actual result in each averaged record (i.e. the Result in Fig. 1C) and these were subtracted from the signals that were actually recorded. As a result "clean" responses to magnetic stimulation (Fig. 1D) were obtained. In the article all responses are presented after the results were subtracted in this way.

Magnetic pulses, like any new sensory stimuli, when applied to the awake animal, first evoked an orienting behaviour response. The monkey usually shifted his gaze downward towards his body, and examined the area of stimulation by hand. After a few (5–10) pulses this orienting behavior became less expressive: the monkey became accustomed to the stimuli and finally paid no attention to these. In spite of continuing stimulation he even fell asleep. Often, after the first magnetic pulses were applied, the monkey became drowsy and fell asleep even faster than this occurred without stimulation. This sleep-promoting effect of abdominal magnetic

stimulation was related to the hypnogenic effect of the electric stimulation of the small intestine reported earlier in cats (Kukorelli and Juhasz 1976, 1977).

As already mentioned, in EEG recorded in wakefulness results were only found as responses to magnetic stimulation (Fig. 1A). In Fig. 2 (A–E, left column) five examples of "cleaned" averaged EEG responses recorded in wakefulness during 1.5 s after magnetic stimuli are shown. Each record presents results of one experiment. The number of averaged trials is shown under each record.

With development of sleep only small saccades in a downward direction could still be recorded in response to magnetic pulses. These saccades which often happened even under closed eyes, interrupted slow eye drifts typical of light sleep. At this stage the monkey could open his eyes after the stimulation. Later on, the eyes stayed closed, there were no more saccadic eye movements in response to stimulation, and only slow downward eye deviations could be recorded after the magnetic pulse. Finally the eyes reached an upward position. This position of the eyes is typical for deep SW sleep if the monkey is sleeping in a sitting pose. At this stage of sleep magnetic stimuli did not evoke any downward eye movements. However, sometimes stimulation provoked further upward drifts. In our monkey, transition from wakefulness to deep sleep was accompanied by upward shifts of eye position. That is why these upward eye inclinations in response to stimulation were regarded as indication that sleep after stimulation had become even deeper.

Eye movements and EEG were always recorded before, during and after stimulation. For analysis of the effects of magnetic stimulation in sleep, only those trials were selected where no downward eye movements indicated arousal and no visible changes in EEG patterns before and soon after stimulation could be observed.

When magnetic stimuli were applied in SW sleep, clear evoked responses could be seen in the EEG even

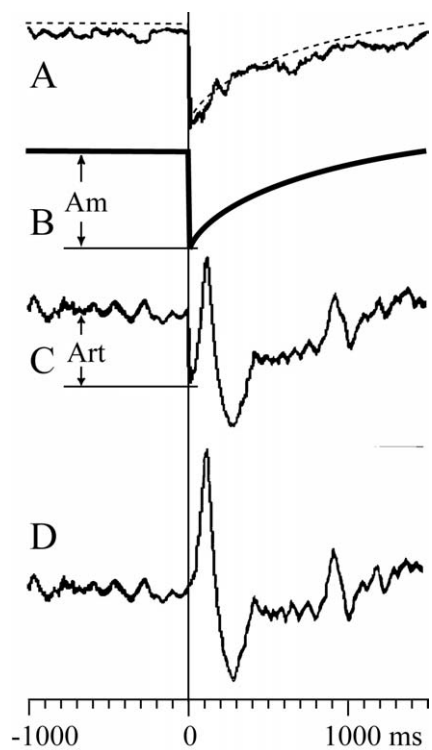

Fig. 1. (A) EEG-signal recorded after magnetic stimulation in wakefulness (solid line), and assumed shape of the artefact of magnetic stimulation (dashed line); (B) Result of magnetic stimulation; (Am) amplitude of the result; (C) EEG-signal recorded after magnetic stimulation in sleep; (Art) amplitude of the result; (D) the shape of the evoked response after subtraction of the result

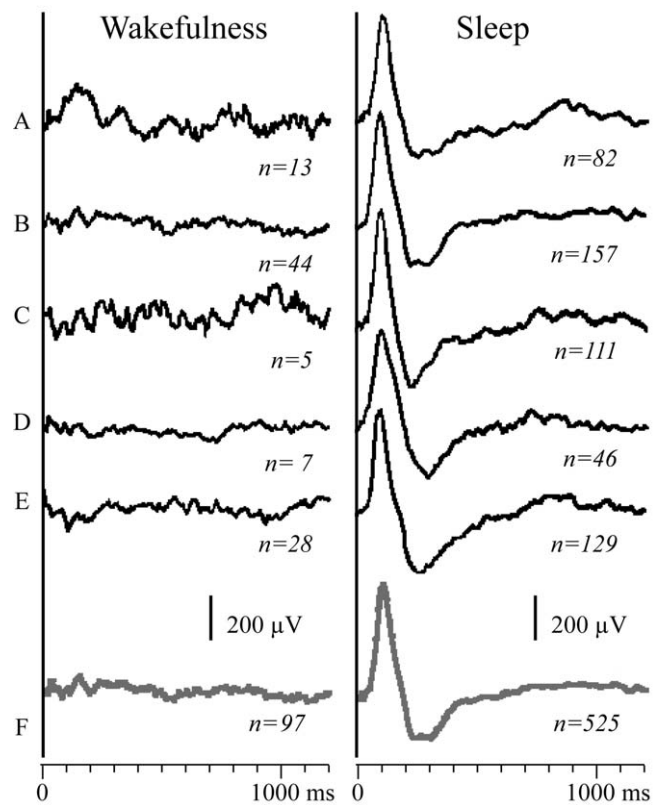

Fig. 2. (A–E) Averaged EEG responses to magnetic stimuli applied in wakefulness (left column) and in SW sleep (right column), recorded in five experiments with one week intervals. The occurrence of stimuli is shown with vertical lines. Numbers of averaged trials are shown under each record. (F) The same responses averaged for all experiments.

without averaging. In Fig. 1C ten averaged original responses to magnetic stimulation in SW sleep are shown. The averaged response includes a short-latency result of stimulation. However, in sleep other response components were also seen with longer latencies of 80–100 ms. Evoked responses to magnetic stimulation in SW sleep were recorded in 5 experiments over one week intervals. Evoked responses were analyzed in nineteen episodes of SW sleep separated by either episodes of rapid eye movement (REM) sleep, or periods of wakefulness. Similar response patterns were always found for the SW sleep condition. Evoked responses averaged for each experiment are shown in Fig. 2 (A–E, right column). In line F (Fig. 2) responses to all magnetic stimuli in SW sleep and in wakefulness which were recorded in five experiments are averaged.

As mentioned, single magnetic stimuli were never applied with intervals of less than 1 minute, and usually these intervals were even longer. As a result, only five episodes of REM sleep were long enough to apply the ten or more stimuli required for comparable averaging. The obtained responses to magnetic stimulation in REM sleep did not differ from those in wakefulness, i.e. these were only the result of stimulation.

Magnetic stimulation is accompanied by an acoustic click. Since cortical evoked responses to acoustic stimuli often increased in sleep (e.g. Juckel et al. 1996, Nordby et al. 1996), it was essential to exclude the possibility that recorded responses were, in fact evoked by the auditory component of the stimulus. Therefore, in two experiments the magnetic coil was moved 40 cm away from the body of the animal. At this distance the coil could not stimulate the tissue but the auditory component was almost the same (or even louder). The coil located this far from the body surface did not produce results. Figure 3A shows an averaged response to 10 purely auditory components of the stimuli in sleep. Indeed, there was a small response, but this was much smaller than the response produced by the coil touching the body surface (Fig. 3B).

Our results showed that: (1) single magnetic pulses applied over the abdominal area of the monkey's body do not wake the sleeping animal; (2) abdominal magnetic stimulation evoked responses in cortical areas during SW sleep; (3) cortical responses to abdominal stimulation disappeared in REM sleep and wakefulness.

With this location of the coil, stimuli could have excited three groups of receptors. First of all, the clicks produced by the coil and the stimulator could have activated the auditory system. Second, magnetic pulses could have excited somatosensory exteroreceptors or fibres located within the skin and muscles under the coil. Third, as was expected, the magnetic pulses could have reached visceral plexuses and ganglia located close to the surface in this part of the body, and visceral receptors located within a depth of 3–4 cm from the body surface.

While acoustic clicks indeed evoked a small response in sleep, this response could be only a minor component of the much bigger evoked potential after magnetic stimulation of the abdominal wall.

In this procedure it was not possible to separate the effects of receptors placed in the skin and muscles and visceral interoreceptors. However, it was shown earlier (e.g. Kitamura et al. 1996, Noachtar et al. 1997) that cortical responses to pure somatosensory stimuli are reduced in sleep compared with their amplitude in wakefulness. With our location of stimulating coil and recording cortical electrodes, no evoked responses were recorded in wakefulness. Therefore, it is very unlikely that such strong responses in sleep were evoked by the activation of the somatosensory cortex in response to skin and muscle magnetic stimulation. However, we can not rule out that information transmitted by the nerve fibres from the abdominal skin and muscles, and also from the blood vessels spread out in this area could be gated to the cortex during slow wave sleep. One can speculate

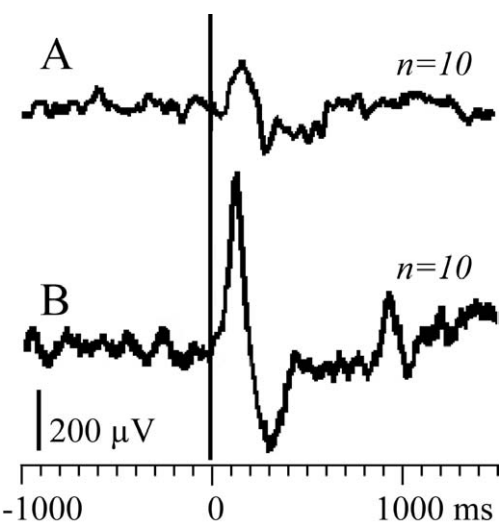

Fig. 3. (A) Evoked response to acoustic component of magnetic stimulus (the coil was removed from the body surface); (B) evoked response to magnetic stimulation in SW sleep. Vertical line – time of stimulation. Number of averaged trials is indicated under the records.

that these fibres carry visceral information that is vital for the recovery of normal skin and muscle functions. Therefore, this visceral but not the somatosensory information is gated during sleep towards the new cortical targets.

Nevertheless, the activity of visceral afferents has become the most likely source of evoked responses to magnetic stimulation applied in sleep. These evoked responses immediately disappeared in wakefulness, and were also not seen in REM sleep. The same was reported for neuronal cortical responses (Pigarev 1994) and for cortical evoked potentials (Kukorelli and Juhasz 1983) in experiments where electric pulses were used for visceral stimulation in cats. Recently we have shown that visceral electrical stimulation evoked cortical responses in the occipital cortex of monkeys during slow wave sleep (Pigarev et al. 2006a). The electric stimulation in these experiments was applied through deeply located electrodes, which entirely precluded the activation of somatosensory afferents. Figure 4A displays the evoked response obtained in our previous study in the monkey's occipital cortex to intraperitoneal electrical stimulation. For comparison Fig. 4B displays the cortical evoked response to abdominal magnetic stimulation from this current study. These evoked responses were standardised by the amplitude of the first positive component.

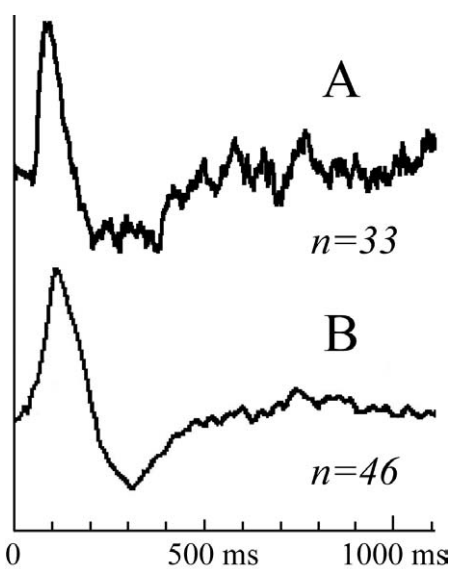

Fig. 4. Comparison of the evoked responses recorded in our previous study on the occipital cortex of monkeys to intraperitoneal electric stimulation (A), and to abdominal magnetic stimulation (B). Numbers of averaged trials are indicated under the records.

Similarity in the manner of these responses is an additional argument in favour of the visceral origin of magnetic evoked responses recorded in this study.

In our study, cortical recording electrodes were placed: one in the occipital area and another four electrodes – the "reference group" – were spread over the frontal area. With these electrodes we expected to have the maximal impact to the recorded responses from the occipital cortex. However, in the event of the synchronous responses of sufficient number of neurons located in other brain areas we could not preclude that these may also have had an impact on the recorded signals. In wakefulness we have not seen any responses to our stimulation. This means that the activity of the somatosensory cortex was not reflected in the signals recorded by our electrodes. The impact on auditory cortex was also minimal (see above). The source of the recorded signals could be in the frontal cortex. However, in wakefulness, we could record visual evoked responses from our electrodes (our unpublished observation). Polarity of these visual evoked responses was the same as polarity of the responses to magnetic stimulation in sleep. If the generator of responses to magnetic stimulation were located in the frontal cortex, it would seem that the polarity should be the opposite. Thus, most likely, the generators of the responses to magnetic stimulation in sleep are located in the occipital cortex. What also points towards the occipital location of the generators, is the strong similarity between responses evoked in this study to magnetic stimulation and in our previous study to electric intraperitoneal stimulation (Pigarev et al. 2006a). In that study both recording electrodes were placed above the occipital cortex and the occipital localisation of the responses was better proved. On the other hand, we would like to stress that the localization of the generators was not the objective of this current study. For us it was sufficient to demonstrate that magnetic stimulation can be used for the research of the distribution of the visceral signals to the cerebral cortex in the sleep-wake cycle.

Another point for discussion is the apparent similarity between the cortical responses described in our studies and the visceral stimulation with "non-specific, evoked" K-complexes. These K-complexes can be provoked by sensory stimulation during first stages of slow wave sleep, but not in REM sleep or wakefulness (for review, refer to Colrain 2005). However, there are at least two factors, which make these effects differ from each other. Provoked K-complexes always follow short latency evoked responses to applied sensory stimulus.

As a result, latency of those evoked K-complexes is rather long – about 400 ms and longer (up to 800 ms), while latencies of our responses were very short – from 50 to 80 ms (see Figs 2, 3, and 4). And the cortical responses to magnetic stimulation were mainly recorded during deep slow wave sleep, whereas the optimal period for the observation of sensory stimuli provoked by K-complexes is within drowsiness stage and very first stages of slow wave sleep. However, we think that both the responses to direct visceral stimulation and that provoked by K-complexes sensory stimulation have a common visceral afferents activity origin. In the first case, we activate these afferents by electrical or magnetic stimulation. In the case of provoked K-complexes we observe the results of the spontaneous activation of visceral afferents. The arguments in favour of this approach are explained in our study of visually triggered K-complex, which is merely described in abstract form (Pigarev et al. 2006b).

The findings presented in this work confirm and extend previous observations concerning a functional link between visceral organs and cortical sensory areas during sleep. It became clear that during sleep, after the propagation of external sensory information to the cerebral cortex is blocked, the gates are opened for the flow of the visceral information. The aim of this reorganisation of brain connectivity is to use the cortical networks for visceral regulation. The need to use the power of cerebral cortex for the visceral regulation is often not immediately obvious. However, the real informational complexity of visceral regulation is highly underestimated. This occurs because the huge flow of the visceral information does not reach our consciousness and can therefore not be explicitly evaluated.

The results of this study also highlighted that magnetic stimulation can be used as a tool for the research of viscero-cortical interaction in the sleep-wake cycle. The option of using magnetic stimulation provides a path for carrying out similar trial studies with human subjects.

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