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Visceral signals reach visual cortex during slow wave sleep: Study in monkeys

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Technical communication

Abstract. Propagation of signals from the gastro-intestinal system towards the occipital cortex within sleep-wake cycle was investigated in three monkeys used in the study of sleep impairment in a chronic MPTP model of parkinsonism. The monkeys differed in motor abilities and sleep structure. One animal (M1) was non-motor disabled and had no sleep alterations. The other two monkeys were severely motor affected, but one (M2) had normal sleep cycles; meanwhile, the other (M3) had no complete sleep cycles. To evaluate the level of sleep and to record cortical evoked responses screw electrodes were implanted over the occipital cortex. Two hours before overnight recordings, two hook electrodes were injected intraperitoneally (under light Ketanest anesthesia) and anchored in gut. Using these electrodes, electric stimulation was applied during slow wave sleep, and in wakefulness. Cortical evoked responses to intraperitoneal stimulation were found indeed during sleep in experiments with M1 and M2. These results show that also in primates with normal sleep pattern visceral information is transferred to the cerebral cortex during slow wave sleep.

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The experiments described in this article were undertaken in order to collect additional evidence *pro* or *contra* the hypothesis that during sleep cerebral cortex can be involved in processing of visceral information for coordination and recovery of those visceral functions, which during wakefulness are under control of the local ganglia of the autonomic nervous system. This hypothesis was first supported by the observations that during sleep neurons in the visual cortex began to respond to the intraperitoneal electrical stimulation of gut and these visceral responses disappeared in wakefulness (Pigarev 1994). Participation of several cortical areas in the processing of visceral information during sleep was later confirmed by the direct observation of synchronization, which was established during particular periods of sleep between firing of the cortical neurons, and periodic myoelectrical activity recorded from the walls of the duodenum and stomach (Pigarev and Bagaev 2002). However, all experiments supporting this hypothesis were performed on cats. We thought it important to demonstrate that propagation of signals from the visceral organs to the cortex during sleep also take place in primates. This article presents results of the first pilot study in this direction. This study was performed in parallel to the main project devoted to the investigation of the sleep pattern in monkeys with an experimental model of parkinsonism after the injections of 1-Methyl,4-phenyl,1,2,3,6-tetrahydropyridine (MPTP). A short explanation of MPTP induction of parkinsonism can be found in Almirall and coauthors (1999). A more deep explanation of this topic is presented in Vila and coauthors (1996). The use of animals with clear symptoms of parkinsonism in the present study was motivated by known observations of different gastrointestinal dysfunctions (gastrointestinal motility disorders, dysphagia, disorders of gastric emptying and constipation) as common and clinically important symptoms in patients with Parkinson's disease (Jost 1997, Pfeiffer 1998). Within the frame of our hypothesis it was possible to expect that such gastrointestinal dysfunctions could be connected with the inability to provide sufficient impact of the cerebral cortex for recovery of these dysfunctions, which could happen as a result of the reduced efficacy of propagation of the visceral signals to the cortex during sleep.

In the present study we have investigated cortical evoked responses to electrical intraperitoneal stimulation applied in sleep and wakefulness in three male eight years old cynomolgus monkeys (*Macaca fascicularis*). All animals were maintained in a special room with controlled temperature (T = 22–24°C) and standard conditions, with a 12 hour-light and darkness cycle (8:00 A.M. – 8:00 P.M. light). Fresh fruits, special food for primates (Old World Primate Diet Expanded, B&K Universal G.J., S.L.) and water were provided *ad libitum*. The IPS guidelines and the guide for the Care and Use of Laboratory Animals adopted and promulgated by NIH, USA, were followed for this experiment.

The three animals were treated with systemic doses of intravenous MPTP (0.3–0.5 mg/kg) with intervals of 3 months. Motor behavior was evaluated according to a disability score that ranged from 0 (normal) to 25 (maximal severity), as previously described (Herrero et al. 1993).

Monkeys clearly showed individual vulnerability to the pro-toxin. One monkey after 4 injections and a cumulative dose of 1.6 mg/kg, did not show any Parkinsonian clinical sign or motor disability. It was classified as a non-motor disabled monkey (M1).

Two other animals showed severe parkinsonism (Motor Score >15) after cumulative doses 2.1 and 4.3 mg/kg, injected over 24 and 27 months respectively. These animals will be referred to as severely affected animals (M2 and M3). Although the motor disability of M2 and M3 was similar, their sleep pattern differed. The medium cumulative dose that received M2 was enough to produce stable parkinsonism but did not disrupt sleep structure severely. The high cumulative dose that received M3 to reach stable parkinsonism strongly disorganized sleep of this animal. The sleep structure of these three monkeys has been reported (Almirall et al. 1999).

Surgical and recording procedures were described in detail in our previous studies (Almirall et al. 1999, Pigarev et al. 1997). Briefly, the monkeys were prepared for chronic recordings of electroencephalogram (EEG), electrooculogram (EOG) and electromyogram (EMG) of the neck muscles. The screw electrodes for EEG recording were located in the occipital bone, over the primary visual cortex. Reference electrodes were implanted in the bone behind the right ear. A band-pass filter (0.1–30 Hz) was used for EEG recordings. For EOG recordings, the pair of screw electrodes was implanted in the bones above and below the left eye.

Two hook electrodes prepared from 200 μm constantan wire, similar to that which were often used for

EMG recordings in humans, were chronically anchored in the neck muscles for EMG recordings and attached to the connector located on the implanted frame (Pigarev et al. 1997).

Two hours before the overnight sleep recordings, two other hook constantan electrodes were inserted intraperitoneally by means of a 60 mm needle for injection (under light Ketanest anesthesia) and anchored in a gut on the right and left side of the body just below the level of stomach.

In this study we did not plan to investigate topography of the cortical representation of particular visceral organs or their different impact in the cerebral evoked responses. For the main goal of this study the stimulating current should cover the maximal surface of the visceral receptors but should not reach the body muscles. Precise location of stimulating electrodes was not important. Using these electrodes, electric stimulation (4 pulses, 0.5 ms duration, 1.5 ms intervals, alternating polarity, 200–400 μA) was applied during sleep and in wakefulness. Intervals between two successive stimulations were not less than one minute. As we could conclude from the observation of the wake animals, these stimuli never reached a threshold of sensation, and the animals absolutely ignored them. However, touching the stomachs of the wake animals, we found that even these weak electric stimuli often triggered the peristaltic waves, which were initiated with the latency of several seconds after the stimulus was applied. Intraperitoneal electrodes were removed from the body after the end of the recording session.

In the previous studies in cats it was shown that cortical neuronal responses to intraperitoneal stimulation could be obtained only during slow wave sleep, but not during rapid eye movement (REM) sleep. That is why in this pilot study, which was strongly limited in time, all intraperitoneal stimuli were applied only during periods of slow wave sleep.

Special care was taken to check that intraperitoneal stimulation did not wake the sleeping animals. EEG, EMG, eye movements and video observation of the face of the animal were monitored before, during and after any intraperitoneal stimulation.

Each animal was used in three experiments with intraperitoneal electrical stimulation. Intervals between the experiments were from 2 to 5 days.

Recordings were performed while animals were sleeping in the primate chair. The chair was placed in the light isolated chamber from 9.00 P.M. till 8.00 P.M. During this entire period, the face of the animal was monitored by the infra-red video camera. EEG, EMG, EOG, and marks of the intraperitoneal stimulation were stored on tape and analyzed off-line.

After the end of the recording sessions which lasted about two weeks with each animal, all recording electrodes and reversible devices for protection of the electrodes and connectors were taken away by a short operation performed under general Ketamine anesthesia, small cuts in the skin were sutured, and soon the animals completely recovered.

To investigate cortical responses to intraperitoneal electrical stimulation, stimuli were applied after the stable slow wave sleep was established according to the visual observation of the animal and EEG, EMG and EOG patterns. More often that happened after midnight. In every experiment, just before or after night sleep session, we recorded cortical activity while the same intraperitoneal electrical stimulation was applied through the same stimulating electrodes but during wakefulness. In total 39, 54 and 46 electrical stimulations were applied during wakefulness in M1, M2 and M3 in three experiments. In the same experiments 77, 76 and 65 stimulations were done during slow wave sleep.

In Fig. 1 cumulative results of this study are presented. Averaged cortical activity recorded in three experiments during electrical intraperitoneal stimulation applied in three monkeys (M1, M2 and M3) in wakefulness (W) and in slow wave sleep (S) is presented. It is seen that during wakefulness there were no responses to visceral stimulation in any animal. However, in monkeys with normal sleep pattern (M1 and M2) there were clear evoked responses to the visceral stimulation during slow wave sleep. In the monkey with severely impaired sleep pattern (M3) we could not record any averaged evoked responses to intraperitoneal stimuli during preserved periods of extremely fragmented slow wave sleep.

These results confirm the previous observation when similar intraperitoneal stimulation evoked neuronal responses in the visual cortex of cats (Pigarev 1994). Results of the present study gave further support to the idea that during sleep information from the visceral organs may reach the cortical level, and the cerebral cortex may be involved in the control of visceral functions.

Evoked responses to the intraperitoneal stimulation were seen only in experiments with M1 and M2. The

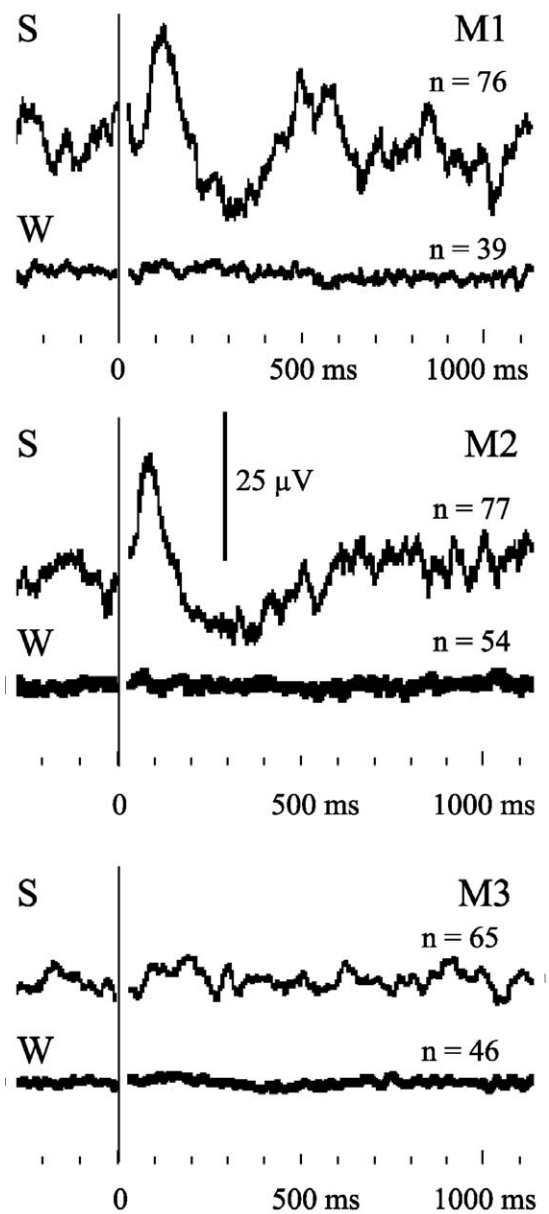

Fig. 1. Averaged cortical EEG recorded in monkeys M1, M2 and M3 during electrical intraperitoneal stimulation delivered in the course of three experiments in wakefulness (W) and slow wave sleep (S). The vertical lines indicate moments of stimulation; (*n*) numbers of the averaged trials.

inability to record visceral responses in M3 certainly does not prove that alteration of a normal sleep pattern after a cumulative dose of 4.3 mg/kg of MPTP in this animal led also to the total impairment of the viscero-cortical propagation during sleep. However, given numerous visceral dysfunctions well known as a component of the Parkinson's disease, study of the viscero-cortical interaction in MPTP treated animals in sleep wake cycle might be an important topic for further investigations.

As it was mentioned, this pilot study was performed in parallel to investigation of sleep in MPTP treated monkeys. As a result, we had no data for the absolutely intact animals. On the contrary, the influence of the MPTP on auditory evoked responses in monkeys was investigated in details (Glover et al. 1988). It was demonstrated that these responses, and especially event related P300 component, first were significantly reduced after MPTP injections. However, these responses started to recover after one month, and became as normal two months after the last injections, while behaviorally animals in that study still demonstrated clear parkinsonism. In the light of the study of Glover and coauthors (1988) we think that cortical responses to visceral stimulation observed in our study reflected the physiological property applicable to the normal monkeys as well.

In the context of our results we would like to remind that intensive investigations of the viscero-cortical interaction were performed in the middle of the previous century. These studies, which were performed in acute experiments under deep anesthesia (for review see, e.g. Musiaschikova and Chernigovskiy 1973), revealed wide visceral projections to most of the cortical areas. Stimulation of these cortical areas led to changes in many visceral parameters studied (i.e. frequency of the intestinal peristaltic activity). However, cortico-visceral interaction could be observed only under deep anesthesia, and strongly reduced, and finally disappeared when the strength of the anesthesia decreased. These effects could not be reproduced later in chronic experiments with wake animals without anesthesia. As a result, cortico-visceral interaction was qualified as an artifact of anesthesia, and results obtained in those studies were forgotten. Now we have shown that cortical responses to visceral stimulation can be recorded in animals without anesthesia, but only during sleep, when viscero-cortical interaction obviously becomes active. Similarity of the evoked responses recorded in our study in sleeping animals, and in the previous investigations performed under anesthesia indicates that most likely the great body of data concerning the cortico-visceral interaction, obtained before, now can be incorporated in the study of sleep.

In this study, first time in primates, it was demonstrated that during slow wave sleep signals from the visceral organs do reach the level of the cerebral cor-

tex. That means that during sleep, after the propagation of external sensory information to the cerebral cortex is blocked, the gates of the cerebral cortex are opening towards the flow of the visceral information. Most likely, the goal of this reorganization of the brain connectivity is to use the cortical networks for the purpose of visceral regulation. The need to use the power of cerebral cortex for the visceral regulation may not be obvious immediately. However, the real informational complexity of the problems connected with regulation and coordination of the visceral activity is strongly underestimated by scientists. That happens because the huge flow of the visceral information that is comparable with the flow of the exteroceptive information does not reach our consciousness during wakefulness and thus cannot be explicitly evaluated.

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