Accuracy in the Recording of Motor Evoked Potentials in Rodents: Reaction Evoked by Transcranial Magnetic Stimulation and Electrical Stimulation

Takashi HIRAOKA

Department of Rehabilitation Medicine, Kawasaki Medical School, Kurashiki 701-0192, Japan

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ABSTRACT. An understanding of the motor conduction pathway and its origin is very important in rehabilitation medicine. With the recent increase of using transcranial magnetic stimulation (TMS), the mechanisms of motor control have been analyzed more directly. Monitoring of motor evoked potentials (MEPs) following magnetic stimulation, especially those from the muscles of the extremities, is useful clinically because central and peripheral motor functions can be evaluated conveniently and noninvasively. Many studies of magnetic MEPs have been reported using monkeys and cats. Although rodents are most frequently and conveniently used for other experiments, there have been only a few reports about magnetic MEPs in rodents. I attempted to record MEP induced by magnetic or electrical stimulation in fifteen Japanese white rabbits and three Wistar rats. However, they could not be recorded in any of the animals by either magnetic stimulation or electrical stimulation using a bipolar electrode. When the electrical stimulation was performed using a monopolar electrode, definite waves of MEP were not recorded in any of the materials except for one rabbit. It is suggested that general anesthesia inhibited the appearance of MEP.

Key words: motor evoked potentials (MEP) — electrical stimulation (ES) — transcranial magnetic stimulation (TMS) — rodents

Clarification of the central nervous system mechanisms that are involved in voluntary movement is very important for development of the physical training of the patients with motor dysfunction such as that caused by cerebrovascular diseases.[1] In most physiological studies related to the mechanism of movement, electrophysiological techniques have been used to evaluate the function of peripheral nerves. With the recent increase of transcranial magnetic stimulation (TMS), the mechanisms of motor control have been analyzed more directly.

With TMS, the brain is percutaneously stimulated by induced current without the use of electrodes. It is so-called electrodeless electrical stimulation. Stimulation of either side of the primary motor area (M1) in the cerebral hemispheres excites the contralateral skeletal muscles, and their excitation is recorded as a motor evoked potential (MEP). Since the first study by Barker *et al* in 1985,[2,3] TMS has been widely used in basic studies of motor function, and in the diagnosis and evaluation of movement disorders because of its simplicity and non-invasiveness.[4] A number of studies on the motor conduction pathway and origins of wave patterns following magnetic

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stimulation have been carried out using monkeys[5-8] and cats.[9,10] However, there have been only a few studies of MEP recordings after TMS in rodents, including rats, due to difficulties encountered in the techniques used.[11,12]

In my laboratory, various TMS studies have been performed in humans,[13-18] but applied to experiments cannot be performed with human subjects due to the ethical limitations in human experiments. To perform experiments involving drug injections and surgical invasion, many animal materials are required, and rodents, which are easily available, are most suitable. Since MEP recording in rodents is considered to be difficult, the author attempted to use the method in this animal study.

MATERIALS AND METHODS

Fifteen male Japanese white rabbits (mean weight, 2.5±0.4 kg) and three male Wistar rats (mean weight, 250±12 g) were used as the experimental animals. Animal treatment and the experimental design were approved by the Animal Experiment Committee of Kawasaki Medical School (approval number, 99-131). The animals were housed under constant 14-hour light and 10-hour dark conditions at 25°C. As food, the Japanese white rabbits were given RC-4 and the rats, MF. The animals were allowed free access to food and water. Of the 15 rabbits, 10 were intravenously injected with 1 ml somnopenthyl (sodium pentobarbital) in a shielded room via the auricular vein to induce anesthesia. General anesthesia was maintained by intramuscular injection of 4 ml/kg of 25% urethane. In the remaining five rabbits, general anesthesia was maintained by inhalation of halothane. After the absence of responses to pain stimulation was confirmed, experiments were performed with the animals in the prone position. Rats were anesthetized by intra-abdominal injection of 0.5 ml/kg somnopenthyl. The room temperature was maintained at 25°C.

(1) Magnetic stimulation

In nine rabbits, of which six were craniotomized, and three rats, magnetic stimulation of the left cerebral motor-sensory area was performed using a circular coil, and MEPs in the right gastrocnemius muscle were recorded using a concentric needle electrode. The electrode was inserted percutaneously into the middle portion of the muscle. The ground was placed under the skin of the right foot. Recording was performed at 10 mv-1 mv/div of amplitude and 3 ms/div of sweep velocity using cutoff filters of 500 Hz for low frequencies and 10 kHz for high frequencies. The apparatus used were a magnetic stimulator (SMN-1100, Nihon Koden Inc.), a circular coil (YM-102B with outer diameter of 124 mm and inner diameter of 48 mm, Nihon Koden Inc.), and an MEP recorder (Neuropack Σ, Nihon Koden Inc.).

As shown in Fig 1, magnetic stimulation of the left cerebral motor-sensory area of the rabbits was performed to induce electric currents in the cephalocaudal direction. The stimulation intervals were not constant, but at least 5 sec. Stimulation was performed at several different sites, while searching for sites where the largest MEP could be recorded. The strength of stimulation was initially at a variation rate of magnetic flux of 1 k Tesla/sec. This was gradually increased up to 2.8 k Tesla/sec. In rats, magnetic stimulation was performed at the same setting.

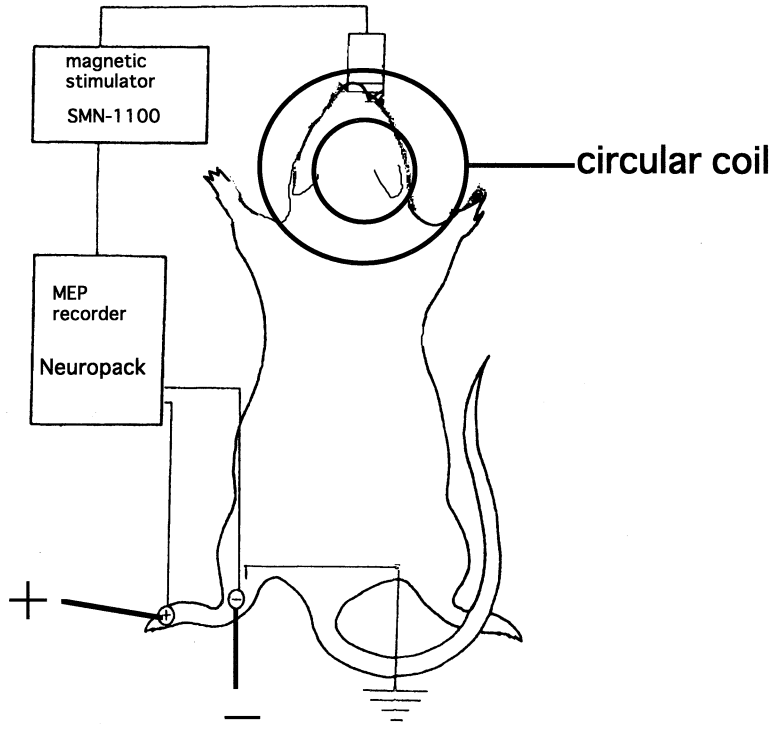

Fig 1. Setup for recording of MEP following TMS

(2) Electrical stimulation

Six rabbits were craniotomized, and two patterns of electrical stimulation were carried out. Anesthesia was achieved by the method described above, and was maintained using halothane in one rabbit and urethane in five rabbits. After a median incision of the scalp, a large area of the cranial bone around the left motor-sensory area was removed using a Luer tool. At first, stimulation was performed on the pia mater in the left cerebral motor-sensory area using a monopolar electrode (diameter of 220 μm, Nihon Koden Inc.). The reference electrode was placed under the scalp. The head was fixed using a stereotaxic instrument for rabbits, after which the left parietal lobe and left frontal lobe were stimulated by moving the electrode in anterio-posterior and bilateral directions by 1 mm. Recording was performed in the right gastrocnemius muscles using a concentric needle electrode, as done in recording magnetic stimulation. Duration of the stimulation was 0.2 ms, and the stimulation frequency was increased in 1 Hz or 2 Hz increments within a range of 1-10 Hz. The stimulus intensity gradually increased from 0 mA to 10 mA at maximum.

Stimulation using a bipolar electrode (diameter of 200 μm IMB-9005, Intermedical Inc.) was also performed in the second experiment. The bipolar electrode was inserted into a depth of 1,300 mm in the motor-sensory area, and stimulation was performed by the method described above.

RESULTS

Magnetic stimulation in the 9 rabbits was performed at a mean of 32.2± 8.7 sites, but no MEP responses were induced from the gastrocnemius muscles irrespective of the changes of systemic anesthesia (Fig 2). Electrical stimulation of 10 Hz using a monopolar electrode induced a wave with a latency of 12 ms and an amplitude of 250 mv in one rabbit, but the same wave pattern could be recorded when the active electrode was moved to the scalp. In the remaining five rabbits, no waves were recorded, although the stimulus intensity increased at maximum (Fig 3). In three rabbits stimulated using a bipolar electrode, no MEP responses were observed. No MEPs were recorded in the rats either.

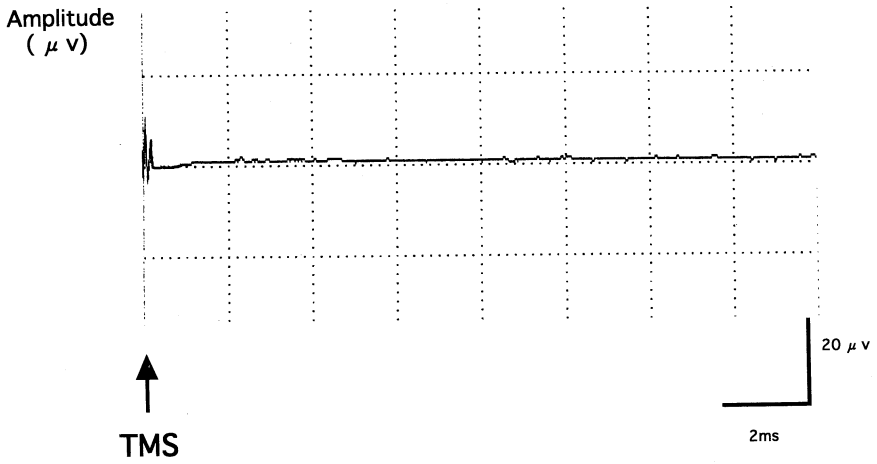

Fig 2. Response following magnetic stimulation using a circular coil

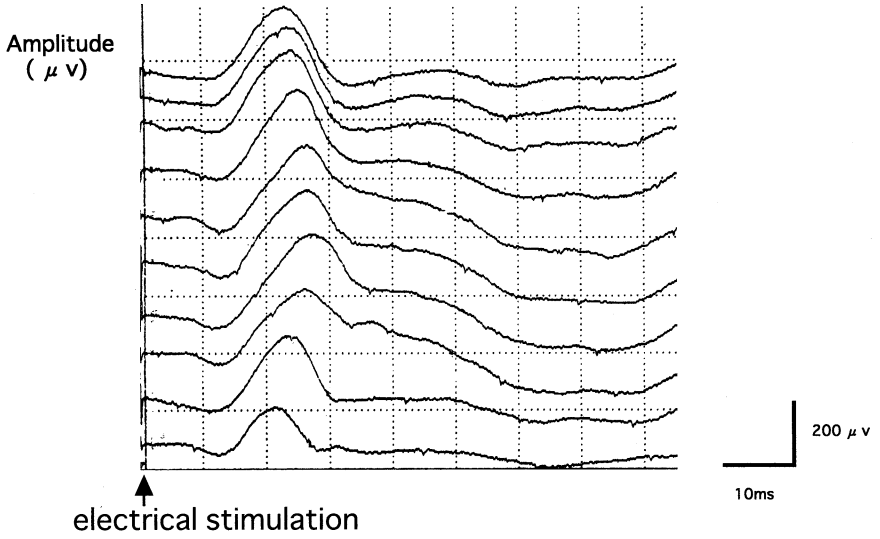

Fig 3. Response in one rabbit following electrical stimulation using a monopolar electrode

DISCUSSION

In the magnetic stimulation and electrical stimulation using a bipolar electrode, MEPs could not be recorded. In one rabbit, an action potential with a latency of 12 ms and an amplitude of 250 mv was recorded by stimulation using a monopolar electrode, but this wave could not be definitely regarded as an MEP because the same waveform could be recorded inspite of the change of stimulation site. The possibility of recording MEP conducted by volume conduction in the distant electrode could not be ruled out. It might be suggested that the rabbit awaked from general anesthesia, when the wave appeared. Nevertheless, the rabbit did not react to pain stimulation. Bolay *et al*[19] and Ueda *et al*[11] reported the recording of MEPs by electrical stimulation using a bipolar electrode in rats. In both studies, most of the amplitudes recorded were 20-30 μV, which are much smaller than those of the MEPs in humans, and too small to be considered reliable. Ueda *et al*[11] also reported that MEPs were recorded by magnetic stimulation in rats. However, since there were large differences in the latency and amplitude between magnetic and electrical stimulation, it is unclear whether the stimulation was performed in the motor area, and their interpretation is considered to be questionable.

In the present study, MEPs could not be clearly recorded in the rodents. One reason might be immature development of the pyramidal tract, since the rodents used in this study are lower mammals than humans and monkeys. Moreover, it was hypothesized that, in Japanese white rabbits, most neurocytes induced by external stimulation act as suppressors of pyramidal cells and suppress the excitation of these cells.

Another hypothesis was the effect of general anesthesia. If anesthesia had been discontinued, MEP might have been recorded, but such an experimental condition would not have conformed to ethical regulations. Since technical and equipment problems connot be ruled out, in the future, experiments will be performed using modified techniques and improved apparatus.

CONCLUSIONS

MEPs were attempted to be recorded by magnetic and electrical stimulation in fifteen Japanese white rabbits and three rats. However, they could not be recorded in any of the animals by either magnetic stimulation or electrical stimulation using a bipolar electrode. No definite waves were recorded in any of the materials except for one rabbit by electrical stimulation using a monopolar electrode. It is suggested that general anesthesia inhibited the appearance of MEPs. In future studies, the investigation will be continued with further consideration of the physiological functions and anatomical structures of Japanese white rabbits, anesthesia, duration of the experiment, the apparatus for stimulation, and experimental techniques.

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