Exp Brain Res (1994) 99:529–532

RESEARCH NOTE

Stuart N. Baker · Etienne Olivier · Roger N. Lemon

Recording an identified pyramidal volley evoked by transcranial magnetic stimulation in a conscious macaque monkey

Received: 11 January 1994 / Accepted: 11 March 1994

Abstract A descending volley in response to non-invasive transcranial magnetic stimulation has been recorded from the pyramidal tract in a conscious monkey and identified by means of a collision test. The short latency of the earliest wave was inconsistent with a trans-synaptically mediated activation of pyramidal tract neurones. Considerable variability in the size of this wave was seen in response to a constant stimulus, and isoflurane anaesthetic was shown to depress it markedly. These results are consistent with direct activation of pyramidal tract neurones at a site close to the cell body.

Key words Pyramidal tract · Magnetic stimulation Motor cortex · Anaesthesia · Monkey

Introduction

The technique of transcranial magnetic stimulation (TMS) of the primary motor cortex has found extensive experimental and clinical uses since its introduction by Barker et al. (1985). It is generally believed that the short latency facilitation of electromyographic activity (EMG) in hand and forearm muscles produced by such stimulation is brought about by the activation of corticospinal neurones, some of which make monosynaptic cortico-motoneuronal (CM) connections in primates. In order to interpret the responses to TMS in man, it is important to determine its site of action, which is a matter of considerable controversy. Different investigators have suggested either that the electric field induced in the brain by TMS activates the corticospinal cells directly (Amassian et al. 1990; Edgley et al. 1990) or that instead intracortical elements are first activated and these discharge the CM cells transynaptically (Amassian et al. 1990; Day et al. 1989). An important factor in determining the site of action appears to be the stimulating coil orientation relative to the head (Amassian et al. 1990).

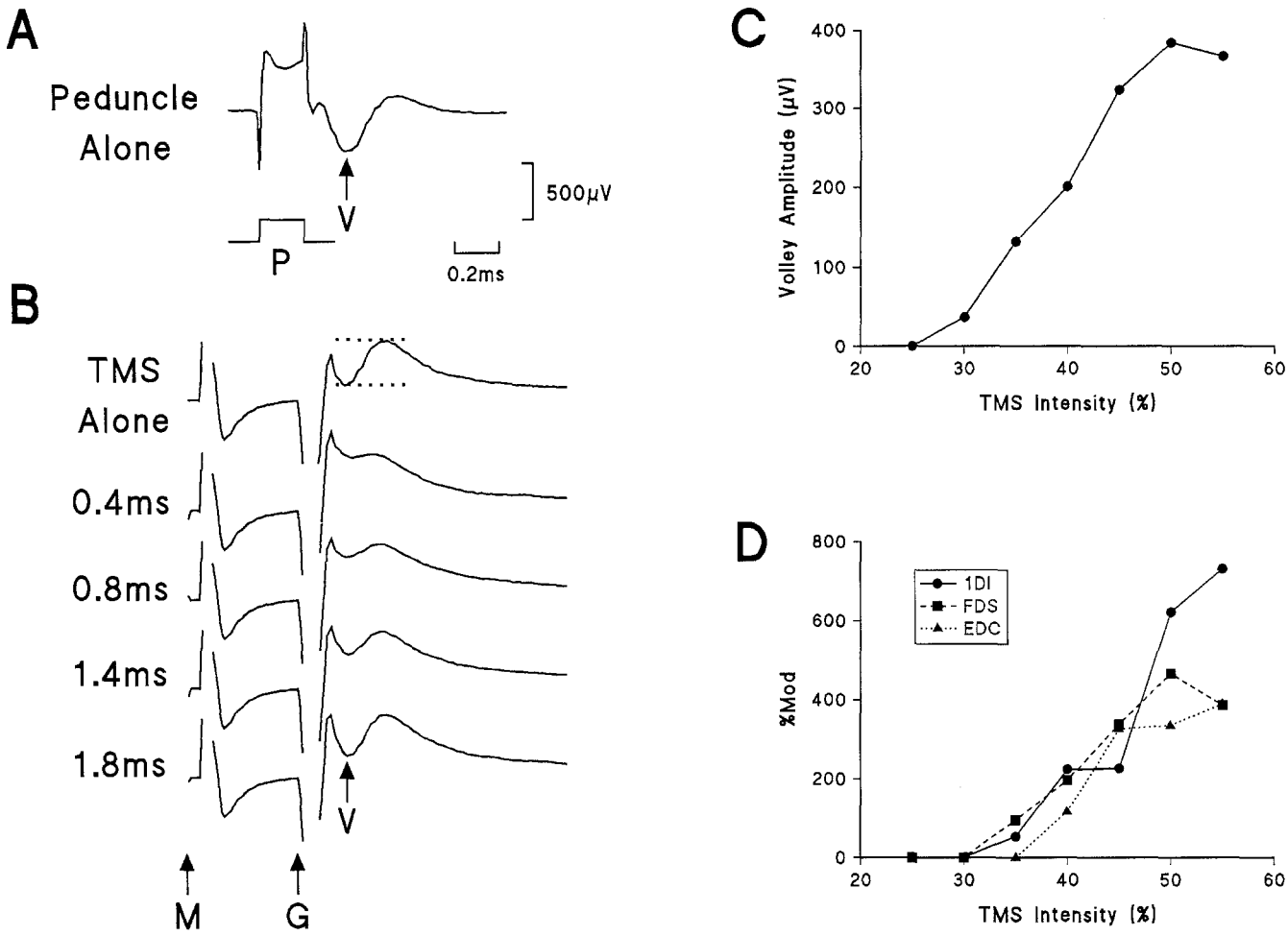
To date, the site of action of TMS has primarily been deduced either from the facilitation of muscle surface EMG activity or single motor units (Day et al. 1989), or from direct recordings of corticospinal volleys in anaesthetised monkeys (Amassian et al. 1990; Edgley et al. 1990) or human patients (Burke et al. 1993). Anaesthesia might be expected to have a profound effect on the dominant mode of activation, either by depressing synaptic function and so preferentially blocking indirect activation or by hyperpolarising cells (Matsumura et al. 1988) so that they would fire only after summation of direct and later indirect excitation had occurred. The issue would therefore be better addressed by recording the corticospinal volley to TMS in a conscious primate. Edgley et al. (1990) provide the only such recording in the literature; however, this is obscured by a large stimulus artefact and is from an unidentified population of fibres. We here report such a recording in a conscious monkey, relatively uncontaminated by the stimulus artefact and identified by means of a collision test.

Materials and methods

Experiments were performed on a female, 2.9-kg *Macaca fascicularis* monkey, trained to perform a lever-pull task for food reward. During two separate operations under full general anaesthesia and aseptic conditions, two fine tungsten electrodes (impedance at 1 kHz 10–20 kΩ) were implanted in the pyramidal tract (PT) and two in the cerebral peduncle, together with a stainless steel headpiece to allow fixation of the head. A full programme of post-operative medication was administered. Electrode location was confirmed in three ways: (1) During the surgery a small craniotomy was made over the motor cortex, and a short-latency, antidromic field potential could be recorded transdurally following stimulation of each electrode. This craniotomy was sealed at the end of the surgery. (2) During recording sessions, stimulation between each of the two pairs of electrodes produced a short-latency facilitation in hand muscle EMG activity similar to that described

S. N. Baker · E. Olivier · R. N. Lemon[1] (✉)
Department of Anatomy, Cambridge University

Present address:
[1] Sobell Department of Neurophysiology, Institute of Neurology, Queen Square, London WCIN 3BG, UK,
Fax no: +44 71 813 3107



previously (Lemon et al. 1986; Flament et al. 1992). (3) At the end of the experimental series, the animal was killed with an overdose of pentobarbitone sodium, perfused through the heart with saline followed by 10% formalin, and the position of the electrode tracks was examined histologically. The tip of the caudal pyramidal electrode was found to be located in the medullary pyramidal tract at the level of the facial nucleus and the rostral electrode, in the corticospinal bundles of the caudal pons; the inter-electrode distance was 5 mm. The peduncle electrodes were located at the level of the rostral lateral geniculate nucleus (posterior electrode) and the rostral substantia nigra (anterior electrode).

Magnetic stimuli were delivered using a Magstim 200 stimulator and figure-of-eight coil positioned over the cortex contralateral to the limb performing the task. The coil was orientated at an incline of approximately 30° to the skull surface, with the handle pointing anteriorly – a position chosen to optimise the stimulus artefact in recordings. The current direction in the central region of the coil was towards the handle, the reverse of that in standard Magstim figure-of-eight coils. Stimuli were either given periodically every 3.2 s or triggered during task performance. Recordings were made differentially from the PT electrodes using a muted amplifier slightly modified from a published design (Millard et al. 1992). The output of this amplifier was filtered d.c. to 40 kHz and digitised at 80 kHz for storage and display on a personal computer. The anterior PT electrode was connected to the inverting input of the amplifier.

EMG recordings were routinely made from up to four intrinsic hand and forearm muscles using surface electrodes. Signals were amplified (gain 500–2 K), high-pass filtered at 30 Hz, and stored on magnetic tape for off-line capture into the computer (sampling rate 5 kHz), where averages of the rectified EMG activity were constructed.

Fig. 1 **A** Volley recorded at the pyramid in response to stimulation of the peduncle electrodes (300 μA, 0.2 ms pulse shown schematically as *P*). The stimulus artefact can be clearly seen. The amplifier was not muted during this recording. Single sweep. (*V* negative inflexion of the volley). **B** Volley recorded at the pyramid in response to transcranial magnetic stimulation (*TMS*), 50% maximal stimulator output. *Top trace* is TMS delivered alone, others show responses to TMS when preceded by electrical stimulation through the peduncle electrodes (same parameters as **A**) at varying intervals, as marked on the *left*. *Dotted lines* show the measurement used to quantify the volley size. *M* start of stimulus. The amplifier was muted from 0.2 ms before point *M* until point *G*. Single sweeps. **C** Variation of volley amplitude with stimulus intensity, measured from averages of 20 sweeps. Stimuli were delivered during task performance and timed to occur at the same point in each trial. Data recorded on a different day from **A** and **B**. **D** Variation with TMS intensity of EMG facilitation in three muscles, same series as **C**. *1DI* first dorsal interosseous, *FDS* flexor digitorum superficialis, *EDC* extensor digitorum communis, *% Mod* percentage modulation, defined as the height of the facilitation peak in the average of rectified EMG above the baseline level, expressed as a percentage of that baseline

Results and discussion

The top trace of Fig. 1B is a typical recording from the pyramidal tract of the volley evoked by TMS. Figure 1A shows the volley recorded at the pyramid following elec-

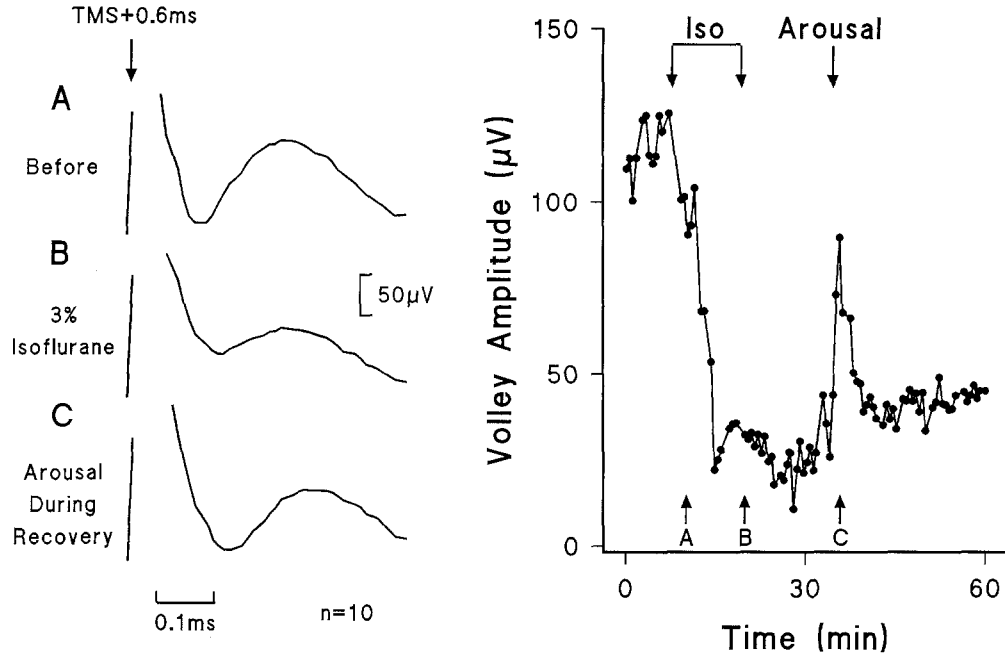


Fig. 2 Effect of isoflurane anaesthesia on volley amplitude. *Right panel* shows the volley amplitude (mean of ten sweeps) as it varied throughout the experiment. Isoflurane (3%) inhalation anaesthetic in a 50:50 mixture of NO_2: O_2 was administered for 10.5 min over the period marked *Iso*. At the time marked *Arousal* a toe pinch was given and a reflex response was obtained for the first time since the cessation of anaesthetic administration. *Left panel* shows the appearance of the volley in means of ten sweeps, corresponding to the points marked on the *right panel* with *arrows A, B* and *C*. *Top arrow* indicates time 0.6 ms after the start of the magnetic stimulus (*TMS*); 40% maximum magnetic stimulator output used throughout

trical stimulation through the electrodes in the cerebral peduncle. A comparison of the two traces shows that the early part of the volley to TMS was probably obscured by the stimulus artefact. The onset of the volley to peduncle stimulation appears to be 0.15 ms before the inflexion marked V on Fig. 1A. The equivalent point V in Fig. 1B is 0.67 ms after the magnetic stimulus, implying that the latency of the pyramidal volley following TMS was approximately 0.52 ms. This value is very close to the onset latency of the antidromic field potential recorded at the cortical surface to electrical stimulation of the pyramid during surgery, which was 0.58 ms. These latencies are therefore consistent with direct activation by TMS of the cortico spinal tract at a cortical site.

Evidence that the direct volley excited by TMS originated from the pyramidal tract was provided by demonstrating that it could be made to collide with a suitably timed, prior electrical stimulus to the peduncle electrodes. This is illustrated in Fig. 1B. Only the volley to TMS is visible in this figure, as the peduncle volley would have fallen during the time when the amplifier was muted, or before the start of the sweep. When the peduncle stimulus preceded the TMS by 0.4 ms, the volley amplitude was substantially reduced. As the interval between the two stimuli was increased, the volley recovered, until it had returned to its control size at an interval of 1.8 ms. This relatively brief collision interval is comparable with that found by Edgley et al. (1990) when the corticospinal volley evoked by TMS was recorded from the spinal cord and collided with electrical stimuli delivered to the pyramid. The incomplete collision of the volley at short interstimulus intervals is probably due to sub-maximal activation of the pyramidal fibres by the peduncle electrodes, which were stimulated with the maximum current which they would pass (300 μA). Similar results were obtained in all seven experimental sessions where a collision test was attempted.

In addition to the early direct (D) wave, later waves were seen in some experimental sessions. When present, they were small (around 10% of the size of the D wave), and could only be clearly discerned in means of 20 sweeps or more. The latency of the first such late wave was 3.1 ms to the first negative inflexion, making the interval between it and the D wave approximately 2.4 ms, a value in agreement with Edgley et al. (1990). As noted by Amassian et al. (1990), when the electrodes are close together and the recorded action potentials have a biphasic shape, some cancellation of potentials will occur in the multi-unit recording; cancellation may be greater for late waves, since their trans-synaptic origin will mean that the individual components of the wave are more temporally dispersed. This could explain the small size of the late waves in these recordings.

Figure 1C shows how the height of the D wave evoked by TMS varied with different stimulus intensities. It can be seen to follow an approximately sigmoidal saturation function. At the highest intensity shown (55% of maximum stimulator output), it is likely that the stimulus artefact began to overlap the negative inflexion of the volley (V in Fig. 1B), causing the apparent reduction in height observed for the 55% point in Fig. 1C. Figure 1D shows the size of the short-latency response of three muscles (measured as the percentage modulation of the background EMG) as a function of stimulus intensity for the same experimental run as Fig. 1C. A comparison of Fig. 1C and 1D shows that the threshold for the D wave is lower than that for a detectable EMG facilitation, but that both D wave amplitude and EMG facilitation saturate at around 50% of maximum stimulator output. This implies that the increasing EMG response is at least partly accounted for

by an increasing D wave for the entire range of stimulus intensities up to saturation. The latencies of the muscle responses were 6.8 ms for the first dorsal interosseous (1DI), 6.1 ms for the flexor digitorum superficialis (FDS) and 5.4 ms for the extensor digitorum communis (EDC).

As illustrated by Fig. 1B, the signal-to-noise ratio in these recordings was high, so that all features of the D wave could be seen in single sweeps. The size of the wave, however, showed considerable variability from sweep to sweep. During the session from which the data in Fig. 1B were taken, stimuli were delivered whilst the monkey performed the task, and the D wave amplitude ranged from a minimum of 236 μV to a maximum of 362 μV, with a mean of 294 μV, (S.D. 17.5 μV) this was considerably larger than the amplifier noise standard deviation of around 5 μV. Similar variability was seen during other sessions. Such variability is consistent with suggestions (Edgley et al. 1990) that the volley is produced by activation at or close to the initial segment of the pyramidal tract neurones, where that the probability of firing is dependent on the level of excitatory drive to the cell at the moment the stimulus is delivered (Brooks and Eccles 1947). This would be expected to produce a more variable volley than if corticospinal axons were stimulated deep to the cortex.

General anaesthesia produces a reduction in cortical activity and hyperpolarisation of pyramidal tract neurones (Matsumura et al. 1988); anaesthetic might therefore be expected to depress a D wave initiated close to the cell soma. The results of an experiment which tested this prediction are shown in Fig. 2. The monkey was first sedated with an intramuscular injection of ketamine hydrochloride (10 mg/kg). Following this, a large volley could still be recorded, consistent with reports that EMG responses to TMS can be obtained in both monkeys and human patients sedated with ketamine (Ghaly et al. 1990; Kothbauer et al. 1993). General anaesthesia was then induced by inhalation of 3% isoflurane in a 50:50 O_2: N_2O mixture; this dramatically reduced the volley amplitude to around one fifth of its control value within 5 min. Following the cessation of the anaesthetic, the volley remained at this depressed level for approximately 5 min, and then recovered slowly. At the time indicated by the arrow on Fig. 2 ("Arousal"), a toe pinch was given to test the depth of anaesthesia; this elicited a reflex response for the first time since withdrawal of the anaesthetic. As can be seen, there was also a powerful but transient facilitatory effect on the volley, before it returned to the previous slow-recovery curve. The volley produced by electrical stimulation of the electrodes in the cerebral peduncle showed no comparable changes with anaesthesia.

In conclusion, this experiment demonstrates for the first time in a fully conscious primate that TMS evokes an early wave of descending activity which can be recorded from the pyramidal tract and identified as corticospinal in origin. The short and consistent latency of this volley, the variability in its amplitude with successive stimuli and its sensitivity to anaesthesia and arousal all suggest that, for the coil type and orientation used, this volley originates at or close to the cell bodies of pyramidal tract neurones.

Acknowledgements Supported by the MRC, Action Research, the Wellcome Trust and the Magstim Company. The authors would like to thank Rosalyn Cummings for technical help, and Dr. Jean Armand for assistance with histology.

References

Amassian VE, Quirk GJ, Stewart M (1990) A comparison of corticospinal activation by magnetic coil and electrical stimulation of monkey motor cortex. Electroencephalogr Clin Neurophysiol 77:390–401

Barker AT, Jalinous R, Freeston IL (1985) Non-invasive magnetic stimulation of the human motor cortex. Lancet 1:1106–1107

Brooks CMcC, Eccles JC (1947) Electrical investigation of the monosynaptic pathway through the spinal cord. J Neurophysiol 10:251–273

Burke D, Hicks R, Gandevia SC, Stephen J, Woodforth I, Crawford M (1993) Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. J Physiol (Lond) 470:383–393

Day BL, Dressler D, Maertens de Noordhout A, Marsden CD, Nakashima K, Rothwell JC, Thompson PD (1989) Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. J Physiol (Lond) 412:449–473

Edgley SA, Eyre JA, Lemon RN, Miller, S (1990) Excitation of the corticospinal tract by electromagnetic and electrical stimulation of the scalp in the macaque monkey. J Physiol (Lond) 425:301–320

Flament D, Hall EJ, Lemon RN (1992) The development of cortico-motoneuronal projections investigated using magnetic brain stimulation in the infant macaque. J Physiol (Lond) 447:755–768

Ghaly RF, Stone JL, Aldrete JA, Levy WJ (1990) Effects of incremental ketamine hydrochloride doses on motor evoked potentials (MEP's) following transcranial magnetic stimulation: a primate study. J Neurosurg Anesthesiol 2:79–85

Kothbauer K, Schmid UD, Liechti S, Rösler KM (1993) The effect of ketamine anesthetic induction on muscle responses to transcranial magnetic cortex stimulation studied in man. Neurosci Lett 154:105–108

Lemon RN, Mantel GWH, Muir RB (1986) Corticospinal facilitation of hand muscles during voluntary movement in the conscious monkey. J Physiol (Lond) 381:497–527

Matsumura M, Cope T, Fetz EE (1988) Sustained excitatory synaptic input to motor cortex neurons in awake animals revealed by intracellular recording of membrane potentials. Exp Brain Res 70:463–469

Millard RE, McAnally KI, Clark GM (1992) A gated differential amplifier for recording physiological responses to electrical stimulation. J Neurosci Methods 44:81–84