HIGHLIGHTED TOPIC | Neural Control of Movement

Noninvasive stimulation of the human corticospinal tract

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Taylor, J. L., and S. C. Gandevia. Noninvasive stimulation of the human corticospinal tract. J Appl Physiol 96: 1496–1503, 2004; 10.1152/japplphysiol.01116.2003.—Spinal tracts can be stimulated noninvasively in human subjects by passing a high-voltage stimulus between the mastoids or by magnetic stimulation over the back of the head. The stimulus probably activates the corticospinal tract at the cervicomedullary junction (pyramidal decussation) and evokes large, short-latency motor responses in the arm muscles. These responses have a large monosynaptic component. Responses in leg muscles can be elicited by cervicomedullary junction stimulation or by stimulation over the cervical or thoracic spine. Because nerve roots are more easily activated than spinal tracts, stimulus spread to motor axons can occur. Facilitation of responses by voluntary activity confirms that the responses are evoked synaptically. Stimulation of the corticospinal tract is useful in studies of central conduction and studies of the behavior of motoneurons during different tasks. It also provides an important comparison to allow interpretation of changes in responses to stimulation of the motor cortex. The major drawback to the use of electrical stimulation of the corticospinal tract is that each stimulus is transiently painful.

magnetic stimulation; motoneuron; cervicomedullary stimulation

SINCE THE DISCOVERY by Fritsch and Hitzig (9) that motor areas of the cerebral cortex could be localized by electrical stimulation, the development of ideas about the control of voluntary movement has been influenced by technical developments related to stimulation and recording in the motor cortex. In 1980, Merton and Morton (27) developed the first “noninvasive” method for human subjects. This method relied on a high-voltage electrical stimulus that allowed sufficient current to pass through the skull to activate the motor cortex. This technique was followed in 1985 by a method based on rapidly changing magnetic fields to activate the cortex through the skull (3). Both techniques excite the cortex and produce rapidly conducting descending volleys in corticospinal axons, thus allowing researchers to examine performance of the major motor pathway in humans. The size of the evoked motor response is influenced not only by the level of cortical “excitability” but also by the “excitability” of the motoneuron pool. The responsiveness of spinal motoneurons depends on multiple descending and afferent inputs, as well as on intrinsic motoneuronal properties, and is therefore difficult to predict. Hence, a method that activates the corticospinal output at a subcortical level is valuable for investigation of the segmental behavior of the motor pathway (e.g., Refs. 6, 11, 40); such a method also allows better interpretation of responses evoked by stimulation of the cortex (e.g., Refs. 10, 14, 18, 33, 37).

Spinal tracts can be stimulated with electrical or magnetic pulses in anesthetized and awake human subjects. During surgery, the spinal cord is stimulated through laminar or epidural electrodes and is used to monitor the integrity of motor and sensory tracts. Spinal stimulation via implanted electrodes is used clinically as a method for pain control. In normal volunteers, stimulation of spinal tracts can be performed noninvasively by electrical or magnetic stimuli applied over the back of the head or by electrical stimulation over the cervical or thoracic vertebrae. In this review, we describe the responses to noninvasive electrical and magnetic stimulation designed to activate the descending motor tracts in the spinal cord; we also discuss some of the pitfalls with these techniques and present some interesting findings.

RESPONSES IN UPPER LIMB MUSCLES

Electrical Stimulation

Initial attempts to activate the spinal tracts percutaneously used electrodes over the cervical enlargement (26, 34). Although this stimulation evoked motor responses in muscles of the upper limb, the latency and size of the responses were not altered by voluntary contraction. During contractions, ongoing input excites the motoneuron pool, causing repeated firing of some motoneurons and bringing others close to the threshold for firing. Thus more motoneurons in the pool are close to threshold and should fire with any given additional synaptic input during voluntary activity than with the muscle at rest. Therefore, the lack of change with voluntary contraction implied that responses to stimulation over the spine were not affected by motoneuronal excitability and so were not evoked synaptically but through stimulation of the motor axons distal to the cell bodies, probably at the vertebral foramina (28). When the stimulus intensity was increased sufficiently (400–
750 V), responses occurred in leg muscles. These were consistent with stimulation of spinal tracts. Although it is likely that spinal tracts innervating the arms were also stimulated with high intensities, any response would have been obscured by the simultaneous supramaximal stimulation of the nerve roots.

Upper limb responses to spinal tract stimulation are only seen when stimuli are delivered as far as possible above the cervical roots. Ugawa et al. (46) demonstrated that motor responses could be evoked in muscles of the upper and lower limbs by passing a high-voltage electrical pulse (50-μs duration, up to 750 V) either across the spinal cord between electrodes on the mastoid processes (Fig. 1A) or along the spinal cord between electrodes in the midline over the skull and upper cervical vertebrae. Responses with the same latency were evoked by a horizontal pair of electrodes sited anywhere from 2 cm above to 4 cm below the bottom of the mastoids. For arm muscles, latencies were 1.8 ms shorter than those to electrical stimulation over the motor cortex and 3–4 ms longer than stimulation of the ventral roots. This behavior was consistent with activation of fast-conducting descending tracts at the level of the pyramidal decussation (or cervicomedullary junction; Refs. 43, 46). The decussation may be a preferred site of activation because axons are more susceptible to stimulation where they bend (1, 21). Stimulation through vertical pairs of midline electrodes evoked responses with a more variable and slightly longer latency (up to 0.7 ms), suggesting a higher stimulation site in the brain stem. Thus, because of the better stability of the responses, the use of horizontal pairs of electrodes has been adopted as standard (46).

When responses are evoked by electrical stimulation at the cervicomedullary junction during voluntary contraction of the target muscles, their latency changes little but their size increases markedly and the threshold intensity of stimulation needed to evoke a response [cervicomedullary motor evoked potential (CMEP)] is decreased (41, 47). This dependence on the excitability of the motoneuron pool indicates that the activation of motoneurons occurs trans-synaptically via stimulation of spinal tracts rather than through direct stimulation of the motoneuronal axons in the spinal roots. In contrast to a stimulus to the motor cortex, which commonly evokes multiple descending volleys and can cause motoneurons to fire more than once (8, 35), cervicomedullary junction stimulation evokes a single descending volley. The antidromic volley from supramaximal peripheral nerve stimulation can completely occlude a CMEP, showing that each motoneuron fires only once (4).

Practicalities and pitfalls of electrical cervicomedullary junction stimulation. Stimulation is commonly carried out using a high-voltage stimulator (e.g., Digitimer 180) with 9-mm-diameter Ag/AgCl electrodes stuck to the skin in the groove between the mastoid process and the occiput (Fig. 1A). A 50- or 100-μs pulse can be used. The longer-duration pulse requires lower voltages. In many subjects, CMEPs can be evoked with available constant-current stimulators (e.g., Digitimer DS7A, 100-μs pulse), which provide up to 400 V. Because large responses (50% maximal M wave; e.g., Ref. 11) can often be evoked even in relaxed muscles, CMEPs do not require averaging. Individual responses are easily measured and can be used to follow even brief changes in motoneuron excitability. In our experience, the stimulus intensities required to evoke CMEPs in relaxed arm muscles are lower for proximal than for distal muscles (Fig. 2). This contrasts with Ugawa et al. (46) who reported more distal muscles as more easily activated.

The most obvious practical difficulty with electrical cervicomedullary stimulation is that it is painful because of activation of local skin afferents. It can also stimulate nearby periph-

Fig. 1. Two methods (A and B) used for stimulation of corticospinal tracts in humans.
CMEP jumps to an earlier latency, and this reactivates spinal tracts. With a high enough intensity, the onset of the toids, is the possibility of stimulating nerve roots in addition to the discomfort (44), but subjects must be prepared for each eral nerves and cause a large sudden contraction of muscles in the head and neck. Larger stimulating electrodes may decrease the discomfort (44), but subjects must be prepared for each stimulus to be transiently painful.

A second problem, even with stimulation between the mastoids, is the possibility of stimulating nerve roots in addition to spinal tracts. With a high enough intensity, the onset of the CMEP jumps to an earlier latency, and this reflects spread of stimulation to the nerve root. In practice, the latency of the response to spinal tract stimulation can be determined at a threshold intensity during weak voluntary contraction. As stimulus intensity is increased, the CMEP increases in size and its latency shortens gradually with the recruitment of larger, faster motoneurons. If, with any increment in intensity, there is an abrupt change in onset latency of \( \sim 1-2 \) ms (usually seen as a “foot” at the start of the potential; see Fig. 2, right), some peripheral axons have been activated and the response reflects a mix of pre- and postsynaptically activated motoneurons. With further increases in stimulus intensity, more and more of the response will arise from peripheral stimulation, as descending activation of the motoneurons is occluded. Stimulus spread is most likely to affect responses in more proximal muscles because of their innervation through higher cervical roots (e.g., biceps brachii through C5-C6), but the problem can occur even with hand muscles (T1 root). Because polarity of stimulation makes little difference to spinal tract responses (46) but depolarization of the peripheral nerve occurs closer to the cathode, siting the anode on the side of the muscles to be recorded can help.

Head position, particularly turning the head, can change the size of CMEPs. This occurs not only with the muscle at rest but also with a constant background EMG. Because the changes in CMEP size occur when motoneuron excitability is clamped by voluntary activation, they probably occur due to movement of the electrodes relative to the point of stimulation rather than via a physiological consequence of altered input from neck or other afferents.

**Magnetic Stimulation**

Stimulation over the back of the head using a double-cone magnetic coil (Magstim 200 stimulator; Fig. 1B) can also activate spinal tracts and evoke motor responses with the same latencies as those evoked by an electrical pulse passed between the mastoids (48). However, responses in the relaxed muscle are often small compared with those evoked with electrical stimulation. Responses are best seen with the coil positioned with its central section over or near the inion and with the current directed downward in the coil. Responses with an appropriate latency are not seen with standard round or flat figure-eight coils; even with the double-cone coil, stimulation is not successful in all subjects. The distance from the inion to the spinal cord at the foramen magnum is \( \sim 7 \) cm and is at the limit of effective stimulation; thus success depends on the anatomy of the individual subject. The probability of obtaining responses in a given muscle in a particular subject can be enhanced by using voluntary contraction to increase the excitability of the motoneuron pool. During strong voluntary contractions of the elbow flexors, the magnetic stimulus can evoke CMEPs of \( >50\% \) of the maximal M wave in biceps brachii and brachioradialis (6).

**Practicalities and pitfalls of magnetic cervicomedullary junction stimulation.** Magnetic stimulation is much less painful than electrical stimulation because current density in the skin is lower, but the stimulus still activates muscles in the head and neck and is accompanied by a loud click. Some subjects find it unpleasant. Relatively low-intensity repetitive stimulation (\( \geq 200 \) stimuli at 1 Hz) over the back of the head, intended to stimulate the cerebellum, has been reported to cause nausea in some subjects (36).

As with electrical stimulation, a major difficulty is unintended stimulation of axons in the motor roots. Like the figure-eight coil, the double-cone coil has a central portion where the magnetic fields generated by current passed around the two component coils add together, so that approximately twice as much current is induced under the center of the coil as under its edges (wings). With the coil on the inion, its lower edges lie on the upper part of the back of the neck (Fig. 1B). Because the nerve roots at the foramina have a lower threshold than the spinal tracts, the current under the wings of the coil can be sufficient to activate them. Therefore, careful positioning of the coil is required. In practice, it is best to start with the coil over the inion and attempt to evoke a response in the weakly contracting muscle. The coil can then be moved by \( 1-2 \) cm to search for the optimal stimulation site. Lower coil positions evoke larger responses but increase the likelihood of root stimulation.

A second, rarely encountered problem with the site of stimulation can occur if the upper edges of the wings of the coil lie too close to the motor cortex. The high intensities of stimulation needed to evoke CMEPs mean that the current induced under the edges of the coil is often above threshold for motor cortical stimulation, and occasionally responses with latencies consistent with motor cortically evoked responses can be seen.
What is Stimulated by Cervicomedullary Junction Stimulation?

Electrical and magnetic cervicomedullary stimulations activate axons in the corticospinal tract. In hand muscles, responses to electrical stimulation of the motor cortex can be occluded by appropriately timed electrical or magnetic stimulation over the brain stem (46, 48). That is, antidromic action potentials collide with and annihilate the action potentials descending from the cortex. This implies that the neurons activated in the motor cortex had also been stimulated at the cervicomedullary level. With longer interstimulus intervals, the responses are facilitated by their interaction at the motoneurons. Similar evidence indicates that corticospinal neurons are also activated to evoke CMEPs in biceps brachii: responses to transcranial magnetic stimulation (TMS) of up to 15% of the maximal M wave are occluded by electrical cervicomedullary stimulation, which evokes potentials of the same size (Ref. 41; Fig. 3). The interaction is complex because of multiple descending volleys evoked by TMS but still demonstrates that many of the same axons are activated by TMS and electrical cervicomedullary junction stimulation.

A significant proportion of the motoneuronal response to cervicomedullary stimulation appears to be monosynaptic for both the hand muscles and the more proximal biceps. In the hand and in biceps, voluntary contraction makes little difference to the onset latency of responses (32, 46). When potentials evoked in the relaxed and contracting biceps brachii by magnetic cervicomedullary stimulation were matched for size, the mean difference was minimal [0.02 ± 0.12 ms, mean ± SD, n = 5 (Ref. 38); Fig. 4B]. This suggests a monosynaptic pathway. If there were multiple synapses in the pathway, increased excitability of each postsynaptic pool of cells should shorten each activation time and reduce the latency. Furthermore, single motor unit studies in biceps show a narrow peak in firing probability after cervicomedullary stimulation (32). The peak is similar in width to that seen with activation of biceps motor units through the Ia reflex pathway and thus implies a largely monosynaptic response (Fig. 4A).

Although cervicomedullary stimulation evokes short-latency corticospinal responses, it also activates other pathways. In patients with benign myoclonic epilepsy and enhanced long-loop reflexes, there are longer latency responses attributed to stimulation of ascending tracts (49). Late responses in a hand muscle were evoked at lower stimulus intensities than the usual short-latency CMEPs and had a latency consistent with long-loop reflexes evoked by stimulation of Ia afferents peripherally. No such responses were seen in normal subjects. However, normal subjects do show large synchronous long-latency responses during strong voluntary contractions of the elbow flexor muscles (39). These responses in biceps and brachioradialis (up to 60% of the maximal M wave) are consistent with transcortical reflex responses and can be evoked by brachial plexus stimulation as well as through cervicomedullary stimulation (Fig. 5). Thus stimulation over the brain stem activates both ascending and descending paths. This raises the question of whether an antidromic volley in Ia afferents contributes to the short-latency motor response. Despite the lower threshold of the ascending pathway, occlusion of motor cortical output by CMEPs of a matched size (see above) suggests that the Ia afferents contribute little. An explanation could be that the ascending pathways are stimulated higher in the brain stem than the descending pathways. Although the corticospinal tract may be susceptible to stimulation where its axons bend at the pyramidal decussation, the ascending pathway crosses above its relay in the cuneate nucleus and, if activated there, could not affect the motoneurons antidromically.

When considering other possible contributions to responses to stimulation over the brain stem, it should be remembered that electrical transmastoid stimulation has been used for many years to activate vestibular afferents [galvanic vestibular stimulation (GVS)]. GVS uses much longer pulses and lower currents so the effect of the brief high-voltage “corticomedullary” stimulation on vestibular afferents is unknown. Although 4-mA pulses of <1 ms are known to give no response in the leg muscles (51), much larger currents of 150–250 mA are used to elicit CMEPs. However, the short-latency responses to GVS are small compared with CMEPs and occur later (~50 ms for GVS response in soleus vs. 22 ms for CMEP in tibialis anterior; Refs. 44, 51).

The cerebellum is also stimulated by an electrical pulse passed between the mastoids (or by magnetic stimulation) and with lower intensity pulses than usually required to stimulate.

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**Fig. 3.** Motor evoked potentials (MEP) elicited by transcranial magnetic stimulation (TMS) are occluded by electrical stimulation at the cervicomedullary junction. The first 3 rows show averaged responses (of 3 trials) from biceps brachii in 1 subject. **Top row:** responses to magnetic stimulation of the motor cortex (MEP). **Second row:** responses to cervicomedullary junction stimulation (CMEP). The amplitude of the responses is 10–15% of the maximal M wave. **Third row:** responses evoked when both stimuli were given at 2 different interstimulus intervals (ISI). **Left:** TMS (solid arrow) was given 5 ms before the electrical cervicomedullary junction stimulus (open arrow), and the response is big. **Right:** interval was 3 ms, and the response is small. **Bottom row:** subtraction of the CMEP from the response to both stimuli. If the responses to the stimuli summed linearly, this subtraction would equal the MEP (dashed line trace). Here, there is facilitation at the ~5-ms ISI; at ~3-ms ISI, there is a reduced response, suggesting collision of the MEP with the antidromic volley evoked at the cervicomedullary junction.
of motor response, it does inhibit the motor cortex briefly (from ~5 to 8 ms after the stimulus). Of course, antidromic volleys in the corticospinal axons themselves will also affect motor cortical excitability directly and through recurrent collateral branches (15, 19).

RESPONSES IN THE LEGS

Responses in tibialis anterior and extensor digitorum brevis have been demonstrated with electrical stimulation at the cervicomedullary junction (44, 46). Similar latency responses can be obtained with magnetic stimulation in some subjects but usually only when responses are facilitated by voluntary activity.

Responses in the same muscles can also be obtained with electrical stimulation over the cervical or thoracic spine (7, 24, 28, 44). Although the technique is less painful than cervicomedullary stimulation, it has not been used often. Ugawa et al. (44) stimulated at two levels with the cathode over the spine of T1 or T4 vertebra and the anode 5 cm above it and obtained responses during voluntary contraction. An estimate of synaptic delay of ~0.5 ms points to mono- or oligosynaptic activation of motoneurons (44) and occlusion of small responses to magnetic cortical stimulation by cervical spine stimulation suggests that the spinaly evoked responses have a corticospinal component (24). Other descending and ascending tracts could contribute to larger responses, although the synapse of Ia afferents in Clarke’s column should reduce the likelihood of antidromic Ia volleys adding to the response.

Magnetic stimulation of the cerebral and thoracic cord has not been successful in evoking responses in the legs, although nerve roots can be stimulated at the vertebral foramina and in the cauda equina (e.g., Refs. 22, 23).

USES FOR CMEP IN HUMAN STUDIES

The motor response to corticospinal tract stimulation at the cervicomedullary junction (CMEP) is used 1) in conduction studies to provide an intermediate site of stimulation, 2) to examine directly the behavior of the corticospinal pathway and the motoneuron pool, and 3) as a comparison, to determine whether changes in the cortically evoked motor evoked potential (MEP) are cortical or spinal in origin.

1) In studies of “central” motor conduction, the CMEP provides evidence for the integrity of the corticospinal tract and can be compared with responses evoked from the nerve roots and from the cortex to localize sites of conduction slowing to
above or below the cervicomedullary junction (foramen magnum; Refs. 29, 43, 50).

2) The CMEP reflects a predominantly monosynaptic response of the motoneurons to stimulation of corticospinal axons and thus provides one of the most direct ways to examine the behavior of motoneurons in awake human subjects. This means that motoneurons can be studied during and after tasks that require voluntary cooperation, either because of their complexity or because they involve maximal or strong voluntary contractions. Because activation occurs through the tract that is most important for voluntary movement in humans, the responses may also give insight into how effective descending input to motoneurons is under varied circumstances. The H reflex is also an extremely useful tool for examining motoneuron behavior but suffers the disadvantage that it can be markedly influenced by presynaptic inhibition of the Ia afferents (17). This can make it difficult to assign changes in H-reflex size to alterations in motoneuron excitability. So far, no presynaptic inhibition of corticospinal inputs to the motoneurons has been described (30). CMEPs can also be examined in muscles where H reflexes are difficult or impossible to evoke. In muscles where both responses can be evoked, their behavior can be compared.

3) Because the size of the MEP depends on the excitability at cortical and spinal levels, CMEPs, which are not influenced by cortical excitability, can help identify the level at which change has occurred. That is, if CMEPs do not change with an experimental intervention but MEPs (of the same size) do, the effect is at a cortical level. H reflexes are used similarly as a probe for motoneuronal changes but have the problems discussed above. Responses to transcortical electrical stimulation (TES) are also used because TES tends to activate the corticospinal axons at a cortical level. The response is less influenced by cortical excitability changes than TMS, which activates corticospinal output indirectly through stimulation of the axons of neurons that provide excitatory synaptic input to the corticospinal neurons (20, 35). This comparison works well when small, near-threshold responses are examined; however, with larger responses, the higher intensity TES also activates the corticospinal neurons indirectly. Thus CMEPs are probably the best comparison response for MEPs. They act through many of the same descending axons to recruit the same motoneurons. However, they are not ideal. The single volley of the CMEP may result in quite different motoneuronal responses to the multiple descending volleys of the MEP.

**SOME FINDINGS WITH CMEPS**

**During Voluntary Contraction**

Voluntary activation of the target muscle has a marked effect on the size of the CMEP. In biceps, a stimulus that evokes a CMEP of >50% of the amplitude of the maximal M wave during a maximal voluntary contraction (MVC) can be barely above threshold with the muscle at rest [see Fig. 3 of Taylor et al. (39)]. The amount of facilitation of the CMEP is influenced by the size of the response at rest, the level of voluntary contraction, and the target muscle (41, 47). Comparison of the facilitation of the CMEP by different levels of voluntary contraction in different muscles could elucidate the recruitment of motoneuron pools by voluntary activity; however, no systematic study has yet been performed.

**After Voluntary Contraction**

After an MVC of more than 5-s duration, the CMEP, when tested with the muscle at rest, is depressed by ~50% and recovers over ~1 min (Fig. 6). This depression is similar after MVCs of 5-s to 2-min duration but varies with the strength of a constant duration contraction (more depression after stronger contractions). We attribute the postcontraction depression of...
the CMEP to a decrease in efficacy of the corticospinal-motoneuronal synapse (Ref. 11, 31; see also Ref. 2). The depression relies on corticospinal activation of the motoneurons because it does not follow 30-Hz peripheral nerve stimulation, which activates motoneurons antidromically as well as synaptically through reflex pathways (11). Depression of the CMEP can be seen with testing during weak voluntary contractions but is abolished during strong contractions. This decreased efficacy of synaptic transmission in the corticospinal pathway can affect voluntary movements. After a 10-s MVC, a subject’s performance of low-force voluntary contractions is impaired with reduced EMG, indicating a decrease in recruitment or firing of motor units and consequently reduced force (31).

**During Muscle Fatigue**

During a fatiguing sustained MVC, maximal motor unit firing rates decrease (e.g., Refs. 5, 25, 53). This implies that the motoneurons become less excitable either because of repetitive firing or through changes in afferent or descending input. One hypothesis is that the firing of the group III and IV muscle afferents, which occurs with muscle fatigue, inhibits the motoneurons. Evidence is provided by a continued depression of H-reflex size when the firing of ischemically sensitive muscle afferents is maintained after the end of an MVC by holding the muscle ischemic (e.g., Refs. 12, 13). Testing with CMEPs shows that the motoneurons do become less responsive during a sustained MVC but recover quickly with rest whether or not muscle ischemia is maintained. This suggests that group III and IV muscle afferents do not directly inhibit the motoneurons during fatiguing contractions (6, 40).

**During Contralateral Voluntary Contractions**

Strong voluntary contractions of one arm or leg can affect muscles on the contralateral side of the body. MEPS, CMEPs, and H reflexes can be used together to try to define these effects. If subjects make a strong voluntary contraction of the wrist flexors of one arm while keeping the other arm relaxed, MEPs in the wrist flexors of the relaxed arm double in size. At the same time, the H reflex decreases by ~50%. CMEPs are unchanged (16). The simplest explanation here is that excitability of the motoneuron pool is unchanged, excitability of the cortex is increased, and there is presynaptic inhibition of the Ia afferent input to the H reflex. This presynaptic inhibition only occurred with a voluntary contralateral contraction and took up to 30 s after the contraction to recover.

**CONCLUSION**

Noninvasive stimulation of the corticospinal tract in human subjects can evoke responses in many muscles of the arms and legs. It provides a way to examine the behavior of the corticospinal pathway at a segmental level during conditions ranging from rest to maximal efforts. Large responses make it suitable for looking at brief as well as longer-lasting changes in motoneuron excitability. It is presently the most appropriate comparison to allow interpretation of changes in the size of the MEP. Used together with MEPS and H reflexes, CMEPs can help define complex changes in the human motor pathway.

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**REFERENCES**


