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Brain Stimulation
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Chapter 51

Cerebellum

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INTRODUCTION

Until recently physiological studies of human cerebellar functions were limited methodologically, although the cerebellum was known to play an essential role in movement execution and motor control by modulation of the primary motor cortex (M1) through cerebellothalamocortical connections (Ito, 1984). However, since the introduction of transcranial stimulation techniques, namely transcranial magnetic (TMS) and electrical (TES) stimulation, we are now able to investigate neural networks by stimulating neural structures transcranially and noninvasively in humans.

TMS or TES was first used to estimate the central motor conduction by recording motor evoked potentials (MEPs) to single-pulse TMS of M1. Afterwards they were used to study motor cortical modulation effects by combining conditioning stimuli with TMS of M1. In such cases, MEPs to single-pulse TMS were used to evaluate motor cortical excitability. A conditioning stimulus preceding or succeeding the test stimulus can either facilitate or inhibit the test response depending on the interstimulus interval (ISI) or the stimulus intensity. This technique has been termed paired-pulse stimulation and was introduced to study facilitatory and inhibitory mechanisms within M1 (Kujirai et al., 1993). However, when the conditioning stimulus is applied to a different brain area, the connectivity between the conditioned area and M1 can be studied. Thus, cerebellar conditioning stimulation enables us to study cerebellar regulatory effects on the contralateral M1. In this chapter we describe this cerebellar stimulation technique and its usefulness as research and diagnostic tools in clinical neurophysiology.

ANATOMY OF CEREBELLOCEREBRAL CONNECTION

Functional connectivity between the cerebellum and cerebral cortices has been studied extensively in animals (Allen and Tsukahara, 1974; Holdefer et al., 2000). The cerebellum receives inputs from the cortex mainly through the middle cerebellar peduncle in terms of the corticopontocerebellar pathway, or through the inferior cerebellar peduncle via climbing fibers from the olive in terms of the corticorubroolivocerebellar pathway (Fig. 51.1). The cerebellar efferent pathway consists of projections from the cerebellum to the motor cortex through the disynaptic dentatothalamocortical pathway. Fibers from the dentate nucleus connect to the ventrolateral motor thalamus via the superior cerebellar peduncle. The motor thalamic cells project further to areas 4 and 6 (Fig. 51.1). The dentatothalamocortical pathway itself is facilitatory. However, Purkinje cells of the cerebellar cortex inhibit the dentate nucleus. Therefore, activation of Purkinje cells result in disfacilitation of the motor cortex. These efferent and afferent cerebellar connections together form the frontopontocerebellothalamocortical loop, and include the Guillain–Mollaret triangle (Fig. 51.1).

CEREBELLAR STIMULATION – METHODOLOGY

This technique uses the paired-pulse paradigm to investigate modulatory effects of the cerebellum on the contralateral motor cortex excitability. The test stimulus is a single-pulse TMS of M1 and the evoked MEP is used as an indicator of the motor cortical excitability. In

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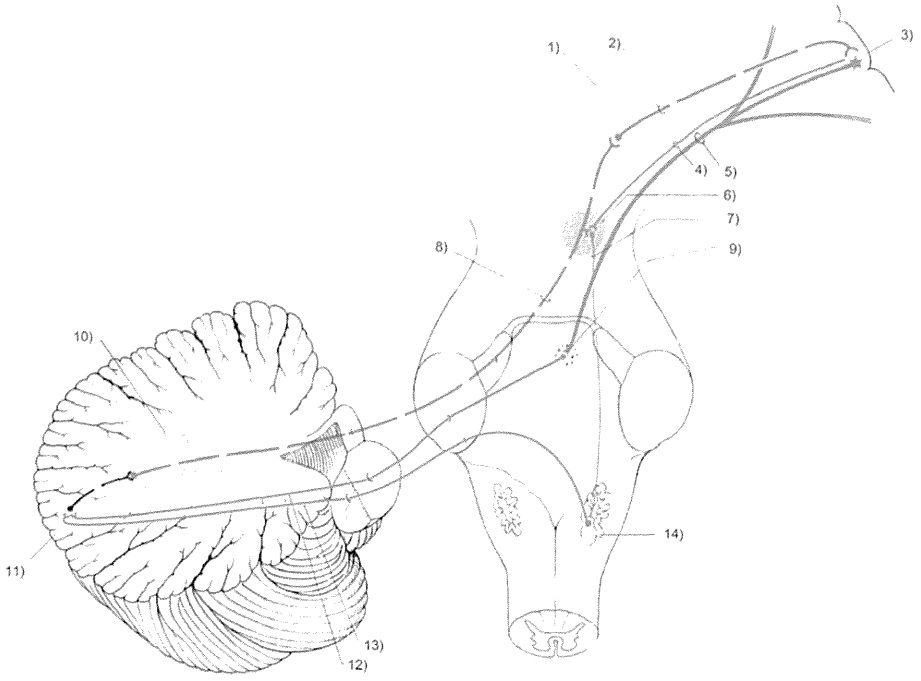

Fig. 51.1. Simplified scheme of the frontopontocerebellothalamocortical loop. Red lines are facilitatory (solid lines: cerebellar afferent pathways; dotted lines: cerebellar efferent pathways); the black line is inhibitory. (1) thalamus, (2) thalamocortical tract, (3) primary motor cortex, (4) corticorubral tract, (5) corticopontine tract, (6) rubral nucleus, (7) rubrotegmental tract, (8) dentatothalamic tract, (9) pontine nuclei, (10) dentate nucleus, (11) cerebellar cortex, (12) pontocerebellar tract, (13) olivocerebellar tract, (14) olive. (Modified from Bähr M, Frotscher M (2009). Neurologisch-topische Diagnostik, 9th edition. Georg Thieme, Stuttgart.)

clinical practice a round coil can be used for M1 TMS. However, for specific research purposes, we usually use a figure-of-eight coil in order to elicit preferentially certain descending volleys. The conditioning stimulus over the cerebellum precedes the test stimulus at certain intervals. We used either TES or TMS for cerebellar stimulation. In the early experiments, TES was chosen because TMS could not sufficiently activate deeper structures such as the cerebellum. However, after invention of the double-cone coil, magnetic cerebellar stimulation was able to be performed effectively and reliably (Ugawa et al., 1995b; Iwata and Ugawa, 2005; Groiss and Ugawa, 2012).

The ability to activate the cerebellum in humans non-invasively offered new scientific perspectives. Accordingly, several investigations have been performed in this research field in humans. In the next paragraph, therefore, we first give a brief overview for the stimulation setting that is usually sufficient for clinical diagnostics. Then, we describe other special issues on cerebellar stimulation in detail afterwards.

Cerebellar magnetic stimulation experiments are recommended to be conducted by two investigators, each of them holding one coil. The test stimulus coil is placed over the vertex to stimulate M1 and its intensity is usually set to elicit MEPs with an amplitude between 0.5 and 1 mV in the target muscle, commonly a small hand muscle such as the first dorsal interosseus (FDI). For the conditioning stimulation, the center of

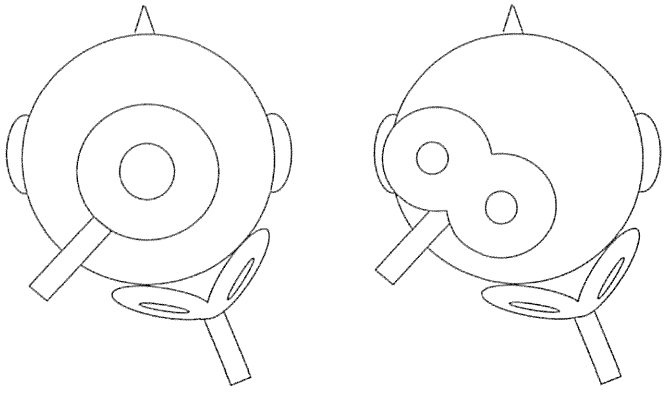

Fig. 51.2. Setup and coil position for cerebellar stimulation experiment. Either a round coil (left) or figure-of-eight coil (right) is used for the motor cortical stimulation. A double-cone coil is positioned over the contralateral cerebellum at about the midpoint between the inion and the posterior mastoid process.

the double-cone coil is placed at the midpoint between the inion and the contralateral mastoid process (Fig. 51.2), and the stimulus intensity is set at 90% of the active brainstem motor threshold (BS-AMT). The current direction is usually chosen to induce upward current in the brain. All stimulation conditions (control condition when the test stimulus is given alone, and paired-pulse conditions when both conditioning and test stimuli are given) should be applied in a randomized order to avoid anticipatory biasing. ISIs of 4–8 ms between conditioning and test stimuli are usually sufficient for clinical investigations.

CEREBELLAR TRANSCRANIAL MAGNETIC STIMULATION IN HEALTHY SUBJECTS

The main cerebellar efferent pathway, the dentatothalamocortical tract, has a facilitatory influence on the contralateral motor cortex (Allen and Tsukahara, 1974). However, when Purkinje cells are activated, the dentatothalamocortical pathway will be inhibited, which results in a disfacilitation of the motor cortex (see Fig. 51.1). Therefore, stimulation of the cerebellum might induce both facilitatory and inhibitory effects on contralateral M1 depending on the activated pathway by the conditioning stimulus. As suppression generally lasts longer and is easier to be detected, the initially observed effect on M1 was solely inhibitory. However, facilitatory effects can also be elicited when certain stimulation parameters, such as stimulus direction and intensity for the conditioning and test stimulus, are all set appropriately.

Cerebellar inhibition

The first study exploring cerebellocortical effects used TES. The electrodes for conditioning cerebellar stimulation were placed at the posterior edge of the mastoids with the anode on the right and the cathode on the left side (Ugawa et al., 1991a). This stimulation site has been proven to be most effective to activate the corticospinal pathways at the level of the pyramidal decussation in humans (Ugawa et al., 1991b). The cerebellar stimulation intensity was 10% below BS-AMT at the level of the pyramidal decussation. The test stimulus was applied to the left M1 with a 9-cm diameter round coil positioned over the vertex and surface electromyogram (EMG) was recorded from the right FDI. Test stimulation intensity was set to elicit EMG responses of 1 mV in the relaxed FDI. Paired stimuli at various ISI ranging from 1 to 15 ms were intermixed randomly with control trials in which the test or conditioning stimulus was applied alone. Cerebellar stimulation at ISIs between 5 and 8 ms significantly suppressed test responses (Fig. 51.3). This phenomenon was termed

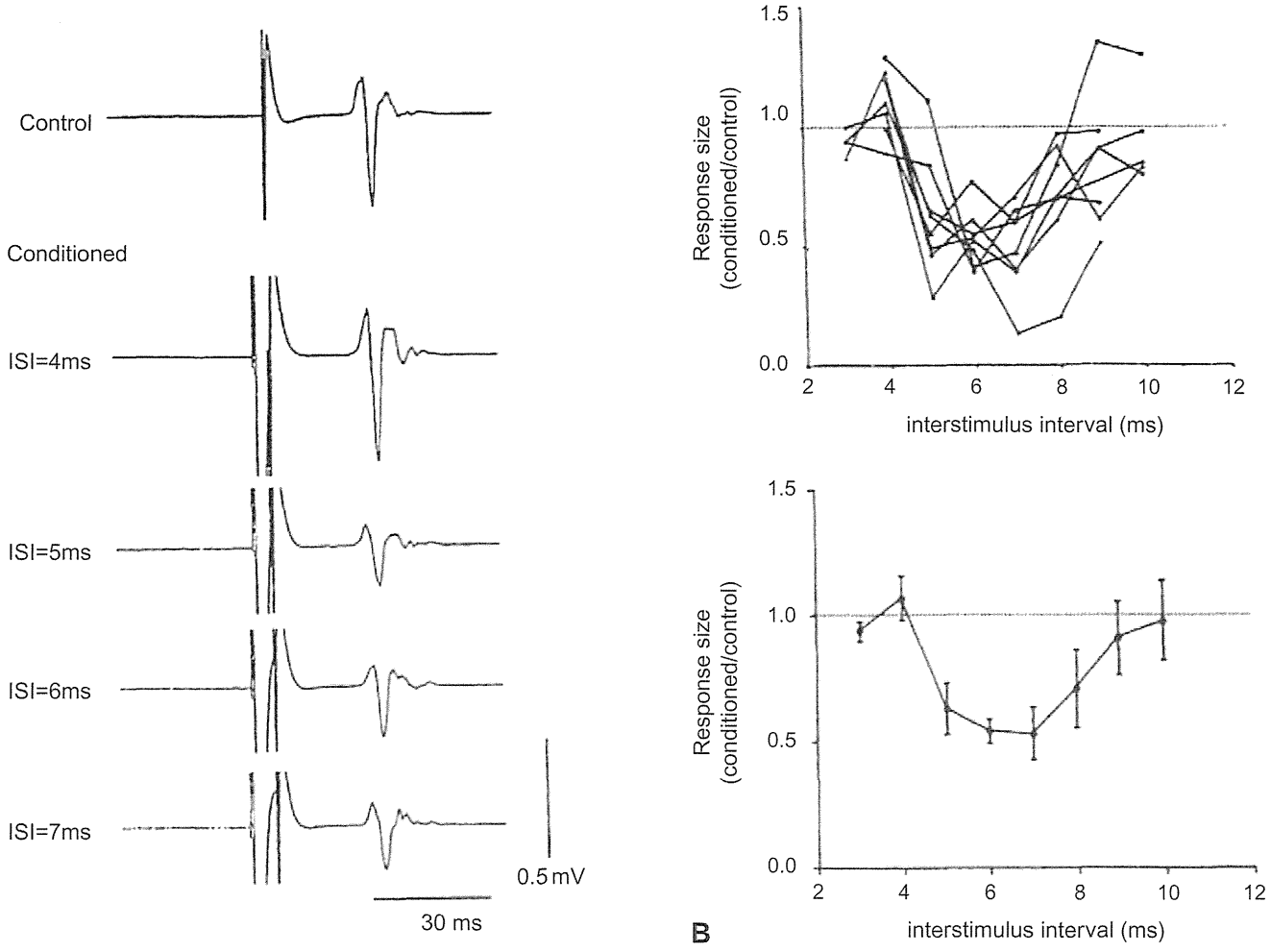

Fig. 51.3. Cerebellar inhibition. (**A**) Representative averaged electromyographic responses from the first dorsal interosseus (FDI) in a healthy subject. Top trace shows the control response to single-pulse transcranial magnetic stimulation (TMS); the lower traces show conditioned responses at interstimulus intervals (ISIs) of between 4 and 7 ms. (**B**) Time courses of cerebellar suppression for single subjects (upper graph) and mean time course (lower graph). Motor evoked potential (MEP) suppression was elicited at 5 ms and lasted for a few milliseconds. (From Ugawa and Iwata, 2005.)

cerebellar inhibition (Ugawa et al., 1991a). The lack of cerebellar inhibition by reversing the TES current directions supports the notion that unilateral cerebellar stimulation actually induced cortical inhibition.

It is well known that electrical or magnetic stimulation of M1 elicits multiple successive descending volleys (Amassian et al., 1987; Day et al., 1989). The initial wave observed is elicited by a direct stimulation of corticospinal neurons and is therefore termed the D-wave. The D-wave is followed by several indirect waves (I-waves) at intervals of about 1.5 ms, which are termed I1-, I2-, and I3-waves, in order of their appearance. The latency difference between D-waves and I3-waves is thus about 4.5 ms. Depending on the current direction induced by TMS, different D- or I-waves are elicited preferentially (Sakai et al., 1997). Posteriorly directed currents in the brain preferentially evoke I3-waves, whereas anteriorly directed currents in the brain preferentially evoke I1-waves. To study which descending volleys are influenced by cerebellar stimulation, a more detailed evaluation was performed by using variously directed test stimulus currents with a figure-of-eight coil placed over M1. Cerebellar inhibition was observed preferentially in I3-waves, peaking at an ISI of 6 and 7 ms, and was not observed in responses to I1-waves (Fig. 51.4) (Ugawa and Iwata, 2005).

Consistently, cerebellar stimulation did not suppress MEPs to TES of M1, which elicits mainly D-waves. These results suggest that cerebellar inhibition act on the target neurons for I3 interneurons in the primary motor cortex, which are probably I2 interneurons. Considering this intracortical delay of about 4–5 ms to activate pyramidal neurons after cortical TMS, cerebellar inhibition observed at ISIs from 6 to 7 ms in fact reflects cortical inhibition 10–11 ms after cerebellar stimulation. This corresponds well to the conduction time mediated transsynaptically from activation of Purkinje cells through the dentatothalamocortical pathway to the contralateral M1 (see Fig. 51.6a).

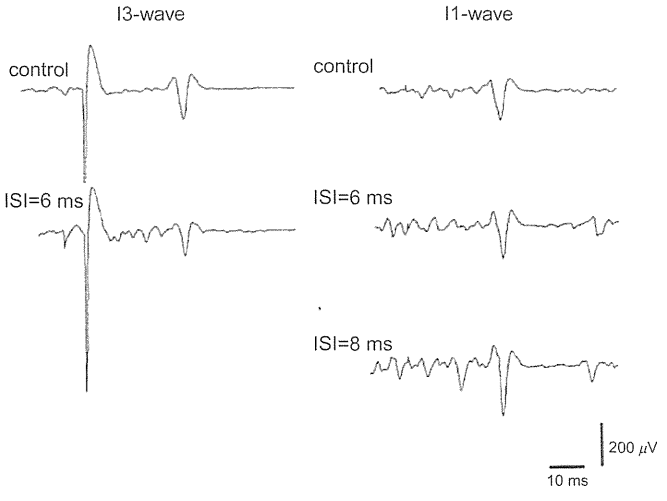

Fig. 51.4. Effects of cerebellar inhibition on I-waves. Top rows show control responses to single-pulse transcranial magnetic stimulation (TMS). Lower rows show small conditioned responses to I3-waves at an interstimulus interval (ISI) of 6 ms (left), whereas I1-waves were not affected at either 6 or 8 ms (right). (From Ugawa and Iwata, 2005.)

These results underline that cerebellar stimulation is highly effective to study cerebellocerebral connections. However, high-voltage TES may be quite painful and thus not appropriate for a clinical routine method. Therefore, with the development of the double-cone coil, which is capable of stimulating deeper structures than common flat coils, TMS was used to replace TES for the conditioning stimulus (Ugawa et al., 1995b).

Conditioning magnetic stimuli were applied at various locations at the back of the head with a double-cone coil. Twelve different positions on the right side were studied for conditioning stimulation. Upward and downward currents directions were also compared. Other stimulation parameters such as intensity or ISI were similar to TES. The test stimulus was applied through a round coil placed over the vertex with an anticlockwise current flow in the brain to stimulate the left M1 optimally. Cerebellar inhibition on the contralateral motor cortex was seen at ISIs between 5 and 7 ms (see Fig. 51.3). Best suppressive effect was observed when the center of the coil was positioned at the midpoint between the inion and the mastoid process contralateral to the stimulated M1, slightly cranial to the foramen magnum level, and an upward current was induced in the brain. However, recent results revealed a considerable interindividual variability of optimal coil placement for brainstem stimulation and suggests careful assessment of the coil position (Shirota et al., 2011). Selection of inappropriate TMS intensity or current directions may activate neighboring structures such as the pyramidal tract in addition to the cerebellum (Ugawa et al., 1991a; Fisher et al., 2009), which necessarily leads to a wrong conclusion. Therefore, the position of the double-cone coil, the current direction and the conditioning stimulus intensity have to be chosen very carefully, and adapted individually to minimize inadvertent stimulation of adjacent structures (Ugawa et al., 1991a; Ugawa, 2009). In conclusion, if performed carefully, magnetic cerebellar stimulation is able to evoke the cerebellar inhibitory effects on contralateral M1 with significantly less pain than the electrical cerebellar stimulation. These results indicate clinical utility of magnetic cerebellar stimulation.

Cerebellar facilitation

In some individuals a slight facilitation was observed before the onset of cerebellar inhibition. As the dentatothalamocortical pathway itself is of facilitatory nature,

detailed experiments were conducted to explore whether or not facilitation could be constantly evoked in humans. The cerebellar stimulation effects on M1 were compared among several stimulation parameters, such as the stimulation intensity, ISIs, and current direction. When the conditioning stimulus intensity was set at just below AMT to activate the corticospinal tract, a short but significant facilitation was elicited at an ISI of 3 ms that lasted for less than 2 ms (Fig. 51.5A) (Iwata et al., 2004). Again, only responses to I3-waves were facilitated; I1-waves were not affected (Fig. 51.5B). The position and polarity of the conditioning stimulus were the same as for cerebellar inhibition, suggesting that facilitation is also produced by activation of the cerebellum. The shorter interval for the cerebellar facilitation suggests a direct activation of the dentate nucleus or its fibers (superior cerebellar peduncle). Considering the intracortical delay of about 4–5 ms to activate pyramidal neurons after cortical TMS, cerebellar facilitation which is observed at an ISI of 3 ms has to reflect a cortical facilitation about 7–8 ms after the conditioning stimulus, which matches the disynaptic conduction time via the dentatothalamocortical pathway (Fig. 51.6B) (Iwata et al., 2004). In conclusion, cerebellar facilitatory effects on the contralateral M1 may usually be masked but can be studied when current direction and the stimulation intensity of both conditioning and test stimulus are carefully adjusted.

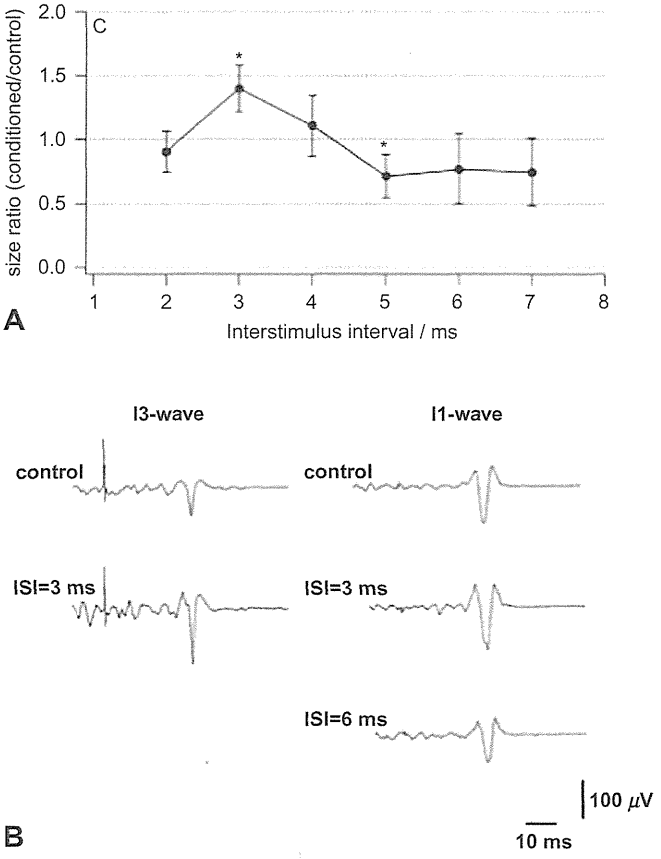

Fig. 51.5. Cerebellar facilitation. (**A**) Mean time course of electromyographic responses. Cerebellar facilitation was evoked at an interstimulus interval (ISI) of 3 ms, whereas inhibition was evoked at an ISI of 5 ms. (From Iwata et al., 2004.) (**B**) Effects of cerebellar facilitation on I-waves. Top rows show control responses to single-pulse transcranial magnetic stimulation (TMS). Lower rows show increased conditioned responses to I3-waves at an ISI of 3 ms (left), whereas I1-waves were not affected at either 3 or 6 ms (right). (From Ugawa and Iwata, 2005.)

CEREBELLAR INHIBITION IN NEUROLOGICAL DISORDERS

Studies on cerebellar inhibition in neurological patients have been important from both methodological and clinical point of view. They clearly confirmed that the effect was mediated effectively by the cerebellothalamocortical pathway, as we will illustrate in more detail in the following section. But what new information can this technique add to clinical neurology? We are not able to determine the exact location of a lesion in ataxic patients based on neurological examination alone, as cerebellar ataxia may be caused by a lesion anywhere within the frontopontocerebellothalamocortical loop. Furthermore, differentiation of cerebellar ataxia from sensory ataxia can sometimes be challenging. Therefore, cerebellar inhibition, which is supposed to reflect functions of the cerebellar efferent pathway, may be useful clinically to differentiate cerebellar efferent ataxia from other, afferent forms of ataxia (Ugawa and Iwata, 2005). Table 51.1 gives a brief overview of the findings of cerebellar inhibition in patients with various neurological disorders.

Cerebellar ataxia

Cerebellar ataxia can develop after a lesion anywhere in the frontopontocerebellothalamocortical loop. Patients presenting with limb ataxia and other clinical cerebellar signs due to different focal lesions and etiologies were studied with cerebellar stimulation. Diseases typically affecting the cerebellar cortex are degenerative disorders such as cerebellar cortical atrophy (CCA), spinocerebellar ataxia (SCA), especially type 6, which often presents with pure cerebellar symptoms, or multiple system atrophy (MSA), especially the cerebellar type. Other diseases affecting the neocerebellum are cerebellar stroke, paraneoplastic diseases such as cerebellitis and paraneoplastic CCA, or intoxication of antiepileptic drugs. All of these diseases showed impaired cerebellar inhibition (Fig. 51.7) (Ugawa et al., 1994b, c, 1997). Similarly, involvement of the dentate nucleus or superior cerebellar peduncle in dentatorubral–pallidoluysian atrophy or Wilson's disease demonstrated abnormal cerebellar inhibition (Ugawa et al., 1997). In contrast,

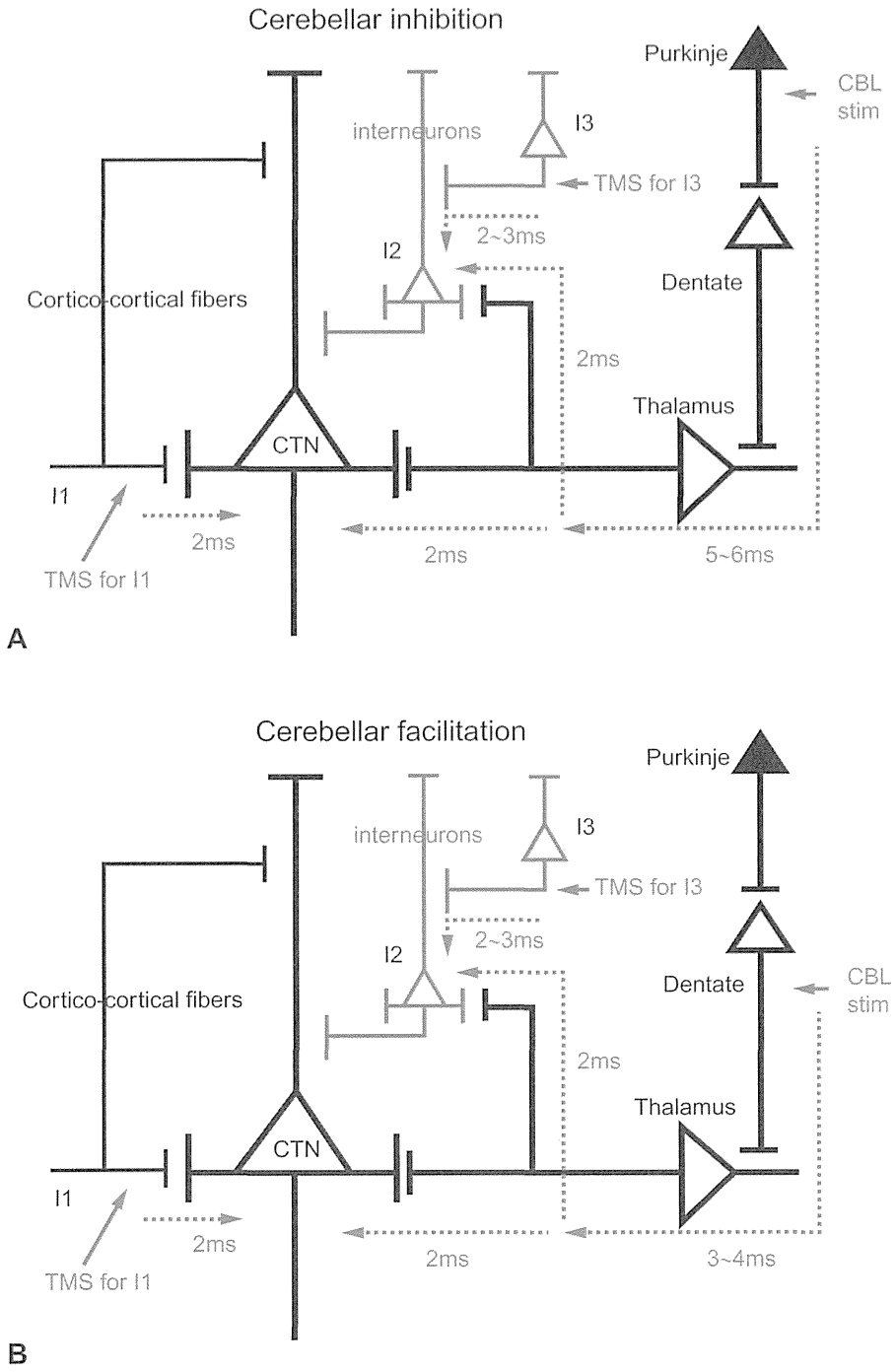

Fig. 51.6. Model of the interaction between cerebellocortical modulation and the cortical interneuronal network for (**A**) cerebellar inhibition and (**B**) cerebellar facilitation. Cerebellar inhibition is supposed to activate Purkinje cells, whereas cerebellar facilitation activates the dentate nucleus. The intracortical delay of 4–5 ms to activate corticospinal tract neurons (CTN) after single-pulse transcranial magnetic stimulation (TMS) of M1 explains the rather short interstimulus interval (ISI) of 5–7 ms for cerebellar inhibition and 3 ms for cerebellar facilitation. CBL, cerebellum. (From Iwata et al., 2004, by kind permission of Springer Science and Business Media.)

ataxic patients with cerebellar afferent pathway involvement (pontine or middle cerebellar peduncular lesions) had normal cerebellar inhibition, although they all showed definite clinical signs indicative of cerebellar ataxia (Ugawa et al., 1995a, 1997). These results confirm that this method truly stimulates the cerebellum. Moreover, it underlines that cerebellar stimulation is able to differentiate lesions at the cerebellum or cerebellar efferent pathways from those at cerebellar afferent pathways.

Cerebellar stimulation in ataxic hemiparesis

For the differential diagnosis of cerebellar symptoms, cerebral vascular diseases (CVDs) are of particular importance, because the cerebellum is often affected

Table 51.1

Cerebellar inhibition in different neurological disorders

Normal cerebellar inhibition	Abnormal (reduced or absent) cerebellar inhibition
No ataxia • Healthy • Parkinson's disease • Motor neuron disease Noncerebellar ataxia • Peripheral nervous system (paraneoplastic sensory neuropathy, Sjögren syndrome) • Spinal tract (tabes dorsalis) • Sensory thalamus • Location unknown (Miller–Fisher–syndrome, hypothyroidism) Cerebellar ataxia (cerebellar *afferent* pathway) • Frontal ataxia (motor cortices, vascular) • Pontine nuclei (vascular) • Medial cerebellar peduncle	Cerebellar ataxia (cerebellar *efferent* pathway) • Neocerebellum a. Degenerative (CCA, spinocerebellar atrophy, multiple system atrophy, dentatorubral-pallidoluysian atrophy) b. Vascular c. Paraneoplastic cerebellitis/CCA d. Intoxication • Dentate nucleus (Wilson's disease, progressive nuclear palsy) • Superior cerebellar peduncle (progressive nuclear palsy) • Motor thalamus (vascular)

CCA, cerebellar cortical atrophy

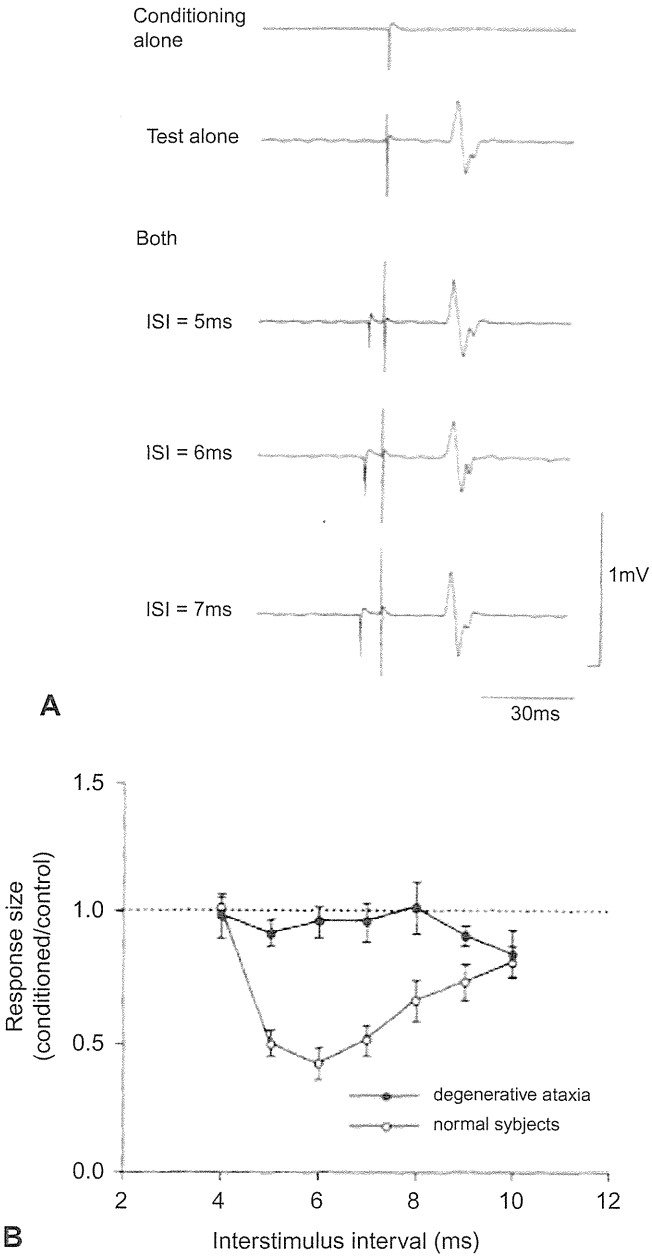

Fig. 51.7. Lack of cerebellar inhibition in patients with degenerative ataxia. (**A**) Representative averaged electromyographic responses from the first dorsal interosseus (FDI) in a patient with degenerative ataxia. Top trace shows the control response to single-pulse transcranial magnetic stimulation (TMS); the lower traces show conditioned responses at interstimulus intervals (ISIs) of between 5 and 7 ms. (**B**) Mean time course of cerebellar suppression for control subjects and patients with degenerative ataxia. No suppression was elicited at any ISIs in the patient group. (From Ugawa et al., 1994c.)

by CVD. Ataxic hemiparesis, for instance, is a lacunar syndrome with ataxia accompanying ipsilateral corticospinal tract impairment. In these patients, ataxia may result from a small lesion anywhere within the frontopontocerebellothalamocortical loop.

We have recently reported on three patients with ataxic hemiparesis, whose lesions were functionally located by cerebellar stimulation (Kikuchi et al., 2012). Consistent with the diagnostic criteria of ataxic hemiparesis, all three patients clinically showed acute-onset ataxia with ipsilateral pyramidal signs, dysmetria disproportional to weakness and minimal to absent cortical signs.

A patient with a small infarction in the posterior limb of the internal capsule had reduced cerebellar inhibition. The posterior limb of the internal capsule contains the corticopontine tract at its anterior portion and the thalamocortical tract at its posterior part. Magnetic resonance imaging (MRI) demonstrated morphologically a lesion at the posterior part of the posterior limb of the internal capsule which suggested a lesion of thalamocortical fibers. This morphological finding fits well with the functional finding of an abnormal cerebellar inhibition (Fig. 51.8).

In the second case, MRI showed a lesion in the lateral thalamus and the cerebellar inhibition was again reduced. The lateral thalamus is the main relay station within the cerebellothalamocortical pathway (the cerebellar efferent pathway) (Fig. 51.8). The morphological finding of cerebellar efferent pathway involvement is again consistent with the functional finding of reduced cerebellar inhibition.

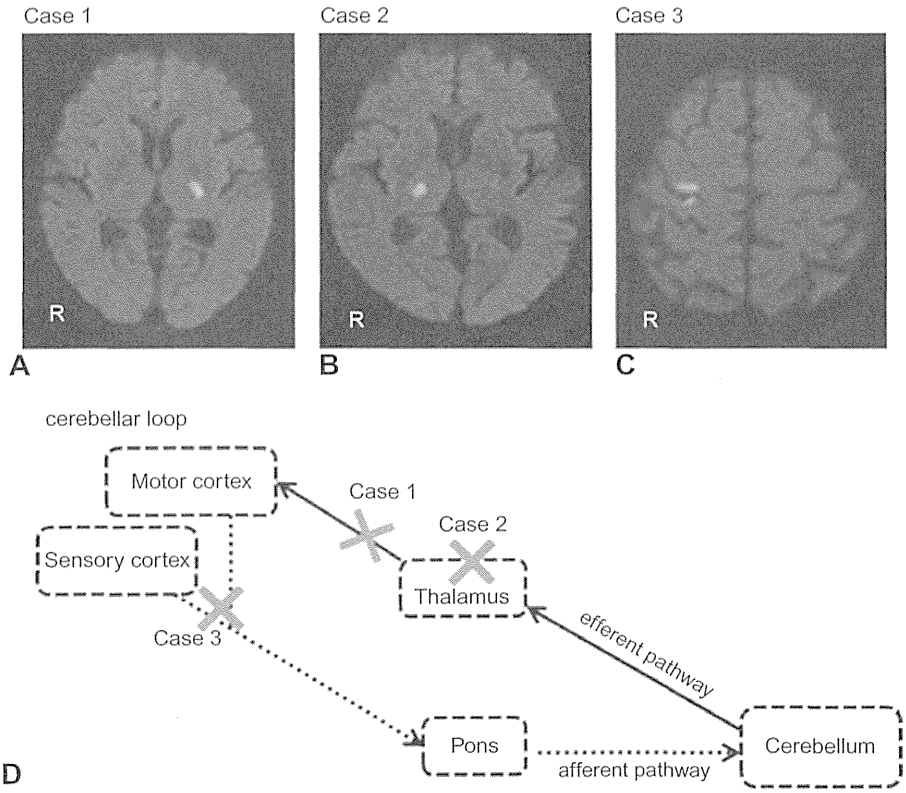

Fig. 51.8. Three exemplary cases with ataxic hemiparesis. Diffusion-weighted magnetic resonance images (upper row) show lesions of the posterior limb of the internal capsule (**A**), motor thalamus (**B**), and sensorimotor cortex (**C**) in the three patients. (**D**) A simplified scheme of the frontopontocerebellothalamocortical loop with the site of lesion in each of the cases. Solid lines indicate the cerebellar efferent pathway and dotted lines the cerebellar afferent pathway. (From Kikuchi et al., 2012, by kind permission of Springer Science and Business Media.)

Normal cerebellar inhibition was elicited in a patient with clinical ataxia due to a lesion at the sensorimotor cortex. As both cerebellar efferent and afferent pathways converge at M1, such a lesion might affect either of them. Which pathway is involved might not always be predictable based on MRI findings. However, normal cerebellar inhibition strongly suggests that the lesion involved only the cerebellar afferent pathway (Fig. 51.8).

Taken together, we conclude that investigations of the cerebellar inhibition not only confirm the knowledge obtained from clinical and anatomical data, but also allow a more differentiated functional focusing in ataxia (Kikuchi et al., 2012).

Noncerebellar ataxia

Clinical ataxia may also be caused by affection of the sensory system. A group of patients with sensory ataxia resulting from sensory neuropathy, spinal tract involvement by tabes dorsalis, or sensory thalamic infarction all had normal cerebellar inhibition (Ugawa et al., 1994b). Furthermore, patients with ataxia of unknown origin, such as Miller–Fisher syndrome and hypothyroidism, also had normal cerebellar inhibition (Ugawa et al., 1994a, 1997; Ugawa and Iwata, 2005). These results also underline the high utility of cerebellar TMS in clinical differential diagnosis.

Parkinsonian syndromes

Patients with idiopathic Parkinson's disease without ataxia show normal cerebellar inhibition (Ugawa et al., 1994b, 1997). Progressive supranuclear palsy (PSP) is a kind of parkinsonian syndrome characterized by supranuclear gaze palsy and postural instability. Patients usually do not show clinical ataxic signs despite frequent pathological cerebellar involvement. However, patients with PSP had significantly reduced cerebellar inhibition (Shirota et al., 2010). This indicates that cerebellar dysfunction may be masked by rigidity or akinesia in patients with PSP. Cerebellar TMS possibly unmasks cerebellar dysfunction in PSP.

Focal dystonia

Recently, cerebellar structural abnormalities were found in patients with dystonia, leading to the idea that the cerebellum might play some role in the pathophysiology of dystonia. Brighina and colleagues (2009) investigated the cerebellothalamocortical pathways in focal

task-specific dystonia, namely patients with writer's cramp and musician's dystonia. Compared with an age-, sex-, and handedness-matched control group, patients with focal dystonia showed significantly reduced cerebellar inhibition. Interestingly, abnormal cerebellar inhibition was found not only on the affected but also on the unaffected side. The authors concluded that Purkinje cell dysfunction might affect the inhibitory drive to the dentatothalamocortical pathways, although the functional role of the cerebellum remains to be determined in the pathophysiology of dystonia (Brighina et al., 2009).

OTHER INVESTIGATIONS

Cerebellar repetitive TMS

A handful of studies have investigated the effects of cerebellar repetitive TMS (rTMS) on contralateral motor cortical excitability, and also behavioral changes in healthy subjects and patients with neuropsychiatric diseases. The results so far have been inconsistent. The first two studies showed an increase of MEP amplitude up to 20 minutes after 1-Hz rTMS, which usually induces suppressive aftereffects. Therefore, suppression of Purkinje cells has been supposed to be induced by cerebellar low-frequency rTMS (Oliveri et al., 2005; Fierro et al., 2007a). Consistently, cathodal transcranial direct current stimulation, which also reduces excitability, leads to a lasting inhibition of cerebellar inhibition although it did not modulate MEP amplitudes elicited by single pulse TMS of M1 (Galea et al., 2009). However, a subsequent study using theta-burst stimulation (TBS) showed contrasting results: intermittent TBS (iTBS), which induces potentiation effects, enhanced MEP amplitudes, whereas continuous TBS (cTBS), which induces suppressive effects, decreased MEP amplitudes (Koch et al., 2008). As iTBS and cTBS also modified short- (SICI) and long-interval (LICI) intracortical inhibition in the opposite directions, it was speculated that TBS might modify γ-aminobutyric acid (GABA)ergic interneurons (Koch et al., 2008). These rTMS studies used a normal figure-of-eight coil in contrast to the double-cone coil used in single-pulse cerebellar stimulation experiments. This difference may explain the above inconsistent results of rTMS experiments. The mechanism of action of cerebellar rTMS is still unclear and needs to be evaluated in further investigations.

Cerebellar rTMS and time perception

Two studies investigated effects of low-frequency cerebellar rTMS on time perception in healthy humans (Fierro et al., 2007b; Koch et al., 2007). The time perception at either millisecond or second range was investigated after 1-Hz rTMS over the lateral cerebellum with a figure-of-eight coil. Both studies found that 1-Hz rTMS over the right or both cerebellar hemispheres resulted in an impairment of millisecond time perception. The results were consistent with an important cerebellar role in time perception demonstrated by neuroimaging studies (Jueptner et al., 1995; Smith et al., 2003). As the aftereffects of 1-Hz rTMS are supposed to be LTD-like plasticity, the authors argued that artificial LTD by 1-Hz rTMS might have interfered with the physiological LTD normally induced by Purkinje cells in time perception tasks (Koch et al., 2007).

Cerebellar TMS and oculomotor function

It is well known that the cerebellum also plays an essential role in oculomotor control. Several studies have investigated effects of TMS on smooth pursuit and saccadic eye movements. Single-pulse TMS over 7 mm lateral and caudal to the inion corresponding to the posterior vermis resulted in hypermetria ipsilateral to the stimulation side during saccades (Hashimoto and Ohtsuka, 1995), accelerated ipsiversive smooth-pursuit eve movements, and decelerated contraversive smooth-pursuit eye movements (Ohtsuka and Enoki, 1998). The authors concluded that the posterior vermis might be involved in the control of both the accuracy of visually guided saccades and smooth-pursuit velocity in a direction-selective manner, consistent with earlier observations in monkeys.

In healthy volunteers, Jenkinson and Miall (2010) studied the saccadic eye movement adaptation after rTMS over the posterior cerebellum. Saccade adaptation was significantly impaired by rTMS over the cerebellum. These results may provide direct evidence for the function of the cerebellar vermis in saccade adaptation in humans (Jenkinson and Miall, 2010).

In contrast to the oculomotor function of the cerebellar vermis, a recent study addressed the question whether the cerebellar hemispheres are also involved in oculomotor function (Panouillères et al., 2012). MRI-guided single-pulse TMS applied to the lateral cerebellum after saccade detection and before the adaptation phase reduced saccade accuracy. However, single-pulse TMS applied during the adaptation phase revealed bidirectional effects on saccadic plasticity. Adaptation aftereffects showed a potentiation of the adaptive lengthening and a depression of the adaptive shortening of saccades (Panouillères et al., 2012). The authors concluded that the cerebellar hemisphere must be involved in both saccade accuracy and saccadic adaptation controls in humans.

METHODOLOGICAL LIMITATIONS

This method is limited to study of the upper limb muscles only. Truncal and lower limb ataxia cause serious gait disturbances or clinical burdens. Unfortunately,

cerebellar inhibition of M1 for lower limb or truncal muscles has not been performed successfully.

Another issue is a technical problem. Cerebellar stimulation sometimes induces antidromic pyramidal tract coactivation when a suprathreshold stimulus is used. This coactivation may affect the cerebellar stimulation experiments (Ugawa et al., 1991a; Fisher et al., 2009; Ugawa, 2009). However, when the threshold is carefully defined using rectified EMG (Hanajima et al., 2007), and current direction and stimulation site are chosen accurately (Shirota et al., 2011), cerebellar TMS has been shown to be a powerful and reliable method to investigate cerebellar function in humans noninvasively (Ugawa, 2009).

SUMMARY, OUTLOOK, AND PERSPECTIVES

Taking all these results together, cerebellar TMS can be regarded as an effective and valuable method to evaluate the cerebellothalamocortical loop function in humans and may be useful for pathophysiological analysis of ataxia. Moreover, growing evidence suggest that cerebellar stimulation might be an unique and valuable tool for studying the cerebellar role in other complex functions such as oculomotor function or higher cognitive functions.

References

Allen GI, Tsukahara N (1974). Cerebrocerebellar communication systems. Physiol Rev 54: 957–1006.

Amassian VE, Stewart M, Quirk GJ et al. (1987). Physiological basis of motor effects of a transient stimulus to cerebral cortex. Neurosurgery 20: 74–93.

Brighina F, Romano M, Giglia G et al. (2009). Effects of cerebellar TMS on motor cortex of patients with focal dystonia: a preliminary report. Exp Brain Res 192: 651–656.

Day BL, Dressler D, Maertens de Noordhout A et al. (1989). Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. J Physiol 412: 449–473.

Fierro B, Giglia G, Palermo A et al. (2007a). Modulatory effects of 1 Hz rTMS over the cerebellum on motor cortex excitability. Exp Brain Res 176: 440–447.

Fierro B, Palermo A, Puma A et al. (2007b). Role of the cerebellum in time perception: a TMS study in normal subjects. J Neurol Sci 263: 107–112.

Fisher KM, Lai HM, Baker MR et al. (2009). Corticospinal activation confounds cerebellar effects of posterior fossa stimuli. Clin Neurophysiol 120: 2109–2113.

Galea JM, Jayaram G, Ajagbe L et al. (2009). Modulation of cerebellar excitability by polarity-specific noninvasive direct current stimulation. J Neurosci 29: 9115–9122.

Groiss SJ, Ugawa Y (2012). Cerebellar stimulation in ataxia. Cerebellum 11: 440–442.

Hanajima R, Wang R, Nakatani-Enomoto S et al. (2007). Comparison of different methods for estimating motor threshold with transcranial magnetic stimulation. Clin Neurophysiol 118: 2120–2122.

Hashimoto M, Ohtsuka K (1995). Transcranial magnetic stimulation over the posterior cerebellum during visually guided saccades in man. Brain 118: 1185–1193.

Holdefer RN, Miller LE, Chen LL et al. (2000). Functional connectivity between cerebellum and primary motor cortex in the awake monkey. J Neurophysiol 84: 585–590.

Ito M (1984). Cerebellum and Neural Control. Raven Press, New York.

Iwata NK, Ugawa Y (2005). The effects of cerebellar stimulation on the motor cortical excitability in neurological disorders: a review. Cerebellum 4: 218–223.

Iwata NK, Hanajima R, Furubayashi T et al. (2004). Facilitatory effect on the motor cortex by electrical stimulation over the cerebellum in humans. Exp Brain Res 159: 418–424.

Jenkinson N, Miall RC (2010). Disruption of saccadic adaptation with repetitive transcranial magnetic stimulation of the posterior cerebellum in humans. Cerebellum 9: 548–555.

Jueptner M, Rijntjes M, Weiller C et al. (1995). Localization of a cerebellar timing process using PET. Neurology 45: 1540–1545.

Kikuchi S, Mochizuki H, Moriya A et al. (2012). Ataxic hemiparesis: neurophysiological analysis by cerebellar transcranial magnetic stimulation. Cerebellum 11: 259–263.

Koch G, Oliveri M, Torriero S et al. (2007). Repetitive TMS of cerebellum interferes with millisecond time processing. Exp Brain Res 179: 291–299.

Koch G, Mori F, Marconi B et al. (2008). Changes in intracortical circuits of the human motor cortex following theta burst stimulation of the lateral cerebellum. Clin Neurophysiology 119: 2559–2569.

Kujirai T, Caramia MD, Rothwell JC et al. (1993). Corticocortical inhibition in human motor cortex. J Physiol 471: 501–519.

Ohtsuka K, Enoki T (1998). Transcranial magnetic stimulation over the posterior cerebellum during smooth pursuit eye movements in man. Brain 121: 429–435.

Oliveri M, Koch G, Torriero S et al. (2005). Increased facilitation of the primary motor cortex following 1 Hz repetitive transcranial magnetic stimulation of the contralateral cerebellum in normal humans. Neurosci Lett 376: 188–193.

Panouillères M, Neggers SFW, Gutteling TP et al. (2012). Transcranial magnetic stimulation and motor plasticity in human lateral cerebellum: dual effect on saccadic adaptation. Hum Brain Mapp 33: 1512–1525.

Sakai K, Ugawa Y, Terao Y et al. (1997). Preferential activation of different I waves by transcranial magnetic stimulation with a figure-of-eight-shaped coil. Exp Brain Res 113: 24–32.

Shirota Y, Hamada M, Hanajima R et al. (2010). Cerebellar dysfunction in progressive supranuclear palsy: a transcranial magnetic stimulation study. Mov Disord 25: 2413–2419.

Shirota Y, Hanajima R, Hamada M et al. (2011). Inter-individual variation in the efficient stimulation site for magnetic brainstem stimulation. Clin Neurophysiol 122: 2044–2048.

Smith A, Taylor E, Lidzba K et al. (2003). A right hemispheric frontocerebellar network for time discrimination of several hundreds of milliseconds. Neuroimage 20: 344–350.

Ugawa Y (2009). Can we see the cerebellar activation effect by TMS over the back of the head? Clin Neurophysiol 120: 2006–2007.

Ugawa Y, Iwata NK (2005). Cerebellar stimulation in normal subjects and ataxic patients. In: M Hallett, S Chokroverty (Eds.), Magnetic Stimulation in Clinical Neurophysiology. 2nd edn. Elsevier, Amsterdam, pp. 197–210.

Ugawa Y, Day BL, Rothwell JC et al. (1991a). Modulation of motor cortical excitability by electrical stimulation over the cerebellum in man. J Physiol 441: 57–72.

Ugawa Y, Rothwell JC, Day BL et al. (1991b). Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. Ann Neurol 29: 418–427.

Ugawa Y, Genba-Shimizu K, Kanazawa I (1994a). Suppression of motor cortical excitability by electrical stimulation over the cerebellum in Fisher's syndrome. J Neurol Neurosurg Psychiatry 57: 1275.

Ugawa Y, Genba-Shimizu K, Rothwell JC (1994b). Suppression of motor cortical excitability by electrical stimulation over the cerebellum in ataxia. Ann Neurol 36: 90–96.

Ugawa Y, Hanajima R, Kanazawa I (1994c). Motor cortex inhibition in patients with ataxia. Electroencephalogr Clin Neurophysiol 93: 225–229.

Ugawa Y, Terao Y, Nagai C et al. (1995a). Electrical stimulation of the cerebellum normally suppresses motor cortical excitability in a patient with ataxia due to a lesion of the middle cerebellar peduncle. Eur Neurol 35: 243–244.

Ugawa Y, Uesaka Y, Terao Y et al. (1995b). Magnetic stimulation over the cerebellum in humans. Ann Neurol 37: 703–713.

Ugawa Y, Terao Y, Hanajima R et al. (1997). Magnetic stimulation over the cerebellum in patients with ataxia. Electroencephalogr Clin Neurophysiol 104: 453–458.

病態解明・新規治療を目指した神経疾患の患者レジストリシステム　第2回

痙性対麻痺：JASPAC

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第2回

痙性対麻痺：JASPAC

瀧山嘉久*

はじめに

遺伝性痙性対麻痺（hereditary spastic paraplegia：HSP）は，臨床的に緩徐進行性の下肢痙縮と筋力低下を主徴とし，病理学的に脊髄の錐体路，後索，脊髄小脳路の系統変性を主病変とする神経変性症候群である。家族性痙性対麻痺（familial spastic paraplegia：FSP）やシュトリンペル・ロラン症候群と呼ばれることもある。最近では，「遺伝性」を明示するために，遺伝性痙性対麻痺と呼ばれることが多い。

随伴症状の有無により，純粋型（pure form）と複合型（complicated form）に分けられ，前者は通常，痙性対麻痺のみを呈するが，時に膀胱直腸症状，振動覚低下，上肢の腱反射亢進を伴うことがある。後者はニューロパチー，小脳性運動失調，脳梁の菲薄化，精神発達遅延，痙攣，難聴，網膜色素変性症，魚鱗癬などを伴う[1,2]。

遺伝形式からは，常染色体優性（AD-HSP），常染色体劣性（AR-HSP），X連鎖性（XL-HSP）に分けられる。頻度としては，AD-HSPが多く，AR-HSPは少なく，XL-HSPは稀である。純粋型はAD-HSPにおいて一般的であり，複合型はAR-HSPやXL-HSPに認められやすい。

従来はHarding[3]が提唱した臨床像と遺伝形式からみた分類法が受け入れられていたが，今日では原因遺伝子座あるいは原因遺伝子そのものが発見された順に，遺伝形式とは関係なくナンバリングされた分子遺伝学的分類（SPG1～SPG72）がなされている。最近のゲノム解析技術の発達により，今後も新たな原因遺伝子が次々と同定されるものと思われる。

欧米のHSPは，4.3～9.8/10万人の有病率であるとされている[4]。1988年から1989年にかけてのHirayamaら[5]の疫学調査によれば，わが国では0.2/10万人の有病率であると推定されている。また，2008年Tsujiら[6]は，特定疾患の臨床調査個人票の解析から10,487人の脊髄小脳変性症患者の4.7%をHSPが占めると報告している。しかし，これまで，わが国のHSPの実態は不明であり，希少疾患であるためにHSPにはなかなか光が当てられなかった。

本稿では，わが国のHSPについてその分子疫学と病態の解明，および治療法の開発を目的としたプロジェクトである，Japan Spastic Paraplegia Research Consortium（JASPAC）について紹介する。

JASPACの構築

2006年，厚生労働科学研究費補助金難治性疾患克服研究事業　運動失調に関する調査研究班（西澤正豊班長）により，これまで系統的な全国調査がほとんど行われてこなかったわが国のHSPに対して，全国調査とゲノム解析をリンクさせた多施設共同研究体制であるJASPACを立ち上げた（その後，佐々木秀直班長，水澤英洋班長のもとで活動を継続している。事務局は設立当時の自治医科大学神経内科から山梨大学神経内科に異動した）[7]。

JASPACの目的は，全国的なゲノムリソースの収集

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神経疾患の患者レジストリシステム

を行い，大規模ゲノム解析により現在実現し得る最良の遺伝子診断サービスを提供するとともに，わが国のHSPの分子疫学と自然歴を明らかにすること，そして将来的に多くの研究者に幅広く活用されるシステムとしてHSPの病態機序の解明と治療法の開発を目指すことである。

2006～2007年にかけて，最初の活動として全国の神経内科医が常勤している日本神経学会教育施設，教育関連施設と日本小児神経学会評議員の常勤している施設の計1,231カ所を対象に痙性対麻痺患者数，遺伝形式，遺伝子解析希望の有無などのHSPでは初めてと思われるアンケート調査を行った[8]。その結果，565施設から回答（回収率45.9%）があり，204施設が痙性対麻痺患者の診療を行っていた。患者数は計691例で，AD-HSP 220例，AR-HSP 94例，XL-HSP 4例，遺伝形式不明3例，孤発性370例であった。204施設のうち117施設が今後の遺伝子解析を希望しており，その需要が大きいことを確認した。

JASPACの登録システム

JASPACの登録の流れをFig. 1に示す。現在は遺伝歴のある痙性対麻痺症例を対象としているが，そのような患者が遺伝子診断を希望する場合には，主治医がまず事務局へ連絡をする（E-mail：jaspac-med@yamanashi.ac.jp）。連絡を受けた事務局から，①遺伝子解析研究への協力のお願いと説明文書（患者用），②遺伝子解析研究への協力についての同意書，③患者情報提供書（Fig. 2）の3つの書類がEメールにて送られる。次に，検査業務を委託しているエスアールエル社から採血用スピッツ（7 mL管2本）と患者匿名化番号が記載されたJASPAC用伝票が届く。採血終了後，検体と伝票をエスアールエル社が回収する。主治医は書類②と家系図を郵送で，書類③と頭部MRI画像をEメールで事務局へ送る。遺伝子解析が終了すれば，事務局から解析結果が郵送される。

2014年4月4日現在，全国46都道府県202施設からHSP 568家系が登録され，インデックス症例448検体がJASPACに集められている。

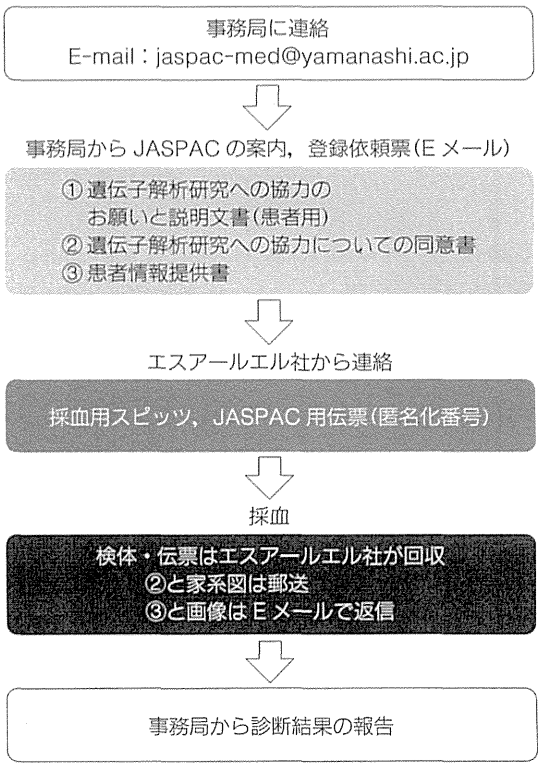
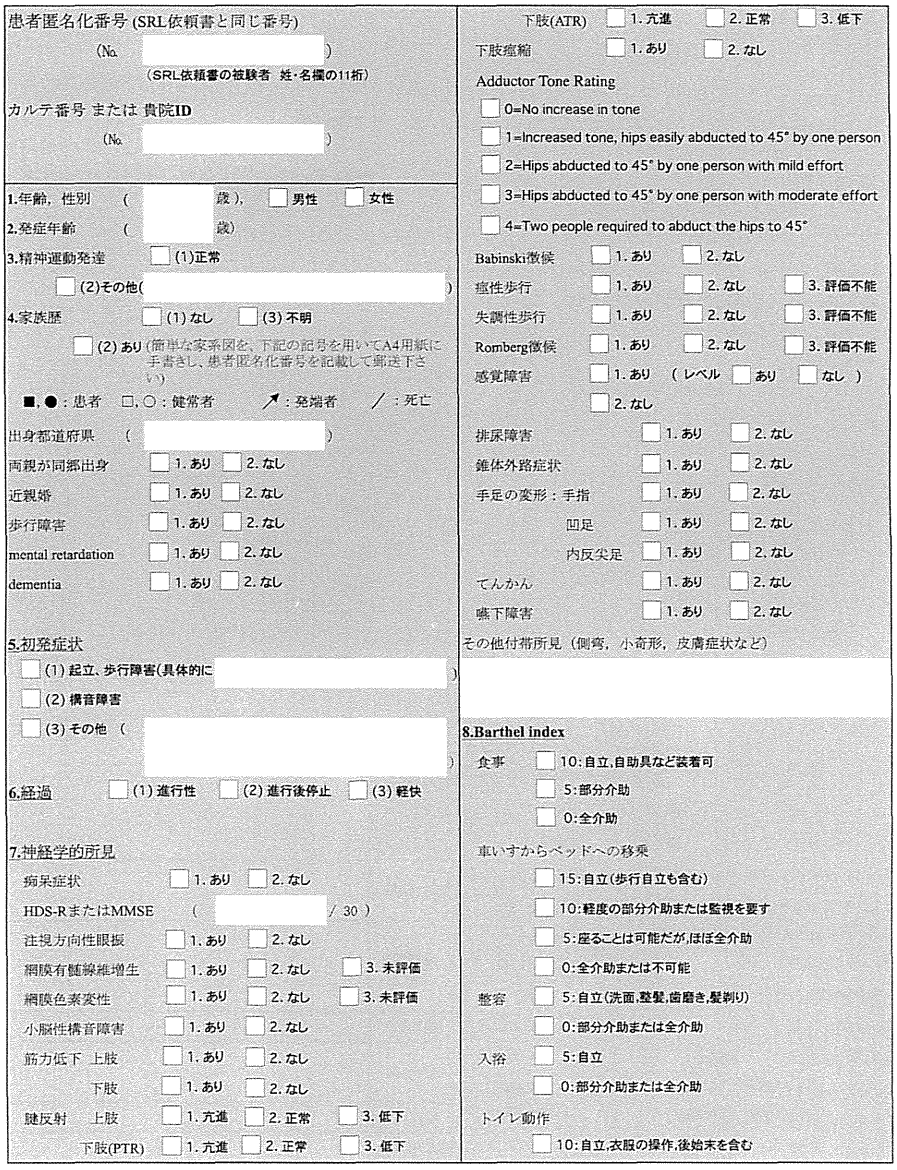


Fig. 1　JASPACの登録システム
事務局にEメールで連絡を入れると必要書類が送られてくる。

わが国のHSPの分子疫学

東京大学神経内科では直接塩基配列決定法（SPG4, SPG31），CGHアレイによるリアレンジメント解析法（SPG1, 2, 3A, 4, 5, 6, 7, 8, 10, 11, 13, 15, 17, 20, 21, 31, 33, 39, 42, ABCD1, alsin, SACS），リシークエンシング・マイクロアレイ解析法（SPG1, 2, 3A, 4, 5, 6, 7, 8, 10, 11, 13, 17, 20, 21, 31, 33, ABCD1）を組み合わせてHSPの網羅的遺伝子解析を行っている（Fig. 3）。自治医科大学神経内科では直接塩基配列決定法により巨大エクソンを持つ*SACS*遺伝子解析を行っている。

痙性対麻痺　患者情報提供書



患者匿名化番号 (SRL依頼書と同じ番号)
(No.)
(SRL依頼書の被験者 姓・名欄の11桁)
カルテ番号 または 貴院ID
(No.)

1.年齢，性別 (歳)， 男性 女性
2.発症年齢 (歳)
3.精神運動発達 (1)正常
(2)その他( )
4.家族歴 (1) なし (3) 不明
(2) あり (簡単な家系図を、下記の記号を用いてA4用紙に手書きし、患者匿名化番号を記載して郵送下さい)
■, ●：患者 □, ○：健常者 ↗：発端者 ／：死亡
出身都道府県 ( )
両親が同郷出身 1. あり 2. なし
近親婚 1. あり 2. なし
歩行障害 1. あり 2. なし
mental retardation 1. あり 2. なし
dementia 1. あり 2. なし

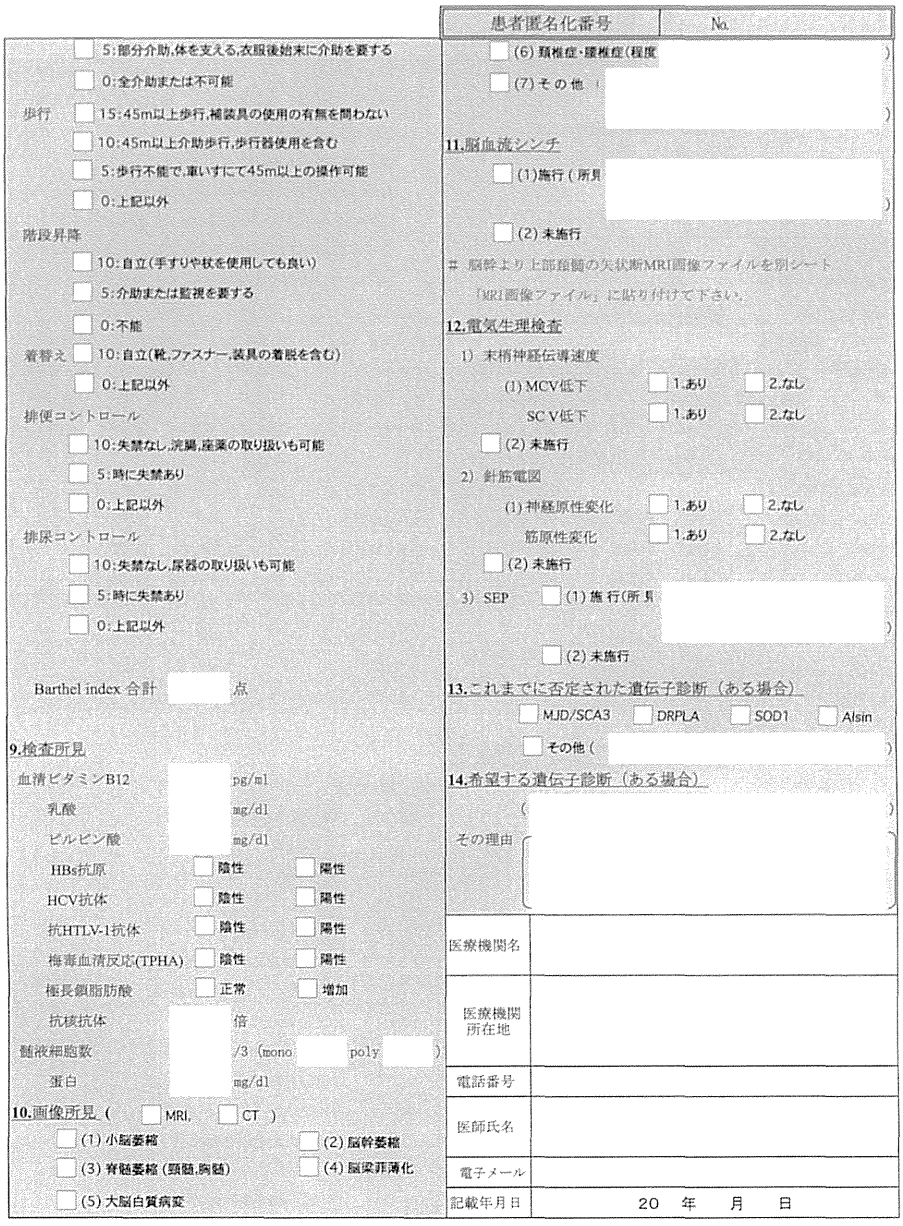
5.初発症状
(1) 起立、歩行障害(具体的に)
(2) 構音障害
(3) その他 ( )
6.経過 (1) 進行性 (2) 進行後停止 (3) 軽快

7.神経学的所見
痴呆症状 1. あり 2. なし
HDS-RまたはMMSE (/ 30)
注視方向性眼振 1. あり 2. なし
網膜有髄線維増生 1. あり 2. なし 3. 未評価
網膜色素変性 1. あり 2. なし 3. 未評価
小脳性構音障害 1. あり 2. なし
筋力低下 上肢 1. あり 2. なし
下肢 1. あり 2. なし
腱反射 上肢 1. 亢進 2. 正常 3. 低下
下肢(PTR) 1. 亢進 2. 正常 3. 低下
下肢(ATR) 1. 亢進 2. 正常 3. 低下
下肢痙縮 1. あり 2. なし
Adductor Tone Rating
0=No increase in tone
1=Increased tone, hips easily abducted to 45° by one person
2=Hips abducted to 45° by one person with mild effort
3=Hips abducted to 45° by one person with moderate effort
4=Two people required to abduct the hips to 45°
Babinski徴候 1. あり 2. なし
痙性歩行 1. あり 2. なし 3. 評価不能
失調性歩行 1. あり 2. なし 3. 評価不能
Romberg徴候 1. あり 2. なし 3. 評価不能
感覚障害 1. あり (レベル あり なし)
2. なし
排尿障害 1. あり 2. なし
錐体外路症状 1. あり 2. なし
手足の変形：手指 1. あり 2. なし
凹足 1. あり 2. なし
内反尖足 1. あり 2. なし
てんかん 1. あり 2. なし
嚥下障害 1. あり 2. なし
その他付帯所見（側弯，小奇形，皮膚症状など）

8.Barthel index
食事 10:自立,自助具など装着可
5:部分介助
0:全介助
車いすからベッドへの移乗
15:自立(歩行自立も含む)
10:軽度の部分介助または監視を要す
5:座ることは可能だが,ほぼ全介助
0:全介助または不可能
整容 5:自立(洗面,整髪,歯磨き,髪剃り)
0:部分介助または全介助
入浴 5:自立
0:部分介助または全介助
トイレ動作
10:自立,衣服の操作,後始末を含む

Fig. 2　患者情報提供書

JASPACによるわが国のHSPの分子疫学をFig. 4に示す。AD-HSP 206家系中SPG4が最も多く78家系(38%)を占めている。以下，SPG3A 11家系（5%），SPG31 10家系（5%），SPG10 3家系（2%），SPG8 1家系（1%）である。AD-HSPの半数では，既知の遺伝子変異を認めず，遺伝子型が同定できていない。



患者匿名化番号　No.

5:部分介助,体を支える,衣服後始末に介助を要する
0:全介助または不可能
歩行
15:45m以上歩行,補装具の使用の有無を問わない
10:45m以上介助歩行,歩行器使用を含む
5:歩行不能で,車いすにて45m以上の操作可能
0:上記以外
階段昇降
10:自立(手すりや杖を使用しても良い)
5:介助または監視を要する
0:不能
着替え
10:自立(靴,ファスナー,装具の着脱を含む)
0:上記以外
排便コントロール
10:失禁なし,浣腸,座薬の取り扱いも可能
5:時に失禁あり
0:上記以外
排尿コントロール
10:失禁なし,尿器の取り扱いも可能
5:時に失禁あり
0:上記以外
Barthel index 合計　点

9.検査所見
血清ビタミンB12　pg/ml
乳酸　mg/dl
ピルビン酸　mg/dl
HBs抗原　陰性　陽性
HCV抗体　陰性　陽性
抗HTLV-1抗体　陰性　陽性
梅毒血清反応(TPHA)　陰性　陽性
極長鎖脂肪酸　正常　増加
抗核抗体　倍
髄液細胞数　/3 (mono　poly　)
蛋白　mg/dl

10.画像所見 (MRI, CT)
(1) 小脳萎縮
(2) 脳幹萎縮
(3) 脊髄萎縮(頸髄,胸髄)
(4) 脳梁菲薄化
(5) 大脳白質病変
(6) 頚椎症・腰椎症(程度　)
(7) その他 (　)

11.脳血流シンチ
(1)施行 (所見　)
(2) 未施行
脳幹より上部脊髄の矢状断MRI画像ファイルを別シート「MRI画像ファイル」に貼り付けて下さい。

12.電気生理検査
1) 末梢神経伝導速度
(1) MCV低下　1.あり　2.なし
SCV低下　1.あり　2.なし
(2) 未施行
2) 針筋電図
(1) 神経原性変化　1.あり　2.なし
筋原性変化　1.あり　2.なし
(2) 未施行
3) SEP　(1) 施行(所見　)
(2) 未施行

13.これまでに否定された遺伝子診断（ある場合）
MJD/SCA3　DRPLA　SOD1　Alsin
その他 (　)

14.希望する遺伝子診断（ある場合）
(　)
その理由

医療機関名	
医療機関所在地	
電話番号	
医師氏名	
電子メール	
記載年月日	20　年　月　日

Fig. 2　患者情報提供書（続き）

AR-HSPについては，網羅的遺伝子解析では12%しか遺伝子型が同定できなかった。そこで，当初AR-HSPが疑われた88例についてエクソーム解析を行ったところ，19例（22%）でAR-HSPの病原性変異が同定され，SPG11 5例，SPG46 4例，SPG28 2例などであった。AR-HSPは極めてヘテロジニアスな集団であり，

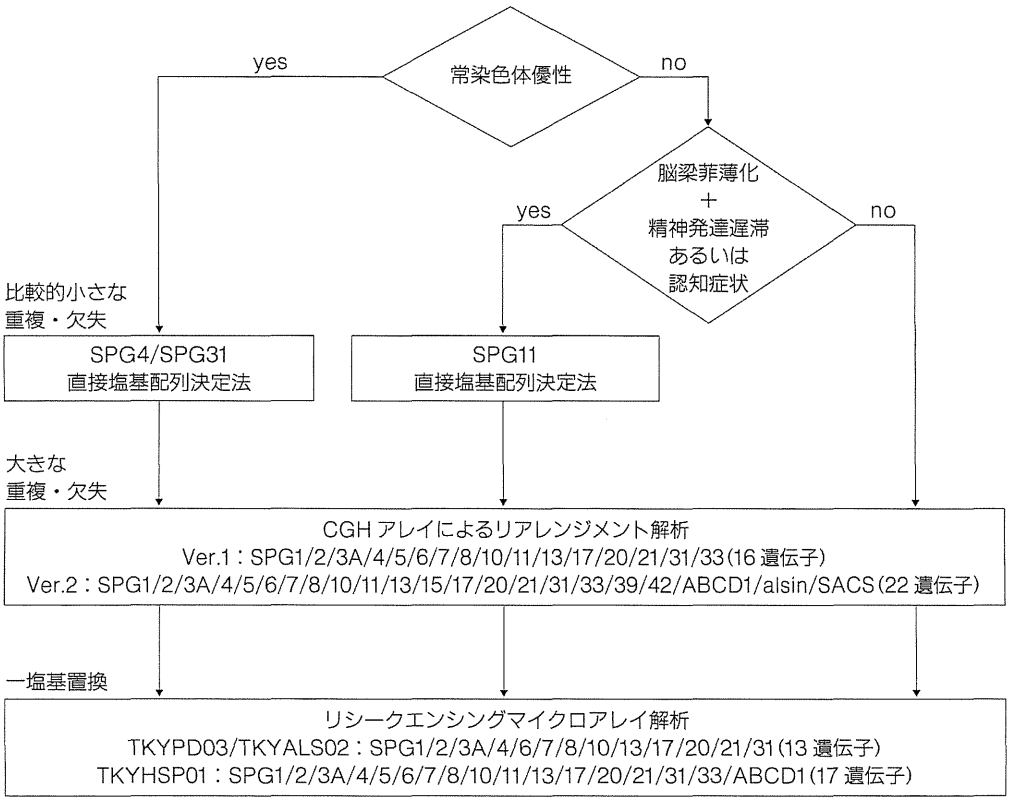


Fig. 3　網羅的遺伝子解析のフローチャート

優性遺伝が考えられる家系では，最初に比較的小さな重複や欠失を示す場合が多い SPG4 と SPG31 について直接塩基配列決定法を行い，変異がない場合には CGH アレイ解析（大きな重複や欠失）から DNA マイクロアレイを用いたリシークエンシング解析（一塩基置換）へと解析を進めている。優性遺伝が否定的な家系では，脳梁の菲薄化と精神発達遅延あるいは認知症状があれば，まず SPG11 の直接塩基配列決定法を行い，次に CGH アレイ，リシークエンシング解析へと進む。そうでない場合は，CGH アレイ，リシークエンシング解析を行っている（東京大学神経内科　石浦浩之らによる）。

ほかに ALS2，ARSACS，SCAR10，PARK9 など痙性対麻痺を呈し得る多くの疾患が含まれていることが判明した[9]。現在，AR-HSP についてはさらに症例数を増やしてエクソーム解析を行っている。

今後の課題として，多くの家系が集積された病型についての遺伝子型・表現型相関と自然歴を検討することが必要である。現在の網羅的遺伝子診断法の問題点として，最近のゲノム解析技術の発展（エクソーム解析や全ゲノム解析）により新しい原因遺伝子が次々に発見されているので，遺伝子診断がそのスピードに追いつかないことが挙げられる。一方で，エクソーム解析を遺伝子診断に導入するにはまだコストが高いと思われる。

JASPAC 研究リソースの活用

JASPAC 研究リソースを活用して，2 つの新規原因遺伝子の同定が行われた。まず，筆者らは視神経萎縮と末梢神経障害を伴う AR-HSP 家系（両親がいとこ婚の兄弟例）について，連鎖解析（ホモ接合体マッピング）

とエクソーム解析を用いて，*C12orf65* 遺伝子変異がその原因であることを見出し，SPG55 として HUGO Gene Nomenclature Committee に登録した[10]。*C12orf65* 遺伝子は小児のリー脳症の原因遺伝子であることが知られていたが[11]，*C12orf65* 遺伝子変異により AR-HSP の表現型を取り得ることが判明した。最近，*C12orf65* 遺伝子変異により，劣性遺伝性のシャルコー・マリー・トゥース病 6 型を起こすことが報告されている[12]。

次に，小脳性運動失調と末梢神経障害を伴う AR-HSP 家系（両親がいとこ婚の兄弟例）について，筆者らは同様の方法でチェディアック・東症候群（Chédiak-Higashi syndrome：CHS）の原因である *LYST* 遺伝子変異が AR-HSP を引き起こすことを見出した[13]。この家系は，CHS に特徴的な白子症，日光過敏症，易感染性を認めず，成人発症であったが，成人型 CHS が AR-HSP の表現型を示すことを知っておくべきであると思われた。

さらに，現在，純粋型 AR-HSP の 2 家系について原因遺伝子の探索が行われており，各々の家系で病原性変異を持つ新しい原因遺伝子を同定しつつある。

HSP は，臨床像が通常，痙性対麻痺とは異なる疾患の原因遺伝子の病原性変異を示すことがあり，また遺伝性ニューロパチーなどの他疾患においてナンバリングされた HSP 原因遺伝子の病原性変異を示すことがあるので，HSP の分子病態を考えるうえでとても興味深い。

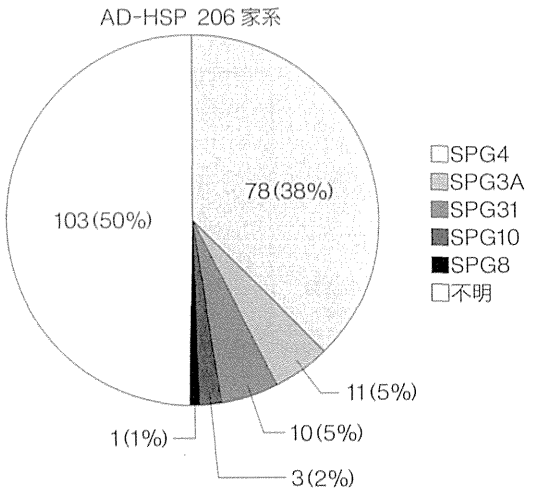


Fig. 4　わが国の AD-HSP の分子疫学
AD-HSP のうち SPG4 が 38%を占めており，最も頻度が高い。AD-HSP の半数では既知の遺伝子変異を認めず，遺伝子型を同定できていない。

HSP の分子病態と今後の展開

HSP の分子病態には，①軸索輸送（SPG30/KIF1A，SPG10/KIF5A，SPG4/SPAST），②小胞体の形状（SPG3A/ATL1，SPG4/SPAST，SPG12/RTN2，SPG31/REEP1），③ミトコンドリア機能（SPG13/HSP60，SPG7/PGN，SPG21/ACP33，SPG55/c12orf65），④ミエリン形成（SPG2/PLP，SPG42/SLC33A），⑤蛋白の折りたたみと小胞体ストレス（SPG6/NIPA1，SPG8/KIAA0196，SPG17/BSCL2，SPG18/ERLIN2），⑥錐体路と他の神経系の成長（SPG1/L1CAM，SPG22/SLC16A2），⑦脂肪酸とリン脂質（SGG28/DDHD1，SPG35/FA2H，SPG39/NTE，SPG54/DDHD2，SPG56/CYP2U1），⑧エンドソーム膜輸送と小胞形成（SPG47/AP4B1，SPG48/KIAA0415，SPG50/AP4M1，SPG51/AP4E1，SPG52/AP4S1，SPG53/VPS37A）の多様な障害が関わっていると推測されている[14]。

今後，JASPAC リソースの活用により HSP の分子病態が詳細に解明され，根本的な治療法が開発されることが望まれる。患者にできる限り正確な医療情報を提供して，将来の治療研究へとつなげるために JASPAC は重要な役割を担っていると考えている。

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文　献

1）McDermott CJ, Shaw PJ: Hereditary spastic paraplegia. Eisen AA, Shaw PJ (eds): Handbook of Clinical Neurology, Vol. 82. Motor neuron disorders and related diseases. Elsevier Science, Amsterdam, 2007, pp327-352
2）瀧山嘉久: 遺伝性痙性対麻痺. 柳澤信夫, 篠原幸人, 岩田誠, 清水輝夫, 寺本　明 (編): Annual Review 神経. 中外医学社, 東京, 2008, pp198-211
3）Harding AE: Hereditary "pure" spastic paraplegia: a clinical and genetic study of 22 families. J Neurol Neurosurg Psychiatry **44**: 871-883, 1981
4）Finsterer J, Löscher W, Quasthoff S, Wanschitz J, Auer-Grumbach M, et al: Hereditary spastic paraplegias with autosomal dominant, recessive, X-linked, or maternal trait of inheritance. J Neurol Sci **318**: 1-18, 2012
5）Hirayama K, Takayanagi T, Nakamura R, Yanagisawa N, Hattori T, et al: Spinocerebellar degenerations in Japan: a nationwide epidemiological and clinical study. Acta Neurol Scand **153**: 1-22, 1994
6）Tsuji S, Onodera O, Goto J, Nishizawa M; Study Group on Ataxic Diseases: Sporadic ataxias in Japan: a population-based epidemiological study. Cerebellum **7**: 189-197, 2008
7）瀧山嘉久, 辻　省次, 佐々木秀直, 服部孝道, 湯浅龍彦, 他: 痙性対麻痺全国共同研究の提案――JASPAC (Japan Spastic Paraplegia Research Consortium). 厚生労働省難病性疾患克服研究事業　運動失調に関する調査及び病態機序に関する研究班　平成17年度研究報告書. 厚生労働省, 2006, pp115-118
8）瀧山嘉久, 嶋崎晴雄, 迫江公己, 欧陽　疑, 滑川道人, 他: 本邦における遺伝性痙性対麻痺の検討――JASPAC 一次アンケート調査より. 厚生労働省難病性疾患克服研究事業　運動失調に関する調査及び病態機序に関する研究班　平成18年度研究報告書. 厚生労働省, 2007, pp101-103
9）辻　省次, 石浦浩之, 高　紀信, 嶋崎晴雄, 三井　純, 他: 常染色体劣性遺伝が疑われた遺伝性痙性対麻痺88例のexome解析. 厚生労働省難病性疾患克服研究事業　運動失調症の病態解明と治療法開発に関する研究班　平成25年度総括・分担研究報告書. 厚生労働省, 2014, pp83-86
10）Shimazaki H, Takiyama Y, Ishiura H, Sakai C, Matsushima Y, et al: A homozygous mutation of *C12orf65* causes spastic paraplegia with optic atrophy and neuropathy (SPG55). J Med Genet **49**: 777-784, 2012
11）Antonicka H, Ostergaard E, Sasarman F, Weraarpachai W, Wibrand F, et al: Mutations in *C12orf65* in patients with encephalomyopathy and a mitochondrial translation defect. Am J Hum Genet **87**: 115-122, 2010
12）Tucci A, Liu Y-T, Preza E, Pitceathly RD, Chalasani A, et al: Novel *C12orf65* mutations in patients with axonal neuropathy and optic atrophy. J Neurol Neurosurg Psychiatry **85**: 486-492, 2014
13）Shimazaki H, Honda J, Naoi T, Namekawa M, Nakano I, et al: Autosomal recessive complicated spastic paraplegia with lysosomal trafficking regulator gene mutation. J Neurol Neurosurg Psychiatry, 2014 Feb 12 [Epub ahead of print]
14）Fink JK: Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. Acta Neuropathol **126**: 307-328, 2013

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Registry System for Rare Neurological Disorders: Perspective of Clinical Trials Based on the Disease Pathomechanisms

Title　**Japan Spastic Paraplegia Research Consortium (JASPAC)**

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Abstract Japan Spastic Paraplegia Research Consortium (JASPAC), a nationwide clinical and genetic survey of patients with hereditary spastic paraplegia (HSP), was started in 2006 as a project of the Research Committee for Ataxic Diseases of the Ministry of Health, Labor, and Welfare, Japan. To date (April 4, 2014), 448 indexed patients with HSP have been registered from 46 prefectures in Japan. We are now performing molecular testing of the HSP patients using Sanger sequencing (SPG4, SPG11, SPG31, and ARSACS), comparative genomic hybridization (CGH) array (SPG1, 2, 3A, 4, 5, 6, 7, 8, 10, 11, 13, 15, 17, 20, 21, 31, 33, 39, 42, ABCD1, alsin, and ARSACS), and resequencing microarray (SPG1, 2, 3A, 4, 5, 6, 7, 8, 10, 11, 13, 17, 20, 21, 31, 33, and ABCD1). In 206 Japanese families with autosomal dominant HSP, SPG4 was the most common form, accounting for 38%, followed by SPG3A (5%), SPG31 (5%), SPG10 (2%), and SPG8 (1%). In 88 patients with autosomal recessive HSP, although SPG11 was the most common form, accounting for 6%, most showed significant genetic heterogeneity. The results of molecular testing will be applicable to patients in terms of improved positive diagnosis, follow-up, and genetic counseling. JASPAC will contribute to elucidating the molecular mechanisms underlying HSP, and will facilitate the development of better treatments for HSP.

Key words **hereditary spastic paraplegia, JASPAC, genetic analysis**