Journal of Clinical Monitoring and Computing (2019) 33:123–132
https://doi.org/10.1007/s10877-018-0141-4

ORIGINAL RESEARCH

Can postoperative deltoid weakness after cervical laminoplasty be prevented by using intraoperative neurophysiological monitoring?

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Received: 2 January 2018 / Accepted: 6 April 2018 / Published online: 17 April 2018

Abstract

Laminoplasty, frequently performed in patients with cervical myelopathy, is safe and provides relatively good results. However, motor palsy of the upper extremities, which occurs after decompression surgery for cervical myelopathy, often reduces muscle strength of the deltoid muscle, mainly in the C5 myotome. The aim of this study was to investigate prospectively whether postoperative deltoid weakness (DW) can be predicted by performing intraoperative neurophysiological monitoring (IONM) during cervical laminoplasty and to clarify whether it is possible to prevent palsy using IONM. We evaluated the 278 consecutive patients (175 males and 103 females) who underwent French-door cervical laminoplasty for cervical myelopathy under IONM between November 2008 and December 2016 at our hospital. IONM was performed using muscle evoked potential after electrical stimulation to the brain [Br(E)-MsEP] from the deltoid muscle. Seven patients (2.5%) developed DW after surgery (2 with acute and 5 with delayed onset). In all patients, deltoid muscle strength recovered to ≥4 on manual muscle testing 3–6 months after surgery. Persistent IONM alerts occurred in 2 patients with acute-onset DW. To predict the acute onset of DW, Br(E)-MsEP alerts in the deltoid muscle had both a sensitivity and specificity of 100%. The PPV of persistent Br(E)-MsEP alerts had both a sensitivity and specificity of 100% for acute-onset DW. There was no change in Br(E)-MsEP in patients with delayed-onset palsy. The incidence of deltoid palsy was relatively low. Persistent Br(E)-MsEP alerts of the deltoid muscle had a 100% sensitivity and specificity for predicting a postoperative acute deficit. IONM was unable to predict delayed-onset DW. In only 1 patient were we able to prevent postoperative DW by performing a foraminotomy.

Keywords Cervical laminoplasty · Postoperative deltoid weakness · Intraoperative neurophysiological monitoring · Muscle evoked potential after electrical stimulation to the brain

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1 Introduction

Laminoplasty, frequently performed in patients with cervical myelopathy, is safe and provides relatively good results. However, motor palsy of the upper extremities, which can occur after decompression surgery for cervical myelopathy, often reduces deltoid muscle strength in the C5 myotome; this scenario is known as *C5 palsy* [1–3]. The condition has a low incidence but can cause severe problems with activities of daily living. Deltoid weakness (DW) has been reported to occur after surgeries performed via both posterior [1, 3, 4], and anterior [5, 6] approaches. Although various etiologies have been proposed [1–3,7–9], a definitive cause has not yet been agreed upon. The goal of the present study was to elucidate the cause of postoperative DW and establish preventative methods.

In recent years, intraoperative neurophysiological monitoring (IONM) has been used to prevent spinal cord injury during surgery of the spine and spinal cord [10–12]. Muscle evoked potential after electrical stimulation to the brain [Br(E)-MsEP], which applies transcranial electrical stimulation to the brain and records evoked potentials from the muscles of the extremities to monitor motor function, has become the most commonly used method [13]. However, there have been few reports on the use of IONM in preventing DW after laminoplasty or laminectomy for cervical decompression [14–20], and only two of these were prospective studies [15, 18]. Although Oya et al. [20] and Bhalodia et al. [21] reported a high sensitivity and specificity of motor evoked potential (MEP) alerts for acute type *C5 palsy*, the ability of IONM to prevent this pathology has not been clarified.

The purpose of the present study was twofold: (i) to investigate prospectively whether postoperative DW can be predicted by performing IONM during cervical laminoplasty; and (ii) to clarify whether it is possible to prevent palsy using IONM.

2 Methods

The present study included the 278 consecutive patients [175 males and 103 females; mean age at surgery, 66.2 years (range 27–87 years)] who underwent French-door cervical laminoplasty for cervical myelopathy under IONM between November 2008 and December 2016 at our institution. The levels of laminoplasty were C3 to C6 or C3 to C7, and the conditions for which laminoplasty was performed were cervical spondylotic myelopathy (206 patients), ossification of the posterior longitudinal ligament (59 patients), and cervical disc herniation (13 patients). All surgeries were performed by experienced spine surgeons (M.A., T.M. and K.M). In all patients, preoperative deltoid muscle strength was > 4 on manual muscle testing (MMT). Patients who demonstrated reduced Br(E)-MsEP of the deltoid muscle intraoperatively underwent foraminotomy. Eight patients who had foraminal stenosis at the C4/5 level underwent prophylactic foraminotomy without having met criteria for an *IONM alarm* (i.e., sign of nerve injury). Patient characteristics are shown in Table 1.

Table 1 Demographic characteristics of the 278 patients

Characteristics	Value
Age at surgery [average, years (range)]	66.2 (27–87)
Sex [n (%)]	
Male	175 (62.9)
Female	103 (37.1)
Diabetes mellitus [n (%)]	52 (18.8)
BMI [average, kg/m^2 (range)]	23.7 (12.1–35.4)
Etiology [n (%)]	
CSM	206 (74.1)
OPLL	59 (21.2)
CDH	13 (4.7)
Laminoplasty level [n (%)]	
C3-6	83 (29.9)
C3-7	195 (70.1)
Foraminotomy [n (%)]	
Prophylactic	
Bilateral C4/5	3 (33.3)
Unilateral C4/5	5 (55.6)
Intervention	
Unilateral C5/6	1 (11.1)
Postoperative DW [n (%)]	
Acute type	2 (28.6)
Delayed type	5 (71.4)
Hospital stay [average, days (range)]	17.3 (13–35)

BMI body mass index, *CSM* cervical spondylotic myelopathy, *OPLL* ossification of posterior longitudinal ligament, *CDH* cervical disc herniation, *DW* deltoid weakness

2.1 IONM techniques

2.1.1 Stimulation

We conducted transcranial electrical stimulation using a method similar to that described by Matsuda and Shimazu [22], in which corkscrew-type electrodes (Nihon Kohden, Inc., Tokyo, Japan) were inserted bilaterally and symmetrically into the outer table of the skull 5 cm lateral and 2 cm anterior to Cz (International 10–20 System of Electrode Placement). Constant-current electrical stimulation was applied using a Neuromaster MEE-1200 Intraoperative Monitoring System (Nihon Kohden, Inc., Tokyo, Japan)

with a train of five pulses at an interstimulus interval of 2 ms. Stimulation frequency was 1 Hz, duration of one pulse was 0.5 ms, and intensity was set to elicit a clear muscle evoked potential (100–200 mA).

2.1.2 Recording

We recorded Br(E)-MsEP from the bilateral deltoid muscles using the Neuromaster Intraoperative Monitoring System (Nihon Kohden, Inc.) with needle electrodes (Nihon Kohden, Inc.). The active electrode was placed in the belly of the deltoid muscle, and the reference electrode was placed at the acromion. Br(E)-MsEP was recorded at a band pass filter of 50–3000 Hz and mean summation time of five times.

2.1.3 Anesthesia

IONM was conducted with patients under total intravenous anesthesia, which was maintained with propofol 3 μg/mL, using target-controlled infusion technique, and remifentanil 0.2–0.3 μg/kg/min. A muscle relaxant (rocuronium 0.6–0.9 mg/kg) was used at the time of induction of anesthesia only, but not otherwise intraoperatively.

2.2 Evaluation

A decrease in deltoid muscle strength postoperatively to <3 on MMT was defined as postoperative DW. An alarm point was defined as reduction of Br(E)-MsEP amplitude to $\leq 30\%$ that of the control waveform [23]. A substantial reduction in Br(E)-MsEP was defined as a reduction to $\leq 30\%$ that of the control waveform at the end of surgery.

In the present study, we analyzed (i) intraoperative changes in Br(E)-MsEP in patients with postoperative DW and (ii) the presence or absence of recovery of Br(E)-MsEP and postoperative DW after foraminotomy in patients with intraoperative reduction in Br(E)-MsEP.

2.3 Surgical technique

The technique for laminoplasty has been described previously [24]. The paravertebral muscles on the left side were detached from the spinous processes and each spinous process was removed at approximately 5 mm from the base of each spinous process. The paravertebral muscles on the right side remained attached to the spinous process and posterior ligamentous complex. The remnants of the spinous processes were in turn split sagittally using a high-speed drill. Lateral gutters were made in the medial one-third of the facet joints. The inner cortex was of a thickness that allowed it to be bend easily. When the lateral gutters were made, the laminae opened out like French doors. Ceramic spacers were fixed between the split spinous processes.

2.4 Statistical analysis

A 2×2 contingency table was created by using postoperative DW (acute and delayed types) and the decrement of Br(E)-MsEP at the end of surgery. We calculated sensitivity, specificity, positive predicted value (PPV), and negative predicted value (NPV) for postoperative DW of Br(E)-MsEP using this contingency table.

3 Results

Seven patients (2.5%) developed postoperative DW. In all patients, deltoid muscle strength recovered to ≥ 4 on MMT 3–6 months after surgery. Onset of paralysis occurred in two patients immediately after surgery (acute type) and in five patients 2–3 days after surgery (delayed type). Both patients who experienced paralysis immediately postoperatively had undergone C4/5 foraminotomy during the procedure. In these patients, Br(E)-MsEP recorded from the deltoid muscle on the side of foraminotomy decreased immediately after the foraminotomy. In five patients who experienced paralysis 2–3 days after surgery, Br(E)-MsEP of the deltoid muscle had not decreased during surgery. One of these five patients with delayed-onset postoperative DW underwent prophylactic C4/5 foraminotomy on the affected side without IONM alarm (Table 2). In addition, Br(E)-MsEP decreased intraoperatively in one patient, who therefore underwent foraminotomy, after which the evoked potential recovered to the level of the control. Postoperative DW did not occur in this case.

3.1 Accuracy of IOMN

For acute paralysis, sensitivity, specificity, PPV, and NPV were 100%. For delayed paralysis, sensitivity was 0%, specificity was 99.3%, PPV was 0%, and NPV was 98.2%. For total paralysis, sensitivity was 28.6%, specificity was 100%, PPV was 100%, and NPV was 98.2% (Table 3).

3.2 Case presentations

3.2.1 Case 1

A 73-year-old male underwent C3–C6 cervical laminoplasty for ossification of the posterior longitudinal ligament.

Table 2 Characteristics of patients with postoperative DW

Case	1	2	3	4	5	6	7
Age (years)	73	42	82	56	76	67	74
Sex	M	M	F	M	F	M	F
Diagnosis	OPLL	CSM	CSM	CSM	OPLL	OPLL	CSM
Level of laminoplasty	C3–C6	C3–C6	C3–C6	C3–C7	C3–C7	C3–C7	C3–C6
DW onset							
Type	Delayed	Delayed	Acute	Delayed	Delayed	Delayed	Acute
Timing (days postoperatively)	3	2	0	3	3	3	0
Substantial decrease in Br(E)-MsEP	None	None	Right deltoid	None	None	None	Left deltoid
Paralyzed muscle	Right deltoid	Left deltoid	Right deltoid	Right deltoid	Left deltoid	Right deltoid	Left deltoid
MMT at onset of palsy	2	2	0	1	2	2	1
MMT at final follow-up	4	5	5	5	5	5	4
Period required for recovery (months)	3	3	6	6	3	6	6
Prophylactic foraminotomy without IONM alarm	No	Bilateral C4/5, C5/6	Right C4/5	No	No	No	Left C4/5

Br(E)-MsEP muscle evoked potential after electrical stimulation to the brain, *CSM* cervical spondylotic myelopathy, *F* female, *IONM* intraoperative neurophysiological monitoring, *M* male, *MMT* manual muscle testing, *DW* deltoid weakness, *OPLL* ossification of posterior longitudinal ligament

Table 3 Relationship between change in Br(E)-MsEP and postoperative DW

	DW (+)	DW (−)	Total
Br(E)-MsEP decrement (+)	2[a]	0	2
Br(E)-MsEP decrement (−)	5[b]	271	276
Total	7	271	278

Br(E)-MsEP muscle evoked potential after electrical stimulation to the brain, *DW* deltoid weakness

[a]Acute type

[b]Delayed type

Although no obvious changes in intraoperative Br(E)-MsEP were observed, MMT on postoperative day 3 revealed decreases in muscle strength of the right deltoid muscle and biceps brachii to 2. A postoperative computed tomography (CT) scan showed narrowing of the C4/5 and C5/6 intervertebral foramina. Three months postoperatively, MMT demonstrated improvements in muscle strength to 4 in both the deltoid and biceps brachii muscles (Figs. 1, 2).

3.2.2 Case 2

A 42-year-old male underwent C3–C6 cervical laminoplasty for cervical spondylotic myelopathy and C4/5 and C5/6 foraminotomy on both sides. A preoperative CT scan showed narrowing of the left C4/5 intervertebral foramen. Br(E)-MsEP amplitudes on the right and left sides at the end of the surgery were 50 and 60%, respectively, relative to the control. On postoperative day 2, MMT demonstrated left deltoid muscle strength to be reduced to 2; however, it normalized by 3 months postoperatively (Figs. 3, 4).

3.2.3 Case 3

An 82-year-old female underwent C3 to C6 cervical laminoplasty for cervical spondylotic myelopathy. A preoperative CT scan showed narrowing of the right C4/5 intervertebral foramen. When we performed a C4/5 foraminotomy on the right side after opening the lamina, Br(E)-MsEP of the right deltoid muscle decreased to 26% of the control level. Immediately after surgery, MMT showed that left deltoid muscle strength was reduced to zero. However, muscle strength had normalized by 6 months postoperatively (Figs. 5, 6).

3.2.4 Case 4

A 62-year-old female underwent C3–C6 cervical laminoplasty for cervical spondylotic myelopathy. A preoperative CT scan showed mild narrowing of the C5/6 intervertebral foramen. Just after opening the split lamina, MsEPs of the left deltoid were shown to be decreased. We therefore performed a C5/6 foraminotomy on the left side, and the evoked potentials recovered. Although the patient experienced

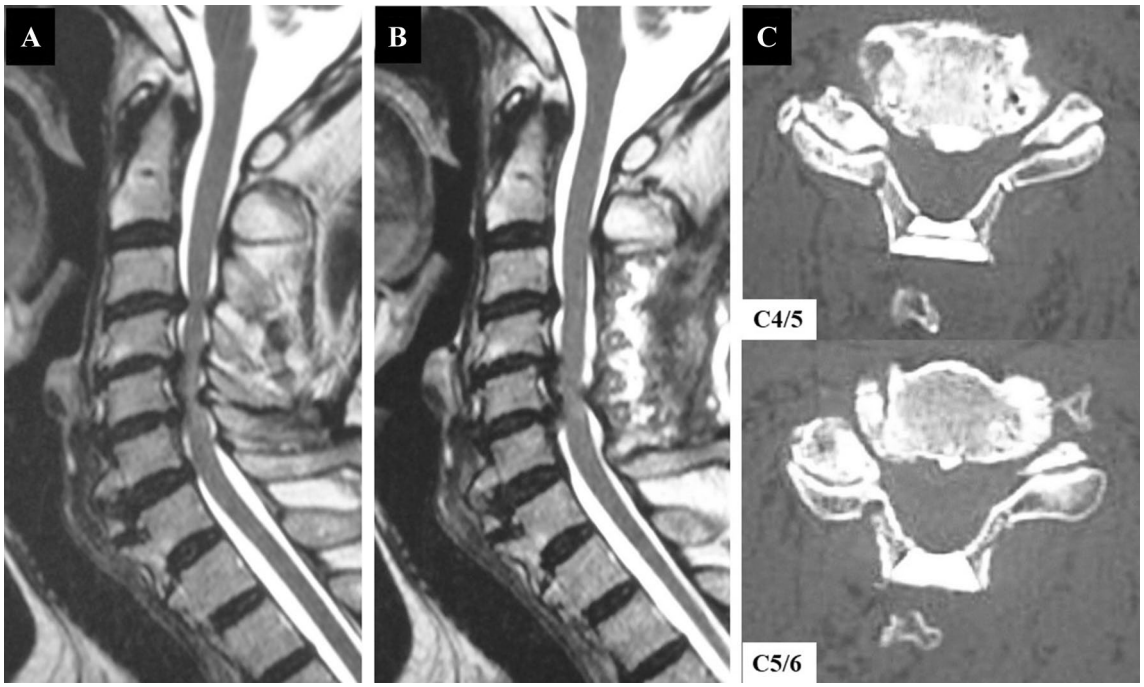

Fig. 1 Case 1. A 73-year-old male with OPLL who underwent C3–C6 laminoplasty. Sagittal view of T2-weighted MRI performed **a** preoperatively and **b** postoperatively. **c** Axial view of postoperative CT scan at intervertebral levels C4/5 (upper frame) and C5/6 (lower frame). There is narrowing of the intervertebral foramina bilaterally at the C4/5 and C5/6 levels. *CT* computed tomography, *MRI* magnetic resonance imaging, *OPLL* ossification of the posterior longitudinal ligament

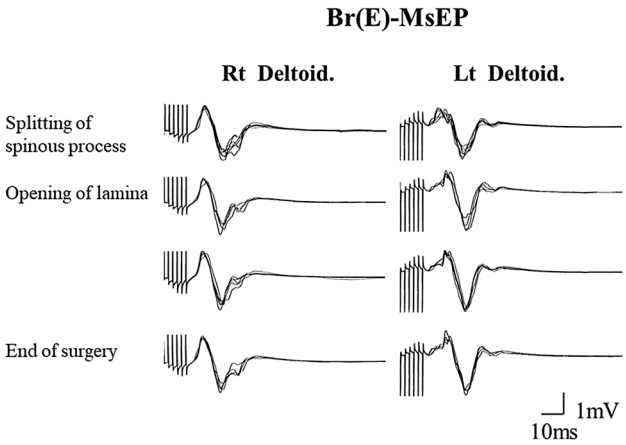

Fig. 2 Case 1. There was no change of Br(E)-MsEP throughout the surgery. However postoperative DW occurred 3 days after surgery at right deltoid muscle (MMT 2) and biceps brachii muscle (MMT 2). Muscle strength recovered to MMT 4 at 3 months after operation. *Br(E)-MsEP* muscle evoked potential after electrical stimulation to the brain, *Lt* left, *DW* deltoid weakness, *MMT* manual muscle testing, *Rt* right

temporary pain postoperatively in the left shoulder, deltoid muscle strength was not reduced (Figs. 7, 8).

4 Discussion

The incidence of postoperative DW after cervical laminoplasty was reported by Imagama et al. [25] to be 2.3%. Various causes of paralysis have been proposed; e.g., tethering or stretching of the nerve roots associated with a posterior shift of the spinal cord after decompression [2, 3, 8], spinal cord ischemia due to impeded blood flow in the radicular artery [2], myelopathies like spinal edema and reperfusion disorders [1, 2, 9], and nerve damage that occurs during a surgical procedure or due to heat from an air drill [2, 7]; however, there is no single definitive theory.

Recently, C5 radiculopathy due to C4/5 intervertebral foraminal stenosis has been mentioned as a possible cause of DW after cervical laminoplasty [26], and one report demonstrated the utility of prophylactic foraminotomy [27].

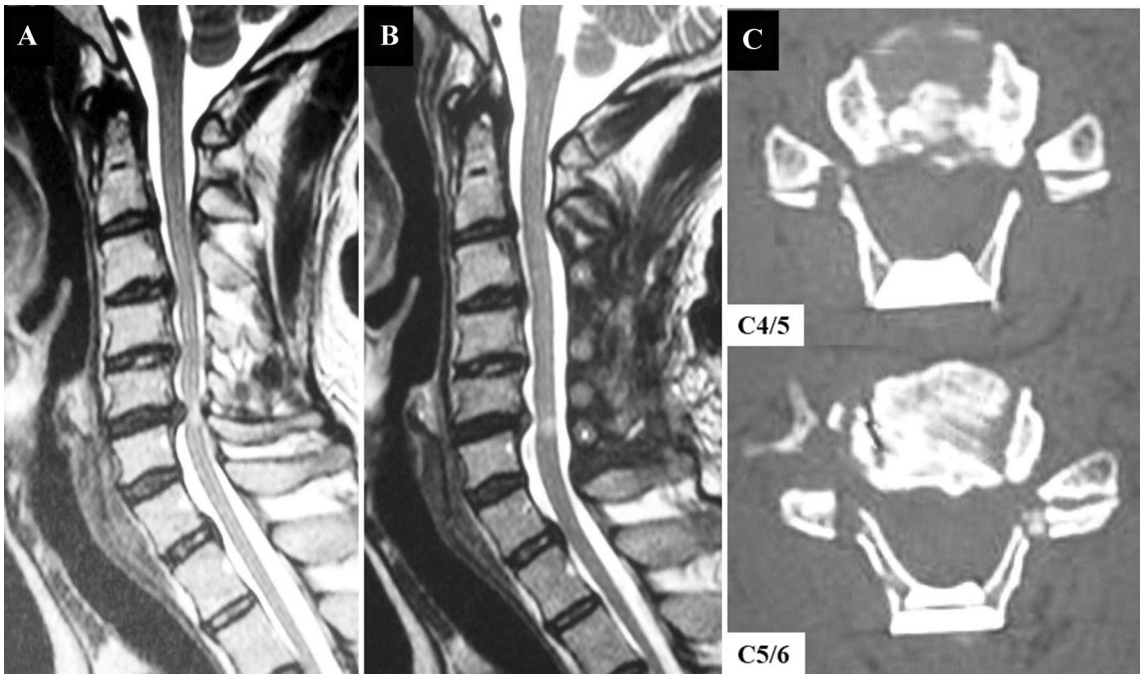

Fig. 3 Case 2. A 42-year-old male with CSM. Sagittal view of T2-weighted MRI performed **a** preoperatively and **b** postoperatively. **c** Axial view of postoperative CT scan at intervertebral levels C4/5 (upper frame) and C5/6 (lower frame). Prophylactic foraminotomy without IONM alarm was performed bilaterally at the C4/5 and C5/6 levels concomitant with C3 to C6 laminoplasty. *CSM* cervical spondylotic myelopathy, *MRI* magnetic resonance imaging, *CT* computed tomography

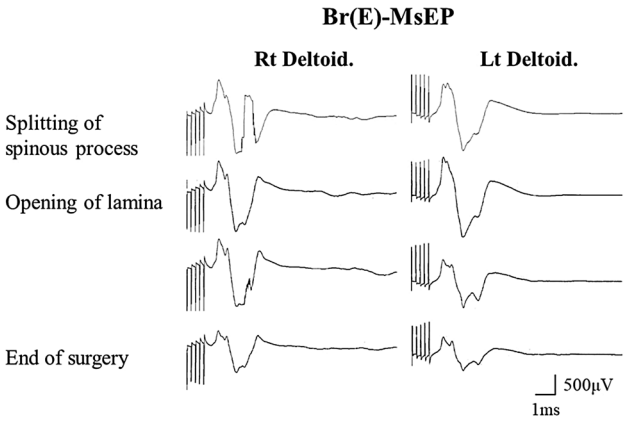

Fig. 4 Case 2. Br(E)-MsEP amplitudes on the right and left sides at the end of the surgery were 50 and 60%, respectively, which is not substantial decrement in evoked potential. On MMT 2 days after surgery, left deltoid muscle strength was reduced to 2 but had normalized by 3 months postoperatively. *Br(E)-MsEP* muscle evoked potential after electrical stimulation to the brain, *Lt* left, *MMT* manual muscle testing, *Rt* right

A few studies have investigated the use of IOMN to prevent postoperative DW. Tanaka et al. [15] suggested that intraoperative nerve damage was not involved because there was no change in intraoperative MEPs in patients who developed postoperative DW, and Yanase et al. [16] found that changes in the MEP waveform patterns in patients with postoperative DW were more frequent than those from individuals in a non-paralysis group. MEPs and spontaneous electromyography were found by Fan et al. [14] to be useful in the detection of intraoperative C5 nerve root injury. Fujiwara et al. [18] found that acute postoperative DW can be predicted using MEPs. Finally, Haghighi et al. [19] did not find somatosensory evoked potentials or MEPs to be useful in the detection of postoperative DW but did note the utility of real-time electromyography activity in predicting postoperative segmental C5 palsy (Table 4).

In the present study, the evoked potentials in one patient were reduced during the surgery but ultimately recovered when we performed a foraminotomy, and the patient's final postoperative muscle strength was not decreased. As far as we know, only two reports [17, 21] besides ours demonstrated the usefulness of IONM in preventing postoperative DW in posterior cervical spine surgery. Bhalodia et al. [21] showed also that amplitude changes resolved following surgical pause and foraminotomy in 6 out of 12 patients with an MEP amplitude reduction of the C5 myotome during cervical spine surgery. Intraoperative tethering injuries to the

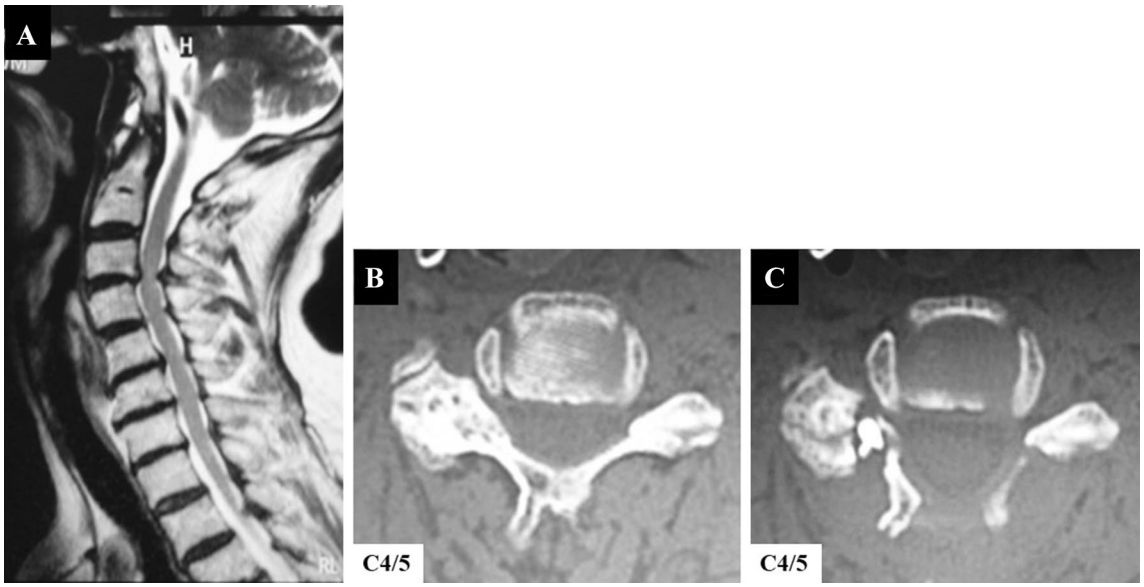

Fig. 5 Case 3. An 82-year-old female with CSM who underwent C3–C6 laminoplasty with foraminotomy at the C4/5 level on the right side. **a** Preoperative sagittal view of T2-weighted MRI. **b** Preoperative and **c** postoperative axial views of CT scan at the C4/5 intervertebral level. A right side foraminotomy was performed. *CSM* cervical spondylotic myelopathy, *MRI* magnetic resonance imaging, *CT* computed tomography

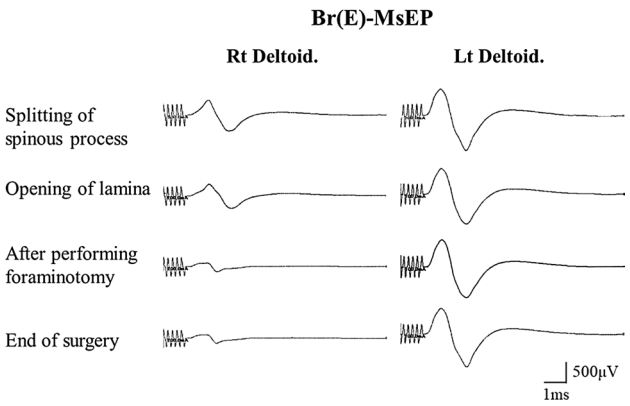

Fig. 6 Case 3. The amplitude of Br(E)-MsEP of the right deltoid muscle was decreased to 26% of the control level after foraminotomy at the C4/5 level on the right. On MMT, right deltoid muscle strength was reduced to 0 immediately after surgery but recovered to 5 by 6 months postoperatively. *Br(E)-MsEP* muscle evoked potential after electrical stimulation to the brain, *Lt* left, *MMT* manual muscle testing, *Rt* right

nerve roots that are detected with IONM may resolve with foraminotomy like our case.

However, it should be pointed out that, in the present study, delayed paralysis occurred 2–3 days following surgery in five patients. Because there was no change in intraoperative Br(E)-MsEP in these individuals, we could not predict the occurrence of paralysis. If the neural damage leading to postoperative DW occurs after rather than during surgery, it would be impossible to detect the palsy even with IONM. Of these five patients, two underwent foraminotomy despite the absence of IONM alarm. Possible causes of their paralysis were: (i) presence of myelopathy, (ii) failed foraminotomy, or (iii) ineffectiveness of foraminotomy in relieving a stretched nerve root. Fujiwara et al. [18] and Lee et al. [26] also reported that foraminotomy was not effective in the prevention of postoperative DW. In addition, in two patients, Br(E)-MsEP was reduced immediately after foraminotomy, and DW occurred immediately after surgery. As such, it is highly likely that an intraoperative nerve root injury occurred in these patients.

There is continued debate over the many criteria for alarm points of Br(E)-MsEP, which include a decrease in amplitude of more than 80% [28], changes in MEP waveform morphology [29], and a multiphasic pattern of MEP [30]. In the present study, a reduction in the amplitude of intraoperative evoked potentials to $\leq 30\%$ of the control waveform ($> 70\%$ decrease) was defined as a substantial reduction, but these values are based primarily on cases of not nerve root disorder but spinal cord paralysis [23]. Alarm criteria of decreases of 50–75% for nerve root injury have been suggested [14–18]. Moreover, Haghighi et al. [19] reported

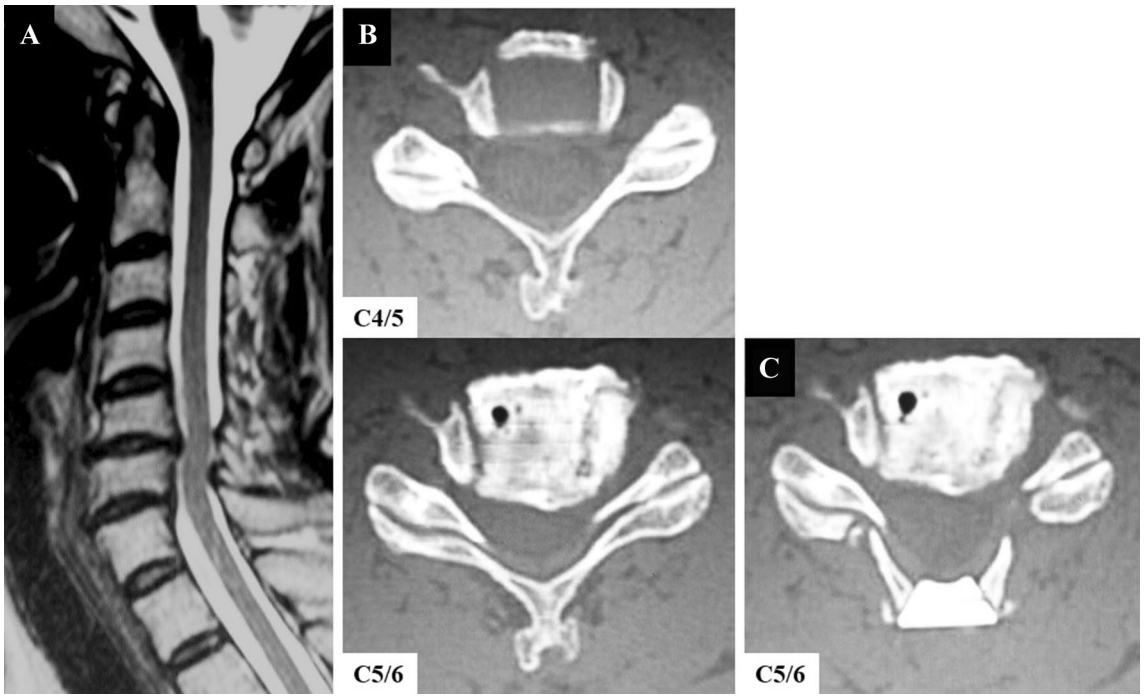

Fig. 7 Case 4. A 62-year-old female with CSM. **a** Preoperative sagittal view of T2-weighted MRI. **b** Preoperative CT axial view at the C4/5 (upper frame) and C5/6 (lower frame) intervertebral levels showing mild narrowing of the C5/6 intervertebral foramen. **c** Postoperative CT axial view at the C4/5 intervertebral levels. A left side foraminotomy was performed. *CSM* cervical spondylotic myelopathy, *MRI* magnetic resonance imaging, *CT* computed tomography

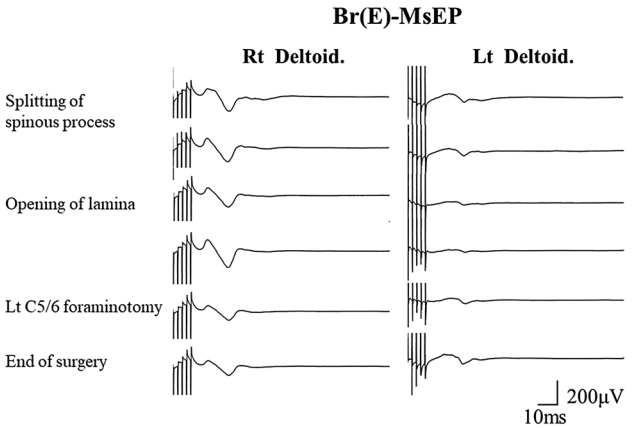

Fig. 8 Case 4. The patient underwent C3 to C6 laminoplasty. Just after the opening lamina, left deltoid Br(E)-MsEP amplitude decreased. Following left side C5/6 foraminotomy, the amplitude recovered. There was no deterioration of muscle strength after surgery *Br(E)-MsEP* muscle evoked potential after electrical stimulation to the brain, *Lt* left, *Rt* right

that the recording of deltoid muscle transcranial MEP was not sensitive to C5 nerve palsy because of possible overlapping nerve root innervation. According to Tsutsui et al. [31], it will be challenging to overcome the ineffectiveness of Br(E)-MsEP monitoring in detecting intraoperative nerve root injury. Additional studies are needed to investigate the possibility of more effective detection methods for nerve root function abnormalities, perhaps by applying other monitoring methods such as monitoring of direct stimulation of the nerve roots.

An important purpose of IONM is to prevent neural injury, but successful detection via IONM does not necessarily contribute to patient satisfaction. In the present study, there was only one case of rescue because of IONM. Neurophysiologic validation of values predicting postoperative DW are necessary.

4.1 Limitations

In this study we applied a single-modality IONM method. Spontaneous electromyography is a supportive tool for interpreting Br(E)-MsEP, and the use of multimodal IONM, including spontaneous electromyography, would have been optimal. Furthermore, MEPs were recorded from the deltoid muscle only. The recording of MEPs from other muscles such as the biceps brachii could have provided more precise information about postoperative motor palsy.

Table 4 Sensitivity and efficacy of IONM for postoperative DW during posterior cervical spine surgery in previous studies

Authors	Total Cases	Palsy		Detected palsy cases by IONM			Rescue case by Foraminotomy
		Type	Number	Br(E)-MsEP	s-EMG	SEP	
Fan et al. [14]	68	Acute	2	2	1	N/A	0
		Delayed	0	0	0	N/A	
Tanaka et al. [15]	162	Acute	0	0	N/A	N/A	0
		Delayed	3	0	N/A	N/A	
Yanase et al. [16]	153	Acute	0	0	N/A	N/A	0
		Delayed	4	3	N/A	N/A	
Nakamae et al. [17]	184	Acute	0	0	N/A	N/A	1
		Delayed	6	0	N/A	N/A	
Fujiwara et al. [18]	160	Acute	3	3	N/A	N/A	0
		Delayed	2	0	N/A	N/A	
Haghighi et al. [19]	100	Acute	5	0	5	0	0
		Delayed	0	0	0	0	
Oya et al. [20]	135	Acute	2	2	0	0	0
		Delayed	1	2	0	0	
The present study	278	Acute	2	2	N/A	N/A	1
		Delayed	5	0	N/A	N/A	

Br(E)-MsEP muscle evoked potential after electrical stimulation to the brain, *IONM* intraoperative neurophysiological monitoring, *DW* deltoid weakness, *s-EMG* spontaneous electromyography, *SEP* somatosensory evoked potential

5 Conclusion

The present study investigated whether IONM could be used to prevent DW after cervical laminoplasty. We were able to prevent postoperative DW using IONM in only 1 patient. However, acute and delayed palsies were not able to be rescued. Furthermore, acute onset of postoperative DW was predicted by IONM, but IONM was unable to predict the occurrence of delayed-onset postoperative DW.

Compliance with ethical standards

Conflict of interest All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee (Wakayama Rosai Hospital ethics committee) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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