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ORIGINAL ARTICLE

Motor unit number estimation of the tibialis anterior muscle in spinal cord injury

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Study design: Case–control study.
Objective: To investigate the number of motor units from the tibialis anterior (TA) muscle in normal subject and its change after SCI.
Setting: China Rehabilitation Research Center, Beijing, China.
Methods: Motor unit number estimation (MUNE) in 45 control subjects (35 young subjects with an average age of 36 years, and 10 elderly subjects with an average age of 65 years) was performed by using adapted multiple point stimulation method (AMPS). Twenty patients with SCI (10 subacute patients with an average age of 39 years, and 10 chronic patients with an average age of 34 years) were also examined for three times in 3 months with the same method.
Results: The mean MUNE value of the TA muscle was 188 ± 20 in the control group, and 40 ± 33 in subacute SCI patients ($P < 0.01$ vs young controls). A continuous increase in the MUNE value was observed in subacute SCI patients during the later follow-up period. In the chronic SCI group, the mean MUNE value was173 ± 29 which was similar to that of young control group.
Conclusions: AMPS could be a useful procedure for quantifying changes of MUNE values after SCI. Changes in functional motor unit number may occur distal to the site of lesion in SCI patients. These phenomena may be caused by neuronal plasticity in motor units, reversible transsynaptic degeneration and/or local functional depression. Owing to the limited sample size and a wide age range of subjects recruited in this study, future study are warranted for revealing detailed changes of MUNE parameters after SCI and exploring the underlying mechanisms.
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Keywords: tibialis anterior; motor unit number estimation; adapted multiple point stimulation method; spinal cord injury

Introduction

Spinal cord injury (SCI) leads to acute functional defects and numerous chronic alterations distal to the site of injury. Some important alterations after SCI may include: the development of dyssynergy between the urinary bladder and external urethral sphincter, reorganization of pathways controlling micturition, spasticity and hyperreflexia in skeletal muscles, chronic pain and sexual dysfunction. As some previous studies[1–3] suggested that these changes are likely due to neurological plasticity or functional reorganization of spinal cord circuitry after SCI, others[4] suggested that transsynaptic degeneration may also be involved. Neuronal structural and/or functional changes caudal to the site of SCI may lead to the failure of neurological regeneration and reorganization.

Motor unit number estimation (MUNE) was introduced by McComas[5] in the early 1970s and has been considered as a direct quantitative electrophysiological measurement of lower motor neurons.[6] For diseases affecting the anterior horn cells, especially the amyotrophic lateral sclerosis, MUNE is believed to be the most sensitive follow-up tool for the evaluation of disease progression.[7] Since McComas's first proposal,[5] various other MUNE methods have been developed and validated. The adapted multiple point stimulation (AMPS) method has several advantages. For example, it is fast and noninvasive, it does not need specific software. AMPS was also proven to be reliable and reproducible, as it minimizes alternation and motor unit (MU) selection bias in estimating thenar MU size and number.[8] The AMPS method combines important elements of the original McComas's technique[5] with the multiple stimulation method, as it can analyze two to three surface-recorded

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MU action potentials (S-MUAP) obtained in response to incremental stimulation at different points. Eric Albrecht and Thierry Kuntzer[9] confirmed the validity of the method in the lower extremity in healthy subjects and in patients with amyotrophic lateral sclerosis or acquired peripheral neuropathies.

To our knowledge, there were only two MUNE study[10,11] of the TA muscle performed without SCI. In addition, the MUNE values of muscles distal to the site of injury in two non-longitudinal MUNE studies of SCI patients were different from each other.[12,13] Therefore, it is unclear whether there are functional MU loss and/or transsynaptic degeneration following SCI. Thus, the aim of this study is to establish a baseline TA MUNE value in a homogeneous healthy control group by using the AMPS technique, then to compare number of MUs among control group and two SCI groups, namely subacute (1–8 months after injury) and chronic (1–4 years after injury) groups and finally to determine if transsynaptic degeneration may occur distal to the site of SCI.

Materials and methods

Subjects and patients

This study included 45 healthy volunteers (controls) from our hospital and 20 SCI inpatients from spine surgery department and urology surgery department.

All volunteers (20- to 75-year-old) were divided into two groups: young and old group. The young controls consisted of 20 men and 15 women at age of 20–57 years (mean, 36 years). The old group consisted of six men and four women at age of 60–75 years (mean, 65 years). They had no neuromuscular symptom and were normal on neurological examination.

Twenty traumatic SCI patients (ASIA A/B) above the T6 segmental level were included in this study. They had no motor function in the L4 myotome. According to the post-injury time period, they were divided into two groups: subacute group ($n=10$) with mean post-injury time of 4 months (range: 1–8 months) and chronic group ($n=10$) with mean post-injury time of 2 years (range: 1–4 years). The average age of subacute and chronic patients were 39 (24–54) and 34 years (18–53), respectively. Patients were excluded if (a) they were in the period of spinal shock, (b) there was any possibility of a second lower spinal lesion or a cauda equina lesion, (c) the existence of diabetes, alcoholism or any other conditions that may cause further central or peripheral neuropathy.[12] The history and neurological examination for each patient were completed before MUNE. All diagnoses were made according to the results from clinical, neuroradiological, laboratory and electrophysiological investigations.

Experimental arrangement

The subjects were seated comfortably in a chair with the examined leg flexed at the hip (90°), the knee (90°) and the ankle in a neutral position.

All studies were performed using a Nihon Kohden electromyography (EMG) machine. S-MUAP and compound motor action potentials (CMAP) were recorded with a gain of 100–200 mV per division and were evoked by 0.02 ms constant current square waves repeated at 0.7 Hz, as proposed by Wang and Delwaide.[8] Surface recordings were filtered with a bandpass from 20 to 5 kHz. The increment of stimulus intensity was 0.2 mA. The active electrode was positioned over the motor point of the TA muscle to maximize the negative peak amplitude and minimize the rise time of the M-potential (approximately 7 cm distal to the tibial tuberosity and 2 cm lateral to the anterior border of the tibia). The reference electrode was positioned over the distal tendon of the TA, and a ground electrode was placed on the tibia. The stigmatic and reference electrodes consisted of disk electrodes with a contact area of 10 mm in diameter (NE-132B).

The left limb was studied in both control and SCI groups. Ten subjects from each group were checked again at 1 week (the young control group), 1 and 3 months (the subacute SCI group) and 3 months (the chronic SCI group), respectively, after participating in the first test. All EMG tests were performed by one examiner who was blinded to the previous result in the later tests.

Electrophysiological methods

The AMPS method and associated algorithms have been described previously.[8,9] The maximal CMAP was obtained by supramaximal stimulation of the peroneal nerve with 0.2 ms constant current square waves at the fibular head site. Measurement of the negative peak area (from the onset of the first negative peak to the first crossing of the baseline) was preferred to peak-to-peak amplitude or negative peak amplitude measurements, as it minimizes the cancellation error.[8] The peroneal nerve was stimulated at various sites and the recordings were made from the TA muscle. Electrical stimulation was first applied at the same fibular head site and then at enough points (usually between 4 and 6 points from the distal fibular head to lateral popliteal space) to obtain 10–15 distinct S-MUAP. At each stimulation point, the stimulus intensity was increased by increments to the level at which the first, second and sometimes the third, subsequent S-MUAP were elicited in an all-or-nothing manner. To limit the variations, only motor axons with distinct thresholds were recruited. Except for the first S-MUAP evoked at a stimulation point, the morphology of the subsequent S-MUAP was not visualized. S-MUAP consisted of negative and positive components, but only the negative peak amplitude of the components was measured as described above. The MUNE value was estimated by dividing the amplitude of the maximal CMAP by the averaged amplitude of S-MUAP.

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the course of this research.

Statistics

All data were reported in the text as means ± s.d. Statistical analysis was performed using paired *t*-tests, one-way analysis

of variance (ANOVA) and the Pearson correlation coefficient. The level of significance was set at $P<0.05$.

Results

Studies in controls

Forty-five healthy subjects attended the first test (test 1). The mean MUNE, CMAP and S-MUAP values of all controls were 188 ± 20, 6.4 ± 1.6 mV and 34 ± 6.0 μV, respectively. There were significant differences in the mean values for S-MUAP, but not CMAP and MUNE, between the young and old groups (Table 1). Specifically, the mean S-MUAP size in the young group was 32 ± 4.8 μV and that in the old group 40 ± 6.4 μV, and the corresponding values for CMAP were 6.1 ± 1.4 and 7.3 ± 1.9 mV, and those for MUNE were 190 ± 20 and 182 ± 21.

There were no significant differences ($P=0.66$) in the MUNE values between the female (190 ± 21) and male subjects (187 ± 20).

One week later, 10 young controls were randomly selected and re-examined. The test-retest correlation coefficient (r) for mean MUNE values was 0.927 ($P<0.01$). Thus, the results of mean MUNE values were highly reproducible in this group by using this method.

Studies in subacute SCI patients

Ten subacute SCI patients attended the first test (test 1), and they were re-examined at 1 (test 2) and 3 months later (test 3). The clinical and electrophysiological data for the subjects are presented in Table 2.

The mean MUNE, CMAP and S-MUAP values for test 1 were 40 ± 33, 1.4 ± 1.1 mV and 35 ± 5.0 μV, respectively, in these patients at 4 ± 1.72 months after SCI. For test 2, the mean MUNE, CMAP and S-MUAP values were 71 ± 37, 2.8 ± 2.2 mV and 33 ± 3.4 μV, respectively. For test 3, the mean MUNE, CMAP and S-MUAP values of those patients were 116 ± 41, 3.6 ± 1.7 mV and 31 ± 4.9 μV, respectively (Table 1) .The results of MUNE and CMAP sizes from the 10 patients who completed all three tests showed significant differences among the three tests ($P<0.05$). In contrast, the mean S-MUAP amplitudes showed no significant change in these patients over the same follow-up period.

Table 1 The results of MUNE, CMAP and mean S-MUAP amplitude for all subjects from the TA muscle

Group	*MUNE*	*CMAP (mV)*	*Mean S-MUAP (μV)*
Controls			
Young group (20–60 years)	190 ± 20	6.1 ± 1.4	32 ± 4.8
Old group (>60 years)	182 ± 21	7.3 ± 1.9	40 ± 6.4*
Subacute SCI group			
Test 1	40 ± 33	1.4 ± 1.1	35 ± 5.0
Test 2 after 1 month	71 ± 37*	2.8 ± 2.2*	33 ± 3.4
Test 3 after 3 months	116 ± 41*	3.6 ± 1.7*	31 ± 4.9
Chronic SCI group			
Test 1	173 ± 29	5.4 ± 1.2	31 ± 4.3
Test 2 after 3 months	177 ± 34	5.5 ± 1.2	31 ± 4.5

Abbreviations: CMAP, compound muscle action potential; MUNE, motor unit number estimation; S-MUAP, surface-recorded motor unit action potential; SCI, spinal cord injury; TA, tibialis anterior.
*$P<0.01$(within groups).

Studies in chronic SCI patients

Ten chronic SCI patients attended the first test (test 1). The same examination was repeated after 3 months (test 2). The clinical and electrophysiological data for the subjects are presented in Table 3.

For test 1, the mean MUNE, CMAP and S-MUAP values of these patients at 27 ± 13.00 months following injury were 173 ± 29, 5.4 ± 1.2 mV and 31 ± 4.3 μV, respectively. For test 2, the mean MUNE value was 177 ± 34, the mean CMAP amplitude was 5.5 ± 1.2 mV and the mean S-MUAP amplitude was 31 ± 4.5 μV (Table 1). Paired t-tests revealed no significant difference in any of the three values between tests 1 and 2.

Comparison among groups

The data from subacute and chronic SCI groups were also compared to that of the young controls. Age of subjects showed no significant difference among the three groups by using ANOVA analysis ($P=0.592$).

The mean CMAP, S-MUAP and MUNE values of subacute SCI (three tests), chronic SCI (two tests) and young control group were analyzed by using ANOVA. The mean MUNE and CMAP sizes of the subacute SCI patients were significantly smaller than those of young controls, as compared with chronic SCI patients ($P<0.01$) by a Tukey's *post hoc* test (Table 1). The mean S-MUAP amplitudes showed no significant difference ($P=0.899$) among the three groups (Table 1).

Discussion

MUNE of TA by AMPS

This study demonstrated that AMPS method is a valid and reliable method for obtaining a MUNE value. The CMAP, which is routinely used in nerve conduction study, is relatively insensitive to the degree of motor neuron loss because of the compensatory effects of collateral reinnervation. On the contrary, MUNE may be more sensitive than CMAP, as MUNE assesses the loss of lower motor neurons as well as the increase in MU action potential size produced by collateral reinnervation.

The TA muscle is a good choice for most MUNE methods, because the deep peroneal nerve is readily accessible to transcutaneous stimulation below the fibular head, and selective recordings from the muscle can be made without contamination from other simultaneously activated muscles such as the peroneus brevis and extensor digitorum brevis.[11] Additionally, as EMG of abductor hallucis and extensor digitorum brevis was not easily elicited in SCI patients,[14] the TA muscle was thus selected for test in this study.

The MUNE value of 190 ± 20 for the TA muscle (on the basis of negative-peak amplitude) is comparable to the results from previous studies which used other MUNE

Table 2 The clinical and electrophysiological data for 10 subacute SCI subjects obtained by the AMPS method from the TA muscle

Subject (no.)	*Age (years)*	*Sex (F/M)*	*Injury level*	*ASIA*[a]	*Duration*[b] *(months)*	*MUNE (no.)*	*CMAP amplitude (mV)*	*Mean S-MUAP amplitude (μV)*
1	33	M	C4	A	4	10	0.31	32
					5	59	1.83	31
					7	106	2.75	26
2	32	M	C8	B	7	17	0.76	46
					8	49	1.48	30
					10	66	1.98	30
3	42	M	C5	A	3	2	0.05	30
					4	21	0.81	39
					7	57	1.64	29
4	48	M	C6	B	5	107	3.43	32
					6	147	5.28	37
					8	164	6.73	41
5	41	M	C6	B	7	84	3.12	37
					8	120	3.73	31
					10	171	5.31	31
6	54	F	T6	B	3	22	0.89	40
					4	72	2.74	38
					6	118	2.72	23
7	39	M	T3	B	4	43	1.53	36
					5	69	2.13	31
					7	149	4.48	30
8	42	F	C4	A	5	40	1.50	37
					6	53	1.74	33
					8	146	5.12	35
9	24	M	T1	A	3	51	1.53	30
					4	70	2.17	31
					6	110	3.29	30
10	33	M	C5	A	1	26	0.88	34
					2	47	1.50	32
					4	71	1.98	28
Mean	39				5.6	76	2.45	33
s.d.	9				2.2	48	1.63	4.8

Abbreviations: AMPS, adapted multiple point stimulation; C, cervical; CMAP, compound muscle action potential; MUNE, motor unit number estimation; SCI, spinal cord injury; S-MUAP, surface-recorded motor unit action potential; T, thoracic; TA, tibialis anterior.
[a]Classification according to ASIA (American Spinal Injury Association, A, sensorimotor complete; B, motor complete, sensory incomplete).
[b]Duration of SCI at the time of recording (in months).

Table 3 The clinical and electrophysiological data for 10 chronic SCI subjects obtained by the AMPS method from the TA muscle

Subject (no.)	*Age (years)*	*Sex (F/M)*	*Injury level*	*ASIA*[a]	*Duration*[b] *(months)*	*MUNE (no.)*	*CMAP amplitude (mV)*	*Mean S-MUAP amplitude (μV)*
1	37	M	C4	A	18	184	5.90	32
					21	157	5.35	34
2	30	F	C3	B	50	146	4.68	32
					53	180	5.22	29
3	53	M	C4	A	21	201	7.23	36
					24	218	7.85	36
4	26	M	C7	A	35	193	6.75	35
					38	226	6.34	28
5	23	M	C3	B	47	212	6.36	30
					50	207	6.83	33
6	35	M	C4	A	18	139	3.20	22
					21	150	3.45	23
7	38	M	C6	B	18	178	5.69	32
					21	172	5.15	30
8	18	M	C7	B	12	129	4.39	34
					15	120	4.31	36
9	53	M	C5	B	24	155	5.11	33
					27	150	5.56	37
10	28	F	T4	A	38	194	5.04	26
					41	186	5.20	28
Mean	34				29.6	175	5.48	31
s.d.	12				13.1	31	1.19	4.3

Abbreviations: AMPS, adapted multiple point stimulation; C, cervical; CMAP, compound muscle action potential; MUNE, motor unit number estimation; SCI, spinal cord injury; S-MUAP, surface-recorded motor unit action potential; T, thoracic; TA, tibialis anterior.
[a]Classification according to ASIA (American Spinal Injury Association, A, sensorimotor complete; B, motor complete, sensory incomplete).
[b]Duration of SCI at the time of recording (in months).

techniques.[10,11] To our knowledge, two MUNE studies of the TA muscle had been reported. Trojaborg and Gooch[10] used the statistical method described by Daube and reported an estimate of 194 ± 5 MUs in the TA muscle in men and women aged between 21 and 80 years (mean age, 49 years). McNeil[11] reported that the MUNE values of the TA muscle at 25% maximum voluntary contractions were 153 ± 46 in 10 young men by the DE-STA method. The difference in MUNE value from these studies was suggested to be due to different recording methods employed.[15]

The S-MUAP size for the young controls was significantly larger than that in the old controls, indicating that each motor neuron innervates more muscle fibers in the latter. Although the difference did not reach significant level, a trend of reduction in MU number was seen in the old controls, indicating that ageing process might be accompanied with MUs loss. Similar loss was also demonstrated in the biceps brachii and the brachialis and thenar muscles in subjects over 60 years of age.[8]

The result of MUNE value was highly reproducible ($r = 0.927$) in the 10 young controls who received a reassessment at 1 week later.

Changes of MUNE values of the TA muscle following SCI

Two important implications may arise from the observed changes in MUNE value of the TA muscle following C3-T6 SCI: (1) the AMPS method appears to be useful in demonstrating the early loss in MUs in SCI; (2) the results of our longitudinal study showed that reversible transsynaptic degeneration of spinal circuits might occur distal to the site of SCI.

All subacute SCI patients showed a significant loss of MUs, as compared to that of young control subjects at the first test. Interestingly, there was a gradual increase in the MUNE values over the follow-up period in subacute SCI patients. Most chronic SCI subjects showed similar number of MUs as that of young controls during the first and the follow-up examination. Therefore, the AMPS method is useful for reflecting the dynamic changes in MUs after SCI.

There were only two reported non-longitudinal MUNE studies of SCI. Yang *et al*.[13] performed the thenar MUNE in 11 patients with cervical SCI using the McComas's method,[5] and found that eight patients had essentially normal MUs both in numbers and contractile properties, and others had variable level of MUs reduction. Some patients showed normal MUs long after the injury. Magnetic resonance imaging scans of the cervical spine provided independent evidences suggesting that patients with low MU values had sustained direct injury to the anterior aspect of the spinal cord at the relevant segmental levels. But there was no evidence of transsynaptic degeneration distal to SCI segmental levels. Hunter and Ashby[12] performed similar studies of MUNE in 11 patients with C4-T6 SCI, and found a reduction (5–125) in the number of functioning MUs in the abductor hallucis muscle. He suggested that transsynaptic degeneration could be a plausible explanation for this finding. However, we found that the MUNE value in the TA muscle decreased abruptly following C3-T6 SCI, even to 0 (no EMG response). Later, the M wave size increased gradually and MUNE value also increased. Although M wave remained smaller than normal size, MUNE value totally recovered to normal level at 1 year or later after SCI. The post-injury time in Hunter's study was from 2 months to 16 years, but mostly around 6 months. Therefore, his result was in line with this finding in subacute SCI patients. The averaged post-injury duration of patients in Yang's study[13] was more than 1 year. However, it may not be suitable for him to choose the thenar muscle for testing, as the thenar motoneurons may sustain direct or secondary injury after C5-6 SCI, other than transsynaptic degeneration.

In subacute SCI patients, we observed a progressive increase in the MUNE value that is accompanied with a gradually increased CMAP value over the follow-up period. The mean S-MUAP value increased slightly at the beginning (35 ± 5.0 μV), but decreased later (33 ± 3.4 μV). There was no significant change in S-MUAP, as compared to that of young controls (32 ± 4.8 μV). As the first examination was usually performed at several months after SCI (mean post-injury time: 4 months), compensatory reinnervations may have occurred during this period. In deed, on the basis of the analysis of the thickness of MU density, single fiber EMG recording study in SCI patients suggested a subsequent enlargement of the remaining MUs during the chronic stage.[16] This phenomenon is similar to that in peripheral neuropathies with Wallerian degeneration.[9] But the latter often showed more prominent changes in the mean S-MUAP value. Changes of the MUNE, CMAP and S-MUAP after SCI in this study were similar to that after stroke.[17] This process may be due to reversible transsynaptic degeneration and/or local functional depression caused by the shamming death of neurons or their functionally inactivation, but rather than a terminal collateral reinnervation of MUs remodeling. Transsynaptic degeneration and/or functional depression secondary to upper motor lesion may precede further MU loss in patients with severe lesions.[17] A progressive increase in the MUNE values might indicate a recovery in lower motor neuron function during the subacute stage of SCI.

Spinal cord injury may trigger multiple forms of plasticity. Synaptic strength could be modified in pre-existing circuits (synaptic plasticity), and new circuits might develop through sprouting and anatomical reorganization, including growth of axonal branches and dendrites. Neurological plasticity could extend from the cervical to the sacral cord.[18] Therefore, the plastic changes in MUs might underlie, at least partially, changes in the MUNE parameters after SCI, which were often presented as S-MUAP. However, neither increase in S-MUAP size nor changes of its value was found during the follow-up period in the subacute and chronic SCI group. Therefore, other unknown factors may also contribute to the alterations in MUNE parameters after SCI.

Conflicting evidence about transsynaptic degeneration of ventral horn neurons caudal to spinal transection or contusion were reported.[19,20] For example, retrograde labeling of soleus motoneurons with unconjugated cholera toxin B showed a 16% decrease in the number of labeled neurons ($P = 0.006$) after chronic (4 months) midthoracic spinal injury in rats.[4] It was suggested that the decreased number

of medium size (area, 545–914 μm^2) motoneurons could be due to cell loss or transsynaptic degeneration, transition of cell size, and/or failure of these motoneurons in the contused animals to transport the tracer. Methodological differences, including counting procedures, and differences in the tracer substances may also be accountable for the differences in the number of labeled cells in a motoneuron pool. Our studies implied that transsynaptic degeneration was not prominent or at least was reversible, as indicated by no significant loss of MUNE values in 1 year or even later time after SCI.

Local neuronal function depression caudal to the lesion site may also be an important factor causing the loss of MUNE. Function of local neuron caudal to SCI may recover gradually from complete depression during spinal shock. The upper motor neuron lesion decreased protein synthesis in the anterior horn cell which may produce a neuropathy. Thus, the distal site of axon and neuromuscular junction may be abnormal, whereas the proximal site may appear healthy. Furthermore, the lack of impulse activity in axon may lead to a decrease in the intra-axonal transport of acetylcholine and other trophic substances. Acetylcholine esterase activity within lumbosacral ventral horn neurons remained suppressed at 90 days after thoracic 11 spinal cord hemisection in adult rats.[21] Therefore, the depression of electric activity in the anterior horn cells, even with normal protein synthesis, may also lead to a loss of MUNE. However, subsequent compensatory neuronal plasticity and/or functional reorganization may help to reduce the deficit, as indicated by a progressive recovery of MUNE.

We are aware that the number of subacute SCI patients was small and their post-injury time was dispersed (1–8 months) in this study. Thus, it is difficult for one to detect detailed changes in S-MUAP after SCI. The mechanisms underlying the observed changes in MUNE after SCI also remain to be established in future studies. Study with a larger sample size would be helpful for evaluating the relevance between the MUNE value and functional outcome. MUNE might be able to predict functional changes in partially preserved area and the segment distal to the incomplete SCI. Similar to the changes in MUNE, the change of motor score (by ASIA neurological standards) in subacute SCI group also seems more prominent than that in the chronic SCI group.

Conclusion

To our knowledge, this is the first longitudinal study of SCI to investigate the dynamic changes of lower motor neuron function by using MUNE. SCI produces numerous functional deficits and alterations distal to the site of injury. The subsequent neurological plasticity may compensate the associated deficits and limit harmful outcomes. Our study suggests that transsynaptic degeneration may occur distal to the site of SCI, which may influence functional reorganization and contribute to the development of spasticity and hyperreflexia in skeletal muscles and chronic pain. The changes of MUNE values in the lower extremities following above T6 SCI might reflect combined changes in sensor, motor or urologic functional outcomes, though future study is needed to provide further information.

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