Research Article

Pattern of startle reflex to somatosensory stimuli changes after spinal cord injury

Yasin Abanoz¹, Yeşim Abanoz¹, Ayşegül Gündüz¹, Murat Uludağ², Nurettin İrem Örnek², Nurten Uzun¹, Halil Ünalın², Meral Kızıltan¹

¹Department of Neurology, Cerrahpaşa School of Medicine, Istanbul University, Istanbul, Turkey, ²Department of Physical Medicine and Rehabilitation, Cerrahpaşa School of Medicine, Istanbul University, Istanbul, Turkey

Introduction: Spinal cord injury (SCI) may cause functional changes at various levels in central and peripheral nervous systems. One of these changes is increased excitability above the lesion such as enhanced auditory startle responses (ASR). Startle response may also be obtained after somatosensory stimulus (startle reflex to somatosensory stimuli, SSS). In this study, we investigated changes of both ASR and SSS in SCI.

Method: We examined ASR and SSS in 14 patients with SCI and 18 age-matched healthy volunteers. SSS responses were recorded from orbicularis oculi (O.oc), sternocleidomastoid (SCM) and biceps brachii (BB) muscles by electrical stimulation of median nerve at the wrist. ASR was evoked by binaural auditory stimuli and recorded from O.oc, masseter, SCM and BB muscles. Probability, latency, amplitude and duration of responses were compared between two groups for each muscle.

Results: Presence of response over O.oc after somatosensory stimuli was decreased in patients compared to controls ($P = 0.004$). There were no differences in SSS responses of other muscles. ASR latency was shorter in masseter, SCM and BB in patients with SCI, but only BB had significantly reduced latency ($P = 0.033$). The duration of O.oc response was longer and the amplitude of SCM was larger in patients with SCI ($P = 0.037$ and $P = 0.015$, respectively).

Conclusion: ASR is enhanced after SCI whereas SSS of eye muscles is hypoactive and pattern of SSS after median stimulation changes in SCI.

Keywords: Spinal cord injury, Auditory startle responses, Startle reflex to somatosensory stimuli

Introduction

The startle reflex (SR) is a defensive response which develops after an unexpected stimulus and includes rapid flexion posture of body and extremities as well as occurrence of autonomic symptoms.¹ Startle reflex is generated in the structures of the caudal brainstem and propagates up to the motoneurons in the upper brainstem and down the spinal cord via reticulobulbar and reticulospinal tracts.

Spinal cord injury (SCI) leads to the destruction of corticospinal tract below the injury and segmental motoneurons at the level of the injury. Although regeneration of the interrupted neurons is possible, it constitutes the minor part of the recovery process after SCI and functional recovery usually follows reorganization of the spared pathways.² Experimental studies in animals have

shown that reorganization can occur at multiple levels, including cerebral cortex, brainstem or spinal cord.³ Viable corticospinal pathways were also shown to re-establish corticospinal transmission.⁴ Additional pathways which serve in functional restoration in rodents are rubrospinal, raphespinal and reticulospinal tracts.² Studies in humans demonstrated development of enhanced auditory startle response (ASR) and other brainstem reflexes after SCI suggesting reorganization of the reticulospinal pathways in humans.⁵⁻⁷

Startle reflex to somatosensory inputs (SSS) which is elicited by electrical stimulation of peripheral nerve or the skin of the body or limbs has been described in healthy volunteers⁸ and in some pathological conditions.⁹ The response recorded over orbicularis oculi (O.oc) muscle was initially defined as somatosensory evoked blink reflex (SBR).¹⁰ Normally, first response is evoked on O.oc, followed by muscles innervated by brainstem, upper extremity muscles and lower extremity

Correspondence to: Ayşegül Gündüz, Department of Neurology, I.U. Cerrahpaşa School of Medicine, Cerrahpaşa Medical Faculty, 34098, K.M.Pasa, Istanbul, Turkey. Email: draysegulgunduz@yahoo.com

muscles. The sequential development of responses is called as response pattern. Response patterns of SSS after median stimulation and ASR are similar and both reflexes are transmitted by reticulospinal tract, but they have different afferent pathways. We hypothesized that SSS may also be exaggerated in patients with SCI providing spared proximal segments of median nerve since efferent pathway of SSS, reticulospinal tract, shows reorganization and enhancement after SCI. Therefore, we planned a study to explore the characteristics and changes of SSS after median nerve stimulation in SCI below the segments forming median nerve and we also include recordings of ASR which has well-defined changes in SCI.

Subjects and method

Subjects

We included 14 patients with SCI. The inclusion criteria were complete SCI which was class A according to American Spinal Injury Association (ASIA) classification and localization within the thoracic or lumbar part of the spinal cord. The control group consisted of age- and sex-matched 18 healthy subjects who did not have any neurological or systemic diseases (M/F: 11/3 vs 10/8, $P = 0.266$). The mean age of patients and healthy subjects were 39.0 ± 9.8 and 35.4 ± 10.7 , respectively ($P = 0.342$).

The study was approved by the local ethical committee and all participants gave informed consent.

Clinical evaluation

We obtained the data regarding the level of the lesion, type of trauma, duration of SCI and all medications used at the time of investigation from the medical records. Level of the lesion was determined according to neurological examination and radiological findings.

Electrophysiological evaluation

All electrophysiological recordings were done with surface silver-silver chloride electrodes using Neuropack Sigma MEB-5504k (Nihon Kohden Medical, Tokyo, Japan). All subjects were examined under the same conditions; sitting on their own wheelchair (for patients) or armchair (healthy subjects) in a quiet room. They were asked to remain awake and relaxed. Both electrophysiological tests were performed in the same session.

Auditory startle response

We delivered monophasic 100 ms, 105-dB hearing level (HL) auditory tone burst stimulus which was produced by Neuropack Sigma MEB-5504k (Nihon Kohden Medical, Tokyo, Japan) bilaterally through earphones.

The stimuli were repeated eight times at random intervals of 2–5 min. The stimulus duration was increased 50 ms for every two stimuli while the subjects were sitting. The recordings were done simultaneously over the unilateral orbicularis oculi (O.oc), masseter, sternocleidomastoid (SCM) and biceps brachii (BB) muscles, while the ground electrode was placed over the sternum. Single sweeps of 500 ms were recorded. Filter settings were 10 and 10,000 Hz.

Startle reflex to somatosensory inputs (SSS)

We used a single electrical stimulus with a duration of 0.2 ms and with an intensity twice the level that evoked a motor response with the maximum amplitude and stimulated median nerve at wrist. The threshold value which is required to evoke a motor response was generally between 20–25 mA. Responses were recorded over the O.oc, SCM, and BB muscles. The ground electrode was placed on the sternum. The stimulus was given randomly as five consecutive bursts with a minimum interval of 20 seconds to prevent habituation. The filter settings were 10-kHz high-cut and 10-Hz low-cut. The analysis time was adjusted as 20–50 ms/div, and the amplitude sensitivity was 200–500 μ V.

Statistical analysis

The mean onset latencies, amplitudes, and durations of responses on each muscle and the presence rates of SSS and response rates of ASR were compared between the two groups. Reflex response was accepted as the response with 50 μ V amplitude from the baseline. Latencies were measured from the onset of the response whereas amplitudes were measured peak-to-peak.

The response rates (probability) were calculated as follows: Number of responses of muscle / Number of total recordings (8) $\times$ 100.

The presence rate was calculated as follows: Number of patients with response / Number of total patients (n) $\times$ 100.

Data analyses were performed using the SPSS 15 software statistical package (SPSS Inc., Chicago, IL, USA). Comparisons were made using the t-test for quantitative data with normal distribution, the Mann-Whitney *U* test for quantitative data with non-normal distribution, and the chi-squared test for qualitative data. Because three electrophysiological parameters (amplitude, latency, and duration) were tested in four muscles (O.oc, SCM, masseter, and BB), a Bonferroni-adjusted significance level of 0.004 was calculated for each electrophysiological investigation to account for the increased possibility of a type I error.

We have conducted a factorial ANOVA using different muscles and groups to disclose the pattern of startle responses.

Results

Clinical findings

All patients suffered from lesions which developed traumatically at the levels between spinal segments of T2–T12. Duration of SCI ranged from 8 months to 15 years. Nine patients were using oral baclofen with doses between 10 and 90 mg, four were on selective serotonin reuptake inhibitors and one patient was taking tizanidine. They used neither intrathecal baclofen nor gabapentin /pregabalin. Detailed clinical findings of all patients are presented in Table 1.

Electrophysiological findings

Auditory startle response

ASR showed normal sequence of muscle activation in both groups. Total ASR probability was not different between both groups, as well ($P = 0.593$). Probability of response over each muscle was similar between groups. Latency of masseter, SCM and BB responses were shorter in patients compared to healthy subjects, however, only BB showed statistical significance ($P = 0.033$). Duration of each response was longer and amplitudes were higher in the SCI group. Amplitude of SCM response was significantly higher in the patient group compared to healthy subjects ($P = 0.015$). Although the duration of O.oc, SCM and BB responses were longer, only O.oc response was significantly longer than controls ($P = 0.037$).

Startle reflex to somatosensory inputs (SSS)

Presence rate of O.oc response (SBR) was reduced in patients compared to healthy subjects ($P = 0.004$). Presence rates of responses of SCM and BB were similar between groups. There was no differences in SSS latencies between two groups for each muscles.

Table 2 shows all findings regarding ASR and SSS.

Discussion

The findings of our study are: 1. patients with SCI had exaggerated ASRs; 2. the probability of SBR was lower in patients with SCI compared to healthy subjects and there were no significant differences in other SSS responses.

An increase in the ASR has previously been observed in patients with SCI.^{5,6} The neural circuitry of ASRs comprises the auditory nerve, cochlear nucleus, nucleus reticularis pontis caudalis and efferent projections in the reticulobulbar and reticulospinal tracts.^{11,12} This circuit is under the neural control from

the basal ganglia via the pedunculopontine nucleus and superior colliculus, cerebellum and cerebral cortex. Normal recruitment pattern of ASR in SCI demonstrated normal neuronal reorganization of this circuit. However, widespread reorganization at all levels including spinal cord, brainstem and cortex which develops probably as a compensatory mechanism leads to a more prominent response in SCM and BB muscles, suggesting increased excitability. Kumru *et al.*⁵ concluded that increased excitability was probably related to the reorganization as well as direct and indirect plastic changes at the level of brainstem. A functional magnetic resonance imaging and transcranial magnetic stimulation study showed that there existed displacement of biceps area and excitability changes occurred in areas distant to the deafferented area.¹³ We previously showed high excitability of trigemino-cervical reflex in patients with SCI and attributed it to the changes which were required to adopt new positioning and to gather information coming from head and neck region for new positioning on earth.⁷ We assume that the increased excitability of ASR may also account for the reorganization for functional recovery as well as for the more efficient use of upper extremities after paraplegia since there are changing requirements after SCI such as use of wheelchair, or adaptation for new positioning.

SSS is elicited by electrical stimulation of a peripheral nerve or the skin of the body or limbs. After median nerve stimulation, afferent pathway is formed by the large diameter somatosensory fibers whereas efferent pathway is the reticulospinal tract. SBR is also triggered by a similar electrical stimulation and recorded over O.oc muscle.¹⁰ Therefore, we think that O.oc response of SSS may correspond to SBR. It was suggested that SBR is a basically programmed blink reflex for protecting eyes and it also reflects the integrity of upper brainstem reticular formation.¹⁴ The peripheral afferent pathway is probably of cutaneous origin, and the final output should be mediated by facial motor neurons; but the polysynaptic central pathways are uncertain. The generation of SBR was suggested to result from the combination of excitatory and inhibitory influences of exteroceptive stimuli, relatively independent of brainstem trigeminofacial interneuronal excitability and mediated by neuronal structures with integration capabilities within the brainstem.^{14,15} We found that probability of SBR was lower in patients with SCI than in healthy controls. First of all, we did not include patients with cervical SCI to prevent misdirecting information as a result of injured central pathway of median nerve somatosensory conduction. Nevertheless, SBR was still

Table 1 Detailed clinical findings of each patient with spinal cord injury.

	Sex	Age	Disease duration (years)	Level of neurological involvement	Oral medication
Patient 1	Male	22	4	T5	Baclofen
Patient 2	Male	24	3	T11	-
Patient 3	Male	48	1.5	T4	Baclofen
Patient 4	Male	40	0.75	T 10	Tizanidine, baclofen
Patient 5	Female	33	5	T2	Sertraline
Patient 6	Male	47	0.5	T12	Citalopram, baclofen
Patient 7	Male	28	0.75	T9-10	-
Patient 8	Male	40	1	T 11	Citalopram, baclofen
Patient 9	Male	49	5	T12	-
Patient 10	Male	41	15	T12	Baclofen
Patient 11	Male	55	9	T12	Baclofen
Patient 12	Female	36	13	T12	Baclofen
Patient 13	Male	46	4.5	T3-4	Citalopram, baclofen
Patient 14	Female	37	11	T10	-

suppressed despite the exaggerated ASR. Exaggerated ASRs which are in accordance with previous literature exclude the possibilities like the suppressive effects of medications used or other factors related to the included patient population. There may be several explanations for the suppression which may be attributed to the afferent pathway of SBR rather than the efferent pathway. First, injured somatosensory pathway may lead to

Wallerian degeneration of pathways originating from upper extremities. However, considering the different anatomical distribution of somatosensory pathways of lower and upper extremities,¹⁶ this probability is very low. Second, decreased proprioceptive inputs from lower extremities may decrease the operating capacity of upper extremity proprioceptive inputs on central generators. However, presence rates of responses of SCM

Table 2 Demographic and electrophysiological findings in patients and healthy subjects.

g	Patients with SCI n=14	Healthy subjects n=18	P
Age	39.0 ± 9.8	35.4 ± 10.7	0.342
Sex (M/F)	11/3	10/8	0.266
SSS			
Presence rate, n (%)			
O.oc (SBR)	8 (57.1)	16 (88.9)	0.040
SCM	7 (50.0)	7 (38.9)	0.530
BB	4 (28.6)	2 (11.1)	0.365
Latency, ms			
O.oc (SBR)	53.3 ± 16.0	52.6 ± 20.3	0.952
SCM	90.5 ± 24.1	94.3 ± 32.8	0.808
BB	90.1 ± 9.8	95.4 ± 48.9	0.823
ASR			
O.oc			
Latency, ms	35.5 ± 14.7	36.7 ± 9.4	0.952
Probability, %	92.8 ± 2.7	99.3 ± 2.9	0.821
Duration, ms	166.5 ± 166.6	63.9 ± 43.1	0.037
Amplitude, µV	481.7 ± 294.9	394.8 ± 210.6	0.511
Masseter			
Latency, ms	50.7 ± 3.8	70.6 ± 22.2	0.067
Probability, %	21.4 ± 37.3	25.0 ± 18.7	0.385
Duration, ms	75.0 ± 7.1	44.5 ± 21.0	0.355
Amplitude, µV	190	115.0 ± 54.2	0.181
SCM			
Latency, ms	59.2 ± 16.5	71.5 ± 19.9	0.120
Probability, %	37.5 ± 35.0	32.3 ± 23.4	0.857
Duration, ms	93.5 ± 55.9	66.0 ± 59.0	0.066
Amplitude, µV	479.5 ± 363.8	173.3 ± 116.4	0.015
BB			
Latency, ms	62.8 ± 12.8	92.8 ± 32.0	0.033
Probability, %	21.1 ± 34.7	24.7 ± 43.4	0.132
Duration, ms	68.3 ± 18.9	40.8 ± 16.1	0.157
Amplitude, µV	536.0 ± 428.7	111.3 ± 70.0	0.110
Total ASR (%)	50.1 ± 28.3	43.0 ± 13.2	0.593

and BB after somatosensory stimuli did not change. Therefore, this theory may not be acknowledged. Lastly, we may speculate that O.oc activity which provides visual information to be gathered probably depends on the stabilization and position on earth. Loss of positioning on earth in patients with SCI leads to a decline in O.oc activity. Previously, response of SCM muscle was shown to be stronger compared to O.oc response after stimulating lower limb in normal individuals.⁸ Authors attributed this finding to the different compositions of afferent volleys after stimulation of lower and upper limbs. After stimulating lower limbs muscle afferents are more determinative and less capable of stimulating O.oc due to dispersion related to the longer distance.⁸ Possibly, upper extremity afferents also become less capable of stimulating O.oc after SCI. In rats, neurons in the trigeminal sensory complex, cervical spinal cord and pontomedullary reticular formation have dual connections with neurons innervating eyelids, both for getting information and for contributing blink reflex.¹⁷ Given the known enhancement of reticulospinal pathway by ASR, hypoactive O.oc response after SCI may be anticipated to result from the decreased transmission between somatosensory pathway of median nerve and neurons innervating O.oc.

Peripersonal space is the space surrounding the subject in which the subject interacts with other people or objects. Excitability of SBR was demonstrated to increase when stimulated hand became closer to the eye within the peripersonal space.¹⁸ Hypoactive SBR in our study may be related to the shrinkage of peripersonal space after SCI which was shown around paraplegic limbs.¹⁹ Changes of peripersonal space after SCI requires further analysis and may affect rehabilitation process.

Regarding clinical correspondence of our findings, exaggerated SR, specifically ASR may be useful to augment voluntary movement in SCI as suggested by Kumru *et al.*⁵ However, somatosensory stimulation seems to be an inappropriate stimulation in this regard. Impaired control of eye closure and loss of sensory modulation of BR may expose eyes to traumas suggesting the need for special care.

There are certain limitations of the study. In some of the reflex studies, authors may use area-under-the-curve to analyze excitability.⁵ However, we are used to analyze amplitude and duration of the reflex response in our experience since amplitude and duration are also accepted as major contributors of the area²⁰ and our previous study demonstrated their sufficiency in analyzing excitability.²¹

In conclusion, pattern of SSS after median nerve stimulation changes after SCI even in the absence of cervical lesion and O.oc becomes less excitable probably due to change of peripersonal space.

Disclaimer statements

Contributors None.

Funding None.

Conflicts of interest None.

Ethics approval None.

References

- 1 Brown P, Rothwell JC, Thompson PD, Britton TC, Day BL, Marsden CD. New observations on the normal auditory startle reflex in man. *Brain* 1991;114(Pt 4):1891–902.
- 2 Fink KL, Cafferty WB. Reorganization of Intact Descending Motor Circuits to Replace Lost Connections After Injury. *Neurotherapeutics* 2016;13(2):370–81.
- 3 Nardone R, Höller Y, Brigo F, Seidl M, Christova M, Bergmann J, *et al.* Functional brain reorganization after spinal cord injury: systematic review of animal and human studies. *Brain Res* 2013;1504: 58–73.
- 4 Hilton BJ, Anenberg E, Harrison TC, Boyd JD, Murphy TH, Tetzlaff W. Re-Establishment of Cortical Motor Output Maps and Spontaneous Functional Recovery via Spared Dorsolaterally Projecting Corticospinal Neurons after Dorsal Column Spinal Cord Injury in Adult Mice. *J Neurosci* 2016;36(14):4080–9.
- 5 Kumru H, Vidal J, Kofler M, Benito J, Garcia A, Valls-Solé J. Exaggerated auditory startle responses in patients with spinal cord injury. *J Neurol* 2008;255(5):703–9.
- 6 Kumru H, Kofler M, Valls-Solé J, Portell E, Vidal J. Brainstem reflexes are enhanced following severe spinal cord injury and reduced by continuous intrathecal baclofen. *Neurorehabil Neural Repair* 2009;23(9):921–7.
- 7 Gündüz A, Uzun N, Örnek Nİ, Ünalın H, Karamemetoğlu ŞŞ, Kızıltan ME. Trigemino-cervical reflex in spinal cord injury. *Neurosci Lett* 2014;580:169–72.
- 8 Alvarez-Blanco S, Leon L, Valls-Solé J. The startle reaction to somatosensory inputs: different response pattern to stimuli of upper and lower limbs. *Exp Brain Res* 2009;195(2):285–92.
- 9 Kızıltan ME, Gunduz A, Apaydin H, Ertan S, Kızıltan G. Auditory startle reflex and startle reflex to somatosensory inputs in generalized dystonia. *Clin Neurophysiol* 2015;126(9):1740–5.
- 10 Miwa H, Imamura N, Kogahara K, Ohori T, Mizuno Y. Somatosensory evoked blink response: findings in patients with Miller Fisher syndrome and in normal subjects. *J Neurol Neurosurg Psychiatry* 1995;58(1):95–9.
- 11 Davis M, Gendelman DS, Tischler MD, Gendelman PM. A primary acoustic startle circuit: lesion and stimulation studies. *J Neurosci* 1982;2(6):791–805.
- 12 Koch M, Kungel M, Herbert H. Cholinergic neurons in the pedunculopontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. *Exp Brain Res* 1993;97(1):71–82.
- 13 Lotze M, Laubis-Herrmann U, Topka H. Combination of TMS and fMRI reveals a specific pattern of reorganization in M1 in patients after complete spinal cord injury. *Restor Neurol Neurosci* 2006;24(2):97–107.
- 14 León L, Casanova-Molla J, Lauria G, Valls-Solé J. The somatosensory blink reflex in upper and lower brainstem lesions. *Muscle Nerve* 2011;43(2):196–202.
- 15 Miwa H, Nohara C, Hotta M, Shimo Y, Amemiya K. Somatosensory-evoked blink response: investigation of the physiological mechanisms. *Brain* 1998;121(Pt 2):281–91.
- 16 Benarroch EE. Spinal and brainstem mechanism of motor control. In: Benarroch EE (ed). *Basic neurosciences with clinical*

- application: Butterworth Heinemann Elsevier, Philadelphia, 2006, pp 571–626.
- 17 Zerari-Mailly F, Dauvergne C, Buisseret P, Buisseret-Delmas C. Localization of trigeminal, spinal, and reticular neurons involved in the rat blink reflex. *J Comp Neurol*. 2003;467(2): 173–84.
- 18 Sambo CF, Forster B, Williams SC, Iannetti GD. To blink or not to blink: fine cognitive tuning of the defensive peripersonal space. *J Neurosci* 2012;32(37):12921–7.
- 19 Scandola M, Aglioti SM, Bonente C, Avesani R, Moro V. Spinal cord lesions shrink peripersonal space around the feet, passive mobilization of paraplegic limbs restores it. *Sci Rep* 2016;6:24126.
- 20 Kumru H, Valls-Solé J. Excitability of the pathways mediating the startle reaction before execution of a voluntary movement. *Exp Brain Res* 2006;169(3):427–32.
- 21 Kiziltan ME, Gunduz A, Apaydin H, Ertan S, Kiziltan G. Auditory startle reflex and startle reflex to somatosensory inputs in generalized dystonia. *Clin Neurophysiol* 2015;126(9):1740–5.