Neurological Sciences (2019) 40:305–310
https://doi.org/10.1007/s10072-018-3624-7

ORIGINAL ARTICLE

# Prepulse modulation and recovery of trigemino-cervical reflex in normal subjects

Meral E. Kızıltan[1] · Pınar Bekdik Şirinocak[2] · Tuba Akıncı[3] · Tuba Cerrahoğlu Şirin[4] · Burcu Nuran Arkalı[1] · Fatma Candan[5] · Ayşegül Gündüz[1]

Received: 7 July 2018 / Accepted: 25 October 2018 / Published online: 5 November 2018
© Springer-Verlag Italia S.r.l., part of Springer Nature 2018

## Abstract

**Objective** In this study, we analyzed the inhibitory control on the trigemino-cervical reflex (TCR), and whether or not prepulse modulation (PPM) has an effect on TCR. Thus, we studied the PPM of TCR. We hypothesized that TCR would presumably be under the modulatory effect after the prepulse stimulus similar to blink reflex (BR). We also studied the recovery of TCR which was previously shown.

**Methods** We included 13 healthy individuals. All subjects underwent recordings of TCR, TCR-PPM, and recovery of TCR. For TCR-PPM, a subthreshold stimulus to second finger 50 or 100 ms before the test stimulus was applied. For recovery of TCR, two stimuli at the infraorbital nerve were applied at 300, 500, and 800 ms interstimulus intervals (ISIs).

**Results** There was an inhibition of bilateral late responses of TCR at the ISIs of both 50 ms and 100 ms. There was no change of latencies. Full recovery of TCR did not develop even at the ISI 800 ms.

**Discussion** We have provided an evidence for the TCR-PPM in healthy subjects for the first time in this study. The prepulse inhibition is attributed to the functions of the pedunculopontine tegmental nucleus. Our study provides a strong indication that there are connections between pedunculopontine tegmental nucleus and trigemino-cervical circuit, which produces TCR.

**Keywords** Prepulse modulation (PPM) · Trigemino-cervical reflex (TCR) · Prepulse inhibition (PPI) · Recovery · Pedunculopontine tegmental nucleus

## Introduction

The prepulse modulation (PPM) is the inhibition or facilitation of a reflex response after the application of a subthreshold stimulus. Till now, the PPM of blink reflex (PPM-BR), the auditory startle reflex (ASR), the masseter inhibitory reflex, and the startle response to somatosensory inputs (SSS) have been investigated [1–3]. It is believed to reflect the filtering activity of brainstem or sensory processing. In experimental conditions in humans, PPM is obtained by stimulating the second finger before the supraorbital electrical stimulation or auditory stimulation at certain interstimulus intervals (ISIs). For the PPM-BR, there is a facilitation of R1 if prepulse stimulus is applied within 50 ms before the supraorbital stimulus, whereas inhibition occurs if prepulse stimulus is applied at longer ISIs (> 75 ms). The ASR can be obtained by recording from facial muscles as well as neck and extremity muscles [4]. From the rostral muscles down to the caudal muscles, probability of eliciting a response after auditory stimulation decreases. Probably due to the reduction in probability, most of the literature on the PPM of ASR covers the response from orbicularis oculi (O.oc) muscle. In the study of Valls-Sole and colleagues [1], authors report recording from masseter and sternocleidomastoid (SCM). Responses on masseter and SCM were also modulated after prepulse stimulus with a time course similar to that of the O.oc response in this study.

✉ Ayşegül Gündüz
draysegulgunduz@yahoo.com

[1] Department of Neurology, Cerrahpaşa School of Medicine, Istanbul University-Cerrahpaşa, Istanbul, Turkey

[2] Department of Neurology, Kocaeli Derince Education and Research Hospital, Istanbul, Turkey

[3] Department of Neurology, Beylikdüzü State Hospital, Istanbul, Turkey

[4] Department of Neurology, Sisli Hamidiye Etfal Education and Research Hospital, Istanbul, Turkey

[5] Department of Neurology, Goztepe Education and Research Hospital, Istanbul Medeniyet University, Istanbul, Turkey

The trigemino-cervical reflex (TCR) is a withdrawal reaction of the head and neck after different types of facial stimuli such as a glabellar tap or an electrical stimulation of supraorbital or infraorbital nerves [5–7]. Recordings are generally obtained from neck muscles or sometimes proximal arm muscles. Studies have reported different types of responses, several excitatory [5, 8], and one inhibitory (exteroceptive suppression) [9]. Leandri and colleagues [8] recorded three consecutive responses on splenius capitis muscle after infraorbital stimulation. Two late excitatory responses had latencies of 50–70 ms and 100–160, respectively [8], whereas an early response was obtained from neck muscles after mainly stimulation of infraorbital nerve [10]. Simultaneous stimulation of infraorbital and supraorbital nerves facilitates the generation of the early response [10]. Other studies also reported generation of late response with a latency of 40–60 ms from the neck or proximal arm muscles after the stimulation of supraorbital or infraorbital nerves [7]. One recent study has shown larger EMG reflexes with shorter latencies and larger kinematic responses were found after stimulating ophthalmic and maxillary skin sites than stimulating the mandibular region [11]. The reflex circuit of TCR involves trigeminal system and cervical spinal cord/bulbar motoneurons [12]. The structures in paramedian pontine reticular formation are possible integrating centers of the reflex. TCR is mainly responsible for defensive reaction of head or for positioning the head in the space in response to facial stimuli. In a previous study, we obtained bilateral and stable responses from the neck muscles after the unilateral infraorbital nerve stimulation in each healthy subject [13].

TCRs are suggested to be a part of the nocifensive complex and, thus, under the control of the "diffuse noxious inhibitory controls" (DNICs) system [14]. Excitability of the TCR was previously analyzed using recovery curves. The reported recovery ratio at 200 ms ISI was around 30% which increased with increasing ISIs. However, it was still below 60% at ISI of 1000 ms [14]. In another study, Serrao et al. [15] investigated the recovery of the late component of TCR after the supraorbital stimulation in healthy subjects and in patients with migraine. One study reported facilitation of the early component after paired stimulation in healthy subjects [8]. To the best of our knowledge, there are no further studies on the TCR recovery.

In this study, we aimed to investigate the inhibitory control on the TCR by applying prepulse stimulus and whether or not PPM has an effect on TCR. We recorded the PPM of the TCR in healthy subjects. We focused on the changes in the second response (with latency around 40–60 ms) because this was the most stable component in our recordings. We hypothesized that if TCR was under inhibitory control, prepulse stimulus would also produce an inhibition. We also studied the recovery of TCR to show that the excitability of TCR was normal in these healthy subjects.

## Subjects and method

**Subjects** We included 13 healthy individuals. Mean age was 37.8 ± 6.1 years (age range 26–48 years). There were four (30.7%) men. None of the participants had any psychiatric, systemic, or neurologic disorders.

The study was approved by the institutional review board, and informed consent was obtained.

**Electrophysiological investigations** All electrophysiological recordings were done using Neuropack Sigma MEB-5504k, Nihon Kohden Medical, Tokyo, Japan. All subjects were examined while they were sitting in an armchair in a quiet room. Surface silver-silver chloride cup electrodes were placed at the level of the C3 and C7 vertebrae for splenius capitis (SC) muscle. The ground electrode was on the clavicle. The filter settings were 3 kHz high-cut and 20 Hz low-cut. Analysis time was as 20 ms/div, and amplitude sensitivity was 100 μV. Recordings were repeated five times and obtained simultaneously over bilateral SC. All recordings in each trial were rectified and averaged for measurements. To determine the perceptive (sensory) threshold, 0.2 mA increase in stimulus intensity was done at each step and the intensity which was recognized by the subject in three successive stimulations was considered threshold. There were three blocks of recordings (baseline TCR, PPM-TCR, TCR recovery) with 20 min pauses between each block. The sequence of blocks was randomly changed in each subject.

**Baseline TCR recordings** For test stimulus, we used transcutaneous electrical stimulus with a duration of 0.5 ms which was applied on the left infraorbital branch of the trigeminal nerve. The intensity of the electrical shocks was 5 times the perceptive threshold of the infraorbital region. We have increased the stimulus intensity up to 50 mA when we could not get a proper response.

**PPM-TCR recordings** For prepulse stimulus, we used transcutaneous electrical shocks with a duration of 0.2 ms which were applied on the left second finger at ISIs of 50 ms and 100 ms before the test stimulus. The test stimulus was the same as in the baseline TCR recordings. We have chosen ISIs of 50 ms and 100 ms since there is a substantial knowledge about the PPM effect on BR at ISIs of 50 ms and 100 ms.

**TCR recovery recordings** We used transcutaneous electrical conditioning stimulus with a duration of 0.5 ms at ISIs of 300 ms, 500 ms, and 800 ms before the test stimulus. Both stimuli were applied at the infraorbital foramen. The intensities of both stimuli were the same as baseline TCR recordings. The choice of ISIs was done according to the previous knowledge of TCR recovery recordings [14, 15].

**Statistical analysis** All the measurements were checked by visual inspection. No trials were rejected (either because of noise or artifact, or a failure to respond). Onset latency, duration, and amplitude of second TCR response were measured using cursors. Area-under-the curve (AUC) was calculated online after placing cursors at onset and end of response.

The following parameters were compared between the recordings at baseline, at the ISI of 50 ms PPM and at the ISI of 100 ms PPM.

i. Onset latency
ii. Amplitude
iii. Duration
iv. AUC

The statistical analysis was performed by the SPSS (Statistical Package for the Social Sciences) for Windows 20 package program. First, we checked the normality of the values using Shapiro-Wilk test. Then, because the distribution was homogenous, we performed factorial repeated measures ANOVA using ISIs as a within-subject factor and side as a between-subject factor. For post hoc analysis, Bonferroni test was used.

For the recovery recordings, we calculated recovery ratio by using the following formula: AUC after test stimulus/AUC after conditioning stimulus × 100. We compared recovery ratio between the ISIs of 300 ms, 500 ms, and 800 ms by factorial repeated measures ANOVA using ISIs as a within-subject factor and side as a between-subject factor.

$p$ value < 0.05 was deemed significant.

## Results

**Baseline TCR recordings** We obtained bilateral late (second) responses in all participants (with latency of 40–60 ms, Table 1).

**PPM-TCR recordings** Comparisons of parameters of late responses showed no difference between latencies after prepulse stimulation, whereas amplitude was reduced by applying a prepulse stimulus with ISI of 50 ms or ISI 100 ms ($p$ = 0.000) compared to the recordings at baseline (Fig. 1a) on both sides. The duration of SC response was also shortened during PPM recordings at ISIs of 50 ms and at ISI 100 ms ($p$ = 0.011) compared to recordings at baseline (Fig. 1b). As expected, AUC was also reduced during PPM recordings at ISI 50 ms or at ISI 100 ms ($p$ = 0.000) recordings (Fig. 1c). There was total inhibition in four and five subjects at ISIs of 50 ms and 100 ms, respectively. There were no differences between sides. Figure 2 shows a representative example of prepulse inhibition (PPI) of TCR.

**TCR recovery recordings** Mean recovery ratio at the ISI of 300 ms was 18.2 ± 27.2%, whereas it was around 30% at ISIs of 500 ms and 800 ms. On the contralateral side, mean recovery ratio was 30.4 ± 37.7% and it was around 50% at 800 ms ISI. There were no side differences. The recovery rates between each ISI was statistically similar ($p$ = 0.152).

## Discussion

The major findings of our study are as follows: (i) there is PPI of the second component of TCR, (ii) prepulse inhibition occurs at both ISI 50 ms and ISI 100 ms, (iii) even after the unilateral stimulation, the prepulse inhibition of late response occurs bilaterally with a similar reduction in magnitude, and (iv) there was no change in latencies.

Bilateral TCR responses with a mean latency of 49–52 ms were elicited after the left-sided infraorbital stimulation in all subjects. After the prepulse stimulation of second finger, the magnitudes of TCRs on both sides were reduced simultaneously. Regarding the PPM-BR after trigeminal stimulation, the magnitude of R2 (late) response to the test stimulus is reduced when another stimulus is applied at a different location at a certain time interval compared to the condition when the stimulus is applied alone. For the stimulation of second finger before the supraorbital stimulation, ISIs beyond 75 ms fully produce inhibition of late responses of BR. This reduction in magnitude supposedly enables the transmission of the first input to the higher centers [16]. Thus, it represents the activity of centers probably in the brainstem which are involved in the sensory processing and filtering activity. Although some studies claimed it also reflected sensorimotor gating, this issue is still controversial. For the second component of TCR, there is a similar pattern of PPI. However, there are two major differences: the TCR PPI seems to develop at least beyond 50 ms and there is no change of latency.

The demonstration of the filtering capacity of the brainstem operating on TCR suggests that filtering activity may be nonspecific. From the psychologic point of view, prepulse inhibition is considered to be a protective mechanism in sake of the first stimulus [16]. Therefore, the first stimulus is presumably expected to be protected against any kind of second stimulus (again trigeminal in our study) in any kind of response (TCR in this case). Second, TCR is a part of head retraction reflex. It provides a defensive reaction in response to facial stimuli [6, 7]. However, it also functions in the orientation of the head in space and in the adaptation of cervical tone in response to any change in the environment [17, 18]. Thus, the alteration of TCR after other types of sensory inputs to the body is required to properly adapt the position of the head and neck in any specific situation.

The PPI is attributed to the functions of pedunculopontine tegmental nucleus. Previous studies of ASR showed inhibition of the O.oc response after the auditory or lower limb tactile

**Table 1** The findings of baseline trigemino-cervical reflex recordings

| | Latency (ms) | Amplitude (μV) | Duration (ms) | AUC (mV ms) |
|---|---|---|---|---|
| Left SC | 50.8 ± 11.6 | 325.0 ± 160.2 | 56.2 ± 22.7 | 1.8 ± 1.2 |
| Right SC | 49.3 ± 16.0 | 401.9 ± 153.9 | 52.7 ± 12.3 | 2.5 ± 1.4 |

The values are given as mean ± SD

*SC*, splenius capitis

prepulse stimulation suggesting a modality-nonspecific common neural tollgate within the brainstem interneurons [19]. The authors concluded that a key relay station would synchronize in a nonspecific way for the surrounding environment to ensure an appropriate PPI within the central nervous system. The reflex circuit of TCR involves trigeminal system and cervical spinal cord/bulbar motoneurons [12]. Hypothetically, TCR is integrated and mediated by cholinergic neurons of the paramedian pontine reticular formation, gigantocellular reticular nucleus, or the intermediate reticular zone [20]. In animals, there are projections from trigeminal sensory complex to the nuclei in the reticular formation [21, 22]. Our study suggests that there should be a connection between the circuit of TCR and pedunculopontine tegmental nucleus, and that the relay station is also reflex-nonspecific and has connections with various brainstem reflexes.

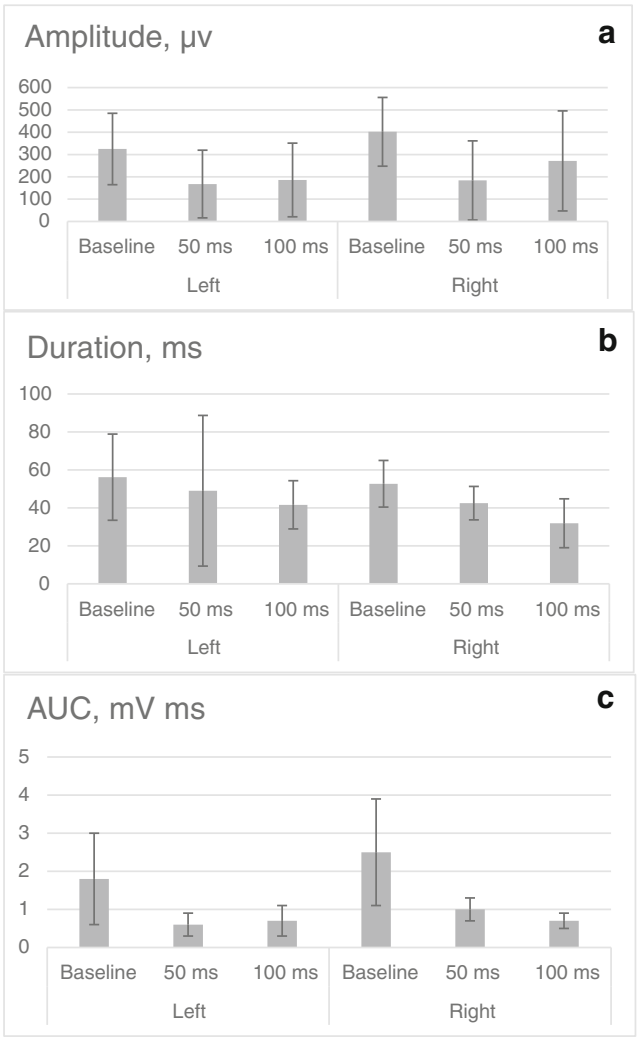


**Fig. 1** Alteration of amplitude, duration, and AUC parameters of TCR responses after prepulse stimuli at 50 and 100 ms interstimulus intervals (data are given as mean ± SD). **a** Amplitude, μv. **b** Duration, ms. **c** AUC, mV ms

Interestingly, regarding PPI, TCR behaves similar to SSS rather than being similar to a segmental reflex. After stimulating a limb, there are two different responses: a startle response originating in the nucleus reticularis pontis caudalis and a withdrawal reaction [3]. The same study showed the SSS was inhibited after a conditioning (prepulse) stimulus, whereas PPI did not have effect on the withdrawal reaction [3]. The authors concluded that the PPI was more effective on polysynaptic reflexes but less effective on monosynaptic reflexes as proposed by Ge and colleagues [23]. They also pointed to another explanation that the PPI had a larger effect on responses generated at brainstem centers rather than on responses integrated in spinal cord interneurons [3]. Our findings confirm that the TCR is governed by polysynaptic connections in the brainstem similar to startle responses. The similar pattern of inhibition may raise the question that the TCR may be a part of startle response. Although there are other similarities such as habituation [14], we cannot directly draw conclusions based on our findings since this issue is beyond our topic. However, recording the muscle recruitment caudally after the trigeminal stimulation as well as the PPI effect on upper and lower extremity responses may be interesting.

We know that the TCR is elicited by the supraorbital branch or ophthalmic and mandibular branches as well as infraorbital nerve [10, 11]. We only investigated the PPM of infraorbital branch of maxillary division. It would be interesting to investigate whether or not TCRs obtained after stimulation of other branches were modulated similarly. Another limitation is the absence of early TCR responses. We did not analyze the early responses because we did not obtain these responses consistently. Although we know simultaneous stimulation of supra- and infraorbital branches facilitates early responses, simultaneous application of three electrical stimuli is technically impossible for us.

The recovery cycle of TCR in our study was very similar to those in previous studies. The BR recovery occurs a bit earlier [14, 15]. We think longer ISIs are required to obtain a full recovery of TCR. We only recorded the recovery of TCR in

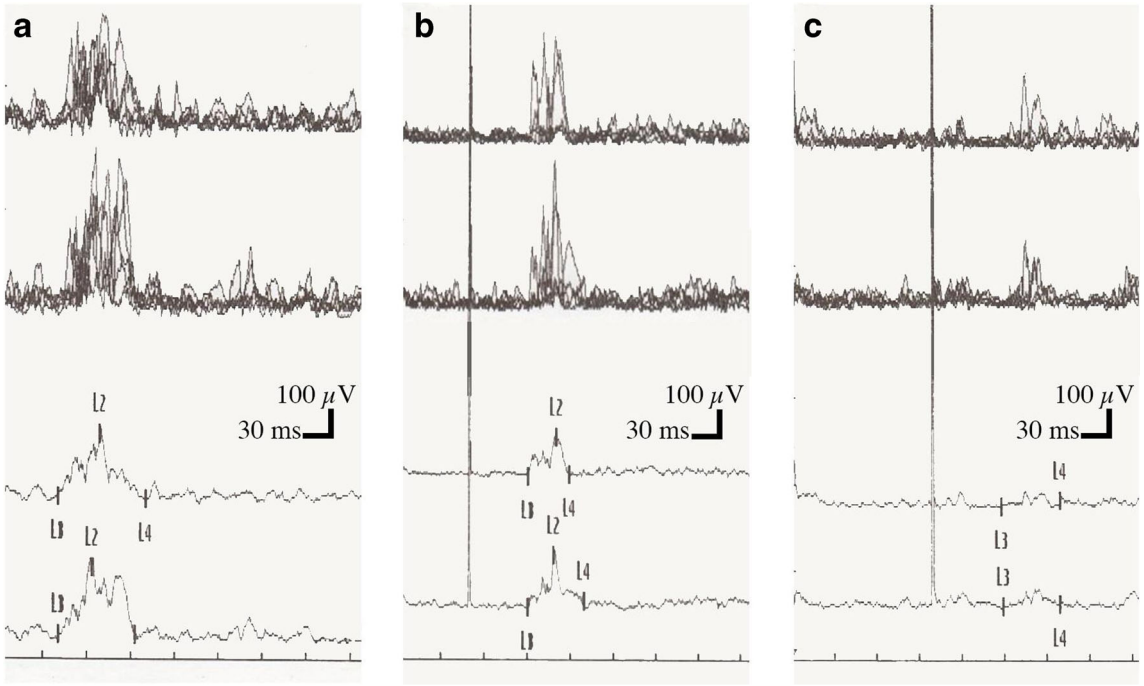


**Fig. 2** A representative example of baseline TCR (**a**), prepulse inhibition of blink reflex at 50 ms (**b**), and at 100 ms (**c**) ISIs in a 44-year-old healthy man (top two, rectified non-averaged recordings; bottom two rectified averaged recordings of left and right splenius capitis)

these healthy subjects to show that excitability of TCR was similar to that reported in previous studies. However, we should accept the fact that omitting the shorter time intervals possibly prevents the verification of facilitations and omitting the longer time intervals prevents the identification of the time required for a full recovery.

In conclusion, we have provided the evidence of the TCR-PPM in healthy subjects for the first time in this study. Further studies covering anatomical attributes and alterations in pathological conditions will be interesting.

**Acknowledgements** The authors thank Zeynep Değirmenci, Sibel Kilinc, Vildan Altop, Mecit Sepetcioglu, and Esat Cetinkaya for excellent technical assistance.

## Compliance with ethical standards

The study was approved by the institutional review board, and informed consent was obtained.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Valls-Sole J, Valldeoriola F, Molinuevo JL, Cossu G, Nobbe F (1999) Prepulse modulation of the startle reaction and the blink reflex in normal human subjects. Exp Brain Res 129:49–56
2. Gómez-Wong E, Valls-Solé J (1996) Effects of a prepulse stimulus on the masseteric inhibitory reflex in humans. Neurosci Lett Apr 208:183–186
3. Alvarez-Blanco S, Leon L, Valls-Solé J (2009) The startle reaction to somatosensory inputs: different response pattern to stimuli of upper and lower limbs. Exp Brain Res 195: 285–292
4. Brown P, Rothwell JC, Thompson PD, Britton TC, Day BL, Marsden CD (1991) New observations on the normal auditory startle reflex in man. Brain 114:1891–1902
5. Di Lazzaro V, Restuccia D, Nardone R, Tartaglione T, Quartarone A, Tonali P, Rothwell JC (1996) Preliminary clinical observations on a new trigeminal reflex: the trigemino-cervical reflex. Neurology 46:479–485
6. Ertekin C, Celebisoy N, Uludağ B (2001) Trigeminocervical reflexes elicited by stimulation of the infraorbital nerve: head retraction reflex. J Clin Neurophysiol 18:378–385
7. Sartucci F, Rossi A, Rossi B (1986) Trigemino cervical reflex in man. Electromyogr Clin Neurophysiol 26:123–129
8. Leandri M, Gottlieb A, Cruccu G (2001) Head extensor reflex evoked by trigeminal stimulation in humans. Clin Neurophysiol 112:1828–1832
9. Nakashima K, Thompson PD, Rothwell JC, Day BL, Stell R, Marsden CD (1989) An exteroceptive reflex in the sternocleidomastoid muscle produced by electrical stimulation of the supraorbital nerve in normal subjects and patients with spasmodic torticollis. Neurology 39:1354–1358
10. Di Lazzaro V, Quartarone A, Higuchi K, Rothwell JC (1995) Short-latency trigemino-cervical reflexes in man. Exp Brain Res 102: 474–482
11. Serrao M, Cortese F, Andersen OK, Conte C, Spaich EG, Fragiotta G, Ranavolo A, Coppola G, Perrotta A, Pierelli F (2015) Modular organization of the head retraction responses elicited by electrical painful stimulation of the facial skin in humans. Clin Neurophysiol 126:2306–2313
12. Serrao M, Rossi P, Parisi L, Perrotta A, Bartolo M, Cardinali P, Amabile G, Pierelli F (2003) Trigemino-cervical-spinal reflexes in humans. Clin Neurophysiol 114: 1697–1703

13. Kızıltan ME, Gunduz A, Kızıltan G, Tekeoğlu A, Sohtaoğlu M (2014) Brainstem and spinal reflex studies in patients with primary progressive freezing of gait. J Neurol Sci 343:51–55
14. Serrao M, Coppola G, Di Lorenzo C, Di Fabio R, Padua L, Sandrini G, Pierelli F (2010) Nociceptive trigeminocervical reflexes in healthy subjects. Clin Neurophysiol 121:1563–1568
15. Serrao M, Perrotta A, Bartolo M, Fiermonte G, Pauri F, Rossi P, Parisi L, Pierelli F (2005) Enhanced trigemino-cervical-spinal reflex recovery cycle in pain-free migraineurs. Headache 45:1061–1068
16. Graham FK Presidential Address, 1974(1975) The more or less startling effects of weak prestimulation. Psycophysiology 12:238–248
17. Prentice SD, Drew T (2001) Contributions of the reticulospinal system to the postural adjustments occurring during voluntary gait modifications. J Neurophysiol 85:679–698
18. Drew T, Prentice S, Schepens B (2004) Cortical and brainstem control of locomotion. Prog Brain Res 143:251–261
19. Leon-Sarmiento FE, Peckham E, Leon-Ariza DS, Bara-Jimenez W, Hallett M (2015) Auditory and lower limb tactile prepulse inhibition in primary restless legs syndrome: clues to its pathophysiology. J Clin Neurophysiol 32:369–374
20. Serrao M, Di Fabio R, Bartolo M, Perrotta A, Tassorelli C, Coppola G, Davassi C, Padua L, Sandrini G, Pierelli F (2011) The contribution of trigemino-cervical reflexes in distinguishing progressive supranuclear palsy from multiple system atrophy. Clin Neurophysiol 122:1812–1815
21. Meredith MA, Wallace MT, Stein BE (1992) Visual, auditory and somatosensory convergence in output neurons of the cat superior colliculus: multisensory properties of the tecto-reticulo-spinal projection. Exp Brain Res 88:181–186
22. Sasaki S, Yoshimura K, Naito K (2004) The neural control of orienting: role of multiple-branching reticulospinal neurons. Prog Brain Res 143:383–389
23. Ge HY, Collet T, Mørch CD, Arendt-Nielsen L, Andersen OK (2007) Depression of the human nociceptive withdrawal reflex by segmental and heterosegmental intramuscular electrical stimulation. Clin Neurophysiol 118(7):1626–1632