

R3 COMPONENT OF THE EYE BLINK REFLEX IN VARIOUS PAINFUL CONDITIONS

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ABSTRACT

• **Objective:** We investigated R1, R2 and especially R3 responses of blink reflex in various painful conditions to understand the eligibility of R3 component in electrophysiological pain studies.

• **Material and Method:** A total of 88 patients and 23 healthy control subjects were included. The patients were classified into 4 groups (diabetic neuropathic pain, tension type headache, lumbar and cervical radiculopathy) based on their painful complaints. By supraorbital nerve stimulation unilateral R1, bilateral R2, bilateral R3 responses were recorded.

• **Results:** R1-R2 response latencies were within the normal range among all patient groups. Prevalence of R3 response

was significantly higher in all patient groups with neuropathic pain. Patients with diabetic neuropathic pain had R2 and R3 responses at higher stimulus intensities. Prevalence of R3 response increased at and above the subjective pain threshold. No significant difference was found in terms of pain thresholds and stimulus intensity among the patient groups and controls.

• **Conclusion:** Short latency and high prevalence of R3 in patients with pain, high stimulus R2-R3 intensity in diabetic neuropathic patients and high stimulus intensity of R3 compared to the subjective pain threshold, may suggest R3 can be used as a neurophysiological marker in nociceptive system dysfunctions.

• **Key Words:** Blink reflex, pain, radiculopathy, diabetic neuropathy Nobel Med 2010; 6(1): 68-73

DEĞİŞİK AĞRILI DURUMLARDA GÖZ KIRPMA REFLEKSİNİN R3 KOMPONENTİ

ÖZET

• **Amaç:** Değişik ağrılı durumlarda göz kırpma refleksinin R1, R2 ve özellikle R3 komponentini inceleyerek, R3'ün elektrofizyolojik ağrı çalışmalarında kullanmaya uygun olup olmadığını araştırdık.

• **Materyal ve Metod:** Toplam 88 hasta ve 23 sağlıklı kontrol çalışmaya alındı. Hastalar ağrı şikâyetlerine göre diyabetik nöropatik ağrı, gerilim tipi baş ağrısı, lomber ve servikal radikülopati olmak üzere 4 gruba ayrıldı. Supraorbital sinir uyarımı ile göz kırpma refleksinin unilaterale R1 ve bilaterale R2, R3 komponentleri kaydedildi.

• **Bulgular:** Tüm hasta gruplarında R1 ve R2 latensleri

normal sınırlar içindeydi. Nöropatik ağrısı olan tüm hasta gruplarında R3 sıklığı fazlaydı. Diyabetik nöropatik ağrısı olan hastalarda R2 ve R3 daha yüksek akım şiddeti ile alınıyordu. Subjektif ağrı eşiği ve üzerinde R3 prevalansı artıyordu. Ağrı eşiği ve akım şiddeti açısından hasta grupları arasında ve kontrol grubunda anlamlı bir fark saptanmadı.

• **Sonuç:** Ağrılı hastalarda R3'ün rastlanma sıklığının fazla ve latansının kısa olması, diyabetik nöropatik ağrılı hastalarda ve subjektif ağrı eşiğiyle karşılaştırıldığında R2-R3'ün yüksek akım şiddeti ile alınması nosiseptif sistem disfonksiyonunda R3'ün nörofizyolojik bir belirteç olarak kullanılabileceğini göstermektedir.

• **Anahtar Kelimeler:** Göz kırpma refleksi, ağrı, radikülopati, diyabetik nöropati Nobel Med 2010; 6(1): 68-73

INTRODUCTION

Pain, being a subjective sensation, various scales are developed to measure the patient's self-reported pain. These tests, although valid and reliable, are dependent on the patients' cooperation and collaboration. Electrophysiological examinations such as nerve conduction studies, somatosensory evoked potentials, micro-neurography, laser-evoked potentials, nociceptive reflexes and functional neuroimaging are useful to demonstrate, locate damage along the peripheral or central sensory pathways objectively.¹ However, some do not assess function of nociceptive pathways, some are time-consuming and difficult, some are available in too few centers and some are expensive. Among the nociceptive reflexes, although its nature is controversial, blink reflex (BR) is a noninvasive, commonly available method. We know that the R3 component of the BR can be evoked by noxious stimulation as well as it can be elicited by innocuous stimuli. Some authors claimed the R3 is a pain-related ultralate reflex that is nociceptive in origin.^{2,3} On the contrary, Maria J. Téllez investigated the presence of the R3 component in two patients with congenital insensitivity to pain and proposed the R3 component of the blink reflex is mainly evoked by stimulating cutaneous A δ fibers rather than by nociceptive fibers and the R3 response cannot be used in the studies of pain to learn about nociceptive processing in the brain stem.⁴ These speculations lead authors to learn more about the unclear origin of the R3 component and nociceptive processing in the brain stem.

Within this respect, we evaluated: (1) R1, R2 and R3 response latencies of BR in various neuropathic painful conditions; (2) The presence of the R3 response in

patients; (3) The prevalence of R3 response with symptomatic duration of pain.

MATERIAL and METHOD

The study comprises 88 patients who referred to the Istanbul University, Cerrahpasa Faculty of Medicine, Neurology Department, EMG Laboratory. Twenty-three healthy people having no neurological complaints and pain were included in the control group. The patients were classified based on their painful complaints (Table1).

According to this classification, Group 1 included patients with diabetic neuropathic pain (DNP), Group 2 included patients with tension-type headache (TTH) only who met IHS criteria, Group 3 included patients with cervical radiculopathy (CR) and Group 4 included patients with lumbar radiculopathy (LR). Neurologic examination was performed and histories were obtained from all patients.

Diabetic neuropathy group: Patients had sensory-motor polyneuropathy, sensorial and autonomic neuropathy in varying degrees. Nerve conduction studies were performed on all the patients. Except for of two patients, all had sensorimotor polyneuropathy findings. Neuropathic pain diagnosed clinically in all patients with diabetes mellitus.

Tension type headache group: Patients fulfilled the diagnostic criteria of the IHS (The International Classification of Headache Disorders, 2nd edition) for TTH. Cranial MR was performed on 22 patients except one, and produced normal findings. EMG was not performed on this patient group. →

Table 1: Painful conditions and patient data						
Patients	M / F	Total	Age range	Mean age	Mean complaint time	Workup
DNP	9 / 14	23	17-62	46,57±11,96		EMG
TTH	5 / 18	23	24-52	40,83±8,76	6,26±4,66	MRI
CR	4 / 17	21	25-58	41,43±10,04	2,82±3,20	MRI, EMG
LR	6 / 15	21	33-60	45,88±6,64	6,48±7,76	MRI, EMG
Total	24 / 64	88				
CONTROL	16 / 7	23	26-59	39,13±8,76	NONE	NONE

M: Male, F: Female, DNP: Diabetic neuropathic pain, TTH: Tension-type headache, CR: Cervical radiculopathy, LR: Lumbar radiculopathy

Table 2: Right/left R1 R2 and R3 response latencies						
Latencies	DNP	CR	LR	TTH	Controls	p
Right						
R1	10,87±1,38	10,75±2,03	10,28±1,52	11,09±1,45	10,33±1,08	.319
R2	31,03±4,05	30,03±3,49	26,81±8,50	30,58±3,26	30,37±3,35	.042
R3	68,96±12,38	63,68±8,30	56,52±8,12	71,99±17,52	55,50±2,12	.000
Left						
R1	10,98±1,31	11,04±2,06	10,38±1,23	10,74±1,19	9,33±0,77	.040
R2	31,59±3,86	31,46±7,96	29,48±2,39	32,05±3,12	29,83±3,50	.237
R3	68,90±10,09	65,59±9,42	55,45±6,78	68,81±11,38	63,45±17,37	.000

Table 3: Comparison of the right/left R3 response rates among the patient groups and to the controls						
	DN	CR	LR	TTH	Controls	p
Right						
P						
Negative	6 ± 26,1	5 ± 23,8	4 ± 19,0	2 ± 8,7	20 ± 87,0	
Positive	17 ± 73,9	16 ± 76,2	17 ± 81,0	21 ± 91,3	3 ± 13,0	0,000
Left						
Left						
Negative	4 ± 17,4	3 ± 14,3	2 ± 9,5	4 ± 17,4	19 ± 82,6	
Positive	19 ± 82,6	18 ± 85,7	19 ± 90,5	19 ± 82,6	4 ± 17,4	0,000

Cervical radiculopathy group: The diagnosis was based on clinical evaluation together with EMG and MRI workup. Patients suffered from varying degrees of bilateral or unilateral radicular pain.

Lumbar radiculopathy group: Lumbar MRI was performed on 20 patients and one of the patients had normal MRI findings. Lumbar disk hernia was detected in 15 patients. There was a narrow lumbar spinal canal in one of the patients. Patients suffered from varying degrees of bilateral or unilateral radicular pain.

Control group: This group included 23 healthy subjects (16 males, 7 females). The range of age was 26-59, while mean age was 39.13±8.76. Patients had no neurological complaint. None of the patients suffered from acute or chronic pain. Neurological examination was normal in all the subjects. Patients and control subjects gave their informed consent prior to their inclusion in the study according to the 1964 Declaration of Helsinki.

Procedures

Patients were ordered to lie down in a supine position on the examination table and close their eyes slightly for recording the blink reflex. Surface electrodes were positioned over the orbicularis oculi muscle at the active pupil level, positioning the reference electrode at 2 cm-lateral and applying bilateral conductor gel (Elefix). Ground electrodes were placed on wrists. Supraorbital nerve was stimulated over percutaneous supraorbital foramen by a cathode electrode. The study was performed using four-channel “Neuropack”^Σ EMG device with filter of 50-3000 Hz, sensitivity of 200 mV/division, analysis time of 200 ms, sweep speed of 200 ms/division, and single stimulus of 0.1 ms.

1) Electrical stimulation of BR was assessed by randomized interstimulus intervaller, starting from 0mA. The interval was adjusted >10 ms to avoid inhibition of delayed motor responses. Individual thresholds for detection (I_0), pain (I_p), R1 component (I_{R1}), R2 (I_{R2}), and R3 (I_{R3}) were determined by applying electrical pulses with de- and increasing stimulus intensity using increments of 0.5mA.

2) Minimum ten different BR responses were superimposed and latencies of unilateral R1, bilateral R2 and consensual R2 (R2C), bilateral R3 and consensual R3 (R3C) responses were recorded.

3) Suppression of synchronous R2 and R3 components was assessed focusing attention on the electrical stimulation and then distracting attention in the patient group and controls. Test stimulus intensity was adjusted to a level clearly below the pain threshold. The procedure was repeated for both eyes.

Biostatistical analysis

SPSS for Windows 10.0 statistics program is used for the data analysis. ANOVA, Kruskal Wallis and chi-square tests were used to compare the data. Pearson test was also used in the correlation test. $p < 0.05$ value was considered to be significant.

RESULTS

A. Right/left R1, R2 and R3 response latencies of BR were compared among the groups and to the controls. We found the right/left R1-R2 response latencies to be within normal range among all patient groups and in the control group (Table 2). The right/left R3 response latencies were significantly shorter in patients with LR and controls compared with other patient groups ($p=0.042$ for the right R2; $p=0.000$ for R3; $p=0.040$ for the left R1 and $p=0.000$ for R3). →

Table 4: Comparison of the patient groups in terms of possible association between the stimulus intensity of the right side R3 response of BR and subjective pain threshold

	DNP		CR		LR		TTH	
	R2	R3	R2	R3	R2	R3	R2	R3
RRp	0,68	0,75	0,28	0,39	0,57	0,11	0,09	0,09

B. R3 response was found to be significantly higher in all patient groups with neuropathic pain compared with the controls (right/left $p=0.000$). The prevalence of R3 component was similar in the patient groups (Table 3).

C. The stimulus intensity necessary to get R2-R3 responses and subjective pain threshold was compared among the patient groups (Table 4). Patients with diabetic neuropathy had R2 and R3 responses at higher stimulus intensities compared with the other patient groups and controls. Prevalence of R3 response increased at and above the subjective pain threshold. Patients with lumbar radiculopathy and controls had R2-R3 responses at lower stimulus intensities and with shorter latencies.

D. Right and left subjective thresholds were compared among the groups and to the controls. Within the regard of subjective pain threshold, no significant difference was found among the patient groups and controls in terms of right/left pain thresholds. No significant difference was found among the patient groups and controls in terms of the stimulus intensity of the right subjective pain threshold (right Pt) and the stimulus intensity of the right subjective pain threshold (left Pt)

E. All patients were divided into two groups and prevalence of the right/left R3 responses was assessed for >2-year and <2-year follow-up periods. The prevalence of R3 responses was found to be increased in the late stages of the pain in patients with DNP. The prevalence of R3 responses was found to be higher in the acute stage of the pain in patients with cervical and lumbar radiculopathy. The prevalence of R3 responses was same in all stages of TTH.

DISCUSSION

Over the past 30 years, several hypotheses have been proposed regarding ultralate R3 component; however, no consensus has been reached yet. We investigated the presence of R3 response in the presence of neuropathic pain, the association of the response with nociception, topographical characteristics and its diagnostic use.

Delwaide and Penders found the R3 response latency to be around 80ms in 1973 and suggested that the

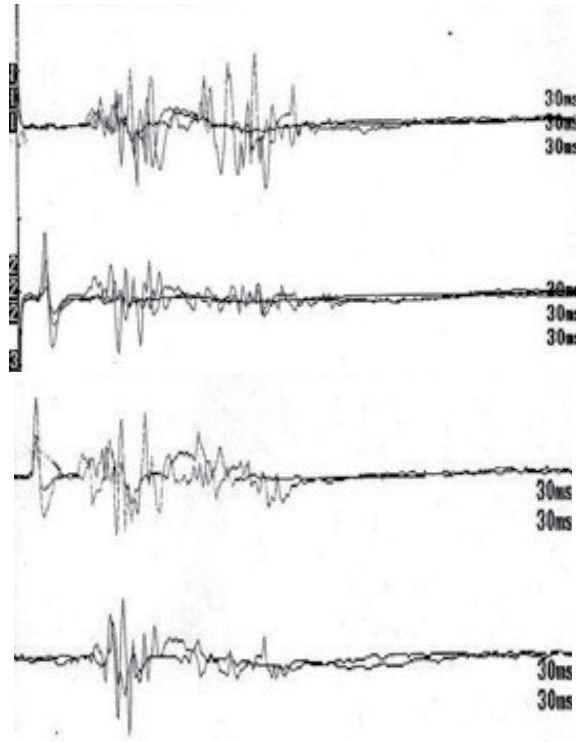


Fig 1 Electrical blink reflex in a 42 year-old woman. Original sweeps from the orbicularis oculi muscle EMG evoked by electrical stimulation of the right and left supraorbital nerve. The R3 component of the blink reflex was clearly elicited.

response was conducted by nociceptive C fibers since the responses were produced by supramaximal stimulation in healthy individuals with a possibly long reflex arc of R3.³ Rossi depressed the ultra-late response by xylocaine and suggested that nonmyelinated C fibers played an important role in the development of R3 response.⁵ In 1996 Ellrich et al obtained R2-R3 responses by laser beam; suggesting that the R1 response arc contained nonmyelinated A β efferents, while R2 reflex arc included A β mechanoreceptors and A δ nociceptors.⁶ The authors also considered that R3 response was activated by a powerful electrical stimulation with a 50-ms longer baseline latency compared to R2 response. So, R3 response was associated with specific nociceptive neurons of trigeminal caudal subnucleus with nociceptive origin. In addition, Fabbri et al. suggested that ultralate response was conducted by C fibers (with nociceptive origin like corneal reflex) when R3 response was depressed by piroxicam, a non-steroidal anti-inflammatory drug.⁷ On the other hand, in 1996 Ellrich and Hopf estimated that R3 component of BR contained A δ fibers.⁶

Studies published by Ellrich in 2001 and 2002 indicate that R3 component of BR contained A δ nociceptors as well as A β fibers.^{2,8}

We found the right/left R1-R2 response latencies to be within normal range among all patient groups and in the control group (Table 2). This suggests that →

individuals do have an unaffected reflex arc. The right/left R3 response latencies were significantly shorter in patients with LR and controls compared to other patient groups ($p=0.000$ for R3).

Studies display R3 latency between 75 and 130 ms, while we found to be 83-84ms. Regardless right/left lateralization, the R3 response latencies were shorter in all patient groups and controls. Earlier studies revealed a R3 response latency of 80ms in healthy individuals via supramaximal electrical stimulation. Rossi et al. saw the R3 only when applying strong electrical pulses and concluded that nociceptive afferents were involved in the reflex arc of the R3, so the conduction was considered to be performed by nonmyelinated C nociceptive fibers.^{3,5,8} R3 component of BR have been studied in atypical facial pain, burning mouth syndrome and migraine which are presenting with neuropathic pain. However, ultralate response latency in BR was not assessed in these studies. The prevalence of R3 responses was assessed in all patients groups and controls in our study (Table 3). R3 response was found to be significantly higher in all patient groups compared to the controls (right/left $P=0.000$). The prevalence of R3 component was similar in the patient groups. High prevalence of R3 component in patients with a painful condition might suggest a relationship between the subjective sensation of pain and the R3 component.

Stimulus intensity of the responses was another investigational parameter (Table 4). We found the stimulus intensity of the right/left R1 response to be lower in all patient groups and controls except in patients with CR. The stimulus intensity of R2-R3 responses was found significantly higher in patients with DNP compared to other patients groups, controls and data in the literature.² On the contrary, the stimulus intensity of the right R2-R3 responses was found significantly lower in patients with LR compared to other patients groups and controls. Review of the available data shows that the stimulus intensity of R3 response was lower in patients with severe migraine, whereas only the stimulus intensity of R1 and R3 responses was lower in patients with burning mouth syndrome and atypical facial pain. This result was interpreted as mechanical allodynia development in trigeminal interneurons with increased hypersensitization in neuropathic pain.⁹⁻¹¹ In our study low stimulus intensity of R2 and R3 responses in the patients with LR may be suggestive of hypersensitization and mechanical allodynia development. R2 and R3 responses were also obtained earlier than R1 responses in patients with LR. This may be explained by the presence of nociceptive afferents in the reflex arc of R3 component since $A\beta$ efferents are more affected in

patients with LR. In addition, complaint time and mean age was higher in these patients, suggesting high potential for mechanical allodynia development. Increased stimulus intensity R2-R3 responses in diabetic patients may be the result of diabetic polyneuropathy.¹² Also it may suggest $A\delta$ nociceptor involvement with a high threshold due to hyperglycemia and trigeminal nerve involvement.¹³

The mean age of patients with DNP and LR was higher compared to other patient groups and controls. It has been reported that prevalence of R3 response is higher in elderly and the stimulus intensity of ultralate responses is found to be lower in patients with burning mouth syndrome.¹⁰ The prevalence of ultralate response with pain increases with every kind of disease with the increasing age if there is an association between R3 response and nociception.^{13,14} In addition, prolonged presence of hyperglycemia in diabetics and compressive pain long before diagnosis in patients with LR may alter the stimulus intensity of R3 responses in these patient groups whose mean age was found statistically high. It also may be associated with nociceptor involvement in neuropathic pain rather than age directly.

Within the regard of subjective pain threshold, no significant difference was found among the patient groups and controls in terms of pain thresholds. Review of the literature revealed pain threshold 14.8 ± 1.5 mA in healthy individuals and this value was consistent with the data of our study.^{2,8} However, in a study conducted by Tommaso et al. in 2002 demonstrated a very low subjective pain threshold.¹¹ This was probably due to heterogeneity of our patient groups and shorter symptom duration compared to the patients with migraine.¹¹ Subjective pain threshold is directly associated with parameters of electrical stimulus. Subjective pain threshold is also directly related to the central processes.

According to our study protocol, the investigation was completed when subjective pain threshold was obtained without allowing electrical stimulation to exceed the pain threshold.¹¹

The association between R2-R3 responses and subjective pain threshold was investigated and compared among the patient groups (Table 4). Meanwhile, a positive correlation was found in patients with DNP and LR. A strong correlation ($r=0.75$) between the stimulus intensity of the R3 response and subjective pain threshold was obtained, while a significantly positive correlation ($r=0.68$) between the stimulus intensity of the right R2 response and subjective pain threshold was attained in patients with DNP. This finding was the evidence suggesting that the stimulus intensity of \rightarrow

R3 response was higher than the subjective pain threshold and the prevalence of R3 response increased when the pain threshold was reached in diabetics compared to other groups. The same correlation was available in R2 responses. As the stimulus intensity increased, the R3 response was obtained rapidly. Higher stimulus intensity may be an indicator of A δ nociceptor involvement in patients with DNP. In addition, high prevalence of trigeminal neuralgia in diabetics may alter the responses in BR. According to the data in the literature, a positive linear correlation is present between R3 response and the pain threshold and R3 response similar to the threshold is interpreted as activation of A δ nociceptive fibers.¹⁵ The literature also shows that the stimulus intensity should be higher to obtain R3 response as the subjective pain threshold is higher. In our study mean pain threshold was high in both patient groups and controls.

Investigating the prevalence of the R3 response respect to the symptom duration was another purpose of our study. We divided the patients into two groups; those with symptom duration of less than 2 years and those with symptom duration of greater than 2 years. When the prevalence of R3 response was evaluated by these stages, we observed that the prevalence of R3 response was higher in advanced stage of the disease in patients with DNP, the prevalence was higher in acute stage in

patients with CR, and LR. The prevalence was similar in all stages in patients with TTH.

These findings may suggest increased involvement in nociceptive fibers with prolonged hyperglycemia and development of trigeminal neuropathy in DNP.¹³ In addition, sprouting of heavily myelinated fibers (A β) affected by the compression with the second neurons in substantia gelatinosa in the acute stage of the disease may lead to sensitization and therefore mechanical allodynia development in patients with LR.^{3,6} Painful bursts are also felt less with the onset of neuroplasticity and development of compensatory mechanisms in chronic stage. Also, increased sensitization of interneurons in trigeminal nerve and episodic-chronic contraction of pericranial muscles are explained by disinhibition of the central opioid system in patients with TTH during pain episodes.

CONCLUSION

The nature of R3 component is still controversial. The findings of our study, short latency and high prevalence of R3 in patients with pain, high stimulus R2-R3 intensity in diabetic neuropathic patients and high stimulus intensity of R3 compared to the subjective pain threshold, may suggest R3 can be used as a neurophysiologic marker in nociceptive system dysfunctions.



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