Review of Psychology
1999, Vol. 6, No. 1-2, 53-78 UDC 159.9

Reflex eyelid blinks – on the applicability in psychophysiological researches

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Eyelids are a constituent part of eye's proctetive system against potentially damaging external stimulation. Eyelid blinks are based on contractions of the musculus orbicularis oculi. These contractions can be spontaneous, "voluntary" and reflex. Reflex contractions are elicited by mechanical and electric stimulation of the eye and facial regions, as well as by intense acoustic stimulatin of the cochlea and light stiulation of the retina. Reflex contractions of the same muscle on different stimuli involve various afferent branches of the reflex arches. Reflex centres related to those contractions vary as well, and they are located in medulla spinalis and in mesencephalon. Research has demonstrated that characteristics of these contractions are not exclusively dependent on physical features of the stimuli. They are largerly influenced by numerous factors causing changes in the functioning of central nerous system. Reflex eyelid blinks can thus serve for research of the functions of nervous system, as they are reflected in overt behavior. This survey tried to emphasize crucial findings of both international and Croatian researchers in this area. These studies provided inshights about the neural basis of blink reflexes and the functioning of central nervous system in general.

Historical framework

It is well known that the basis of eyelid closure are contractions of musculus orbicularis oculi (Figure 1). According to their origin, these muscular contractions can be divided into three groups: a) spontaneous (seemingly with no evident cause), b) reflex (elicited by stimulation of certain receptors), and c) voluntary (cortically controlled, i.e. "deliberate" movements).

Historically, first researh on spontaneous eyelid blinks was reported by Leeuwenhoek (1648; Walter, 1941). Leeuwenhoek investigated the role of fatigue and various psychological factors (such as increased cognitive activity or cognitive passivity) in the frequency of eyelid blinks. His research later inspired numerous investigators, such as Katz (1895), Lans (1902), Poller (1928), Walter (1941), Hall (1945), Collins (1962), Holland & Tarlow, 1972), etc.

Katz (1895) explores the utility of the frequency of spontaneous eyelid blinks as an indicator of the eye fatigue. He presumed that retinal fatigue caused hyperemia of the conjunctiva. This accumulation of blood acts as a stimulus for the ends of nervus trigeminus. When stimulated, this nerve elicits reflex contractions of palpebra. Katz experimented with subjects reading under electrical or gas illumination. He found the increase in frequency of eyelid blinks when the light was weaker.

Lans (1902) finds that spontaneous blinks are reflex activity caused by drying of the cornea and conjunctiva, or by cooling of certain receptors in these tissues. He found that

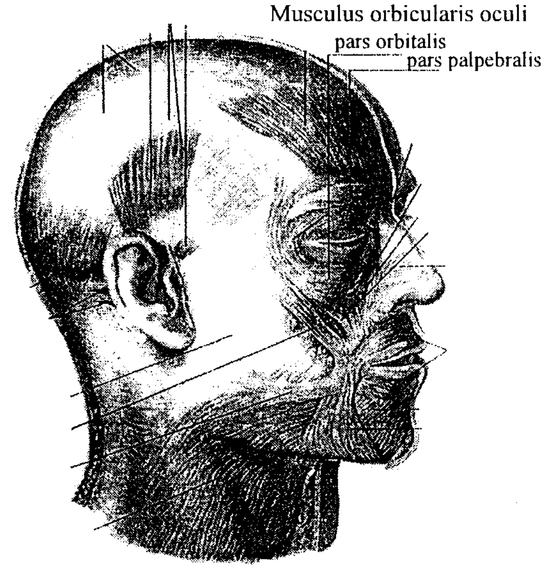

Figure 1. Facial muscles with labeled superior (pars palpebralis) and inferior (pars orbitalis) part of the musculus orbicularis oculi.

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spontaneous blinks stopped when a person was in darkened room of 37°C temperature and maximal humidity (100%).

Poller (1928) reports close attention to particular contents, for example in reading, can decrease the frequency of spontaneous blinks. Walter (1941) demonstrates that increase of CO_2 in the atmosphere up to 30% or more increases the frequency of eyelid blinks. This is an effect similar to the effects of strong air streams over cornea and conjunctiva. Hall (1945) investigates the frequency of spontaneous blinks in healthy and sick subjects, in men and women, and in states of rest and fatigue, trying to determine typical values for particular groups and situations. Collins (1962) reports on positive relations between the level of emotional excitation and the frequency of eyelid blinks. He concludes that the frequency of spontaneous blinks could be useful as an indicator of the level of emotional excitation. Holland and Tarlow (1972) find a decrease in frequency of spontaneous eyelid blinks as a function of the increase in cognitive activity level.

Contemporary research showed that central dopaminergic activity had a very important role in the control of spontaneous blinking. A decrease in blinking frequency was shown in patients who were taking dopamine antagonists (Karson, 1983). To the contrary, frequency of spontaneous blinking increased when dopamine agonists were administered.

At the time of Leeuwenhoek's research on spontaneous eyelid blinks, Descartes (1596-1650) described so called optical blink reflex, elicited by a sudden appearance of intense illumination or some object in the visual field (Wartenberg, 1945).

The growth of knowledge about the reflex activity of muscular orbicularis oculi following the stimulation of retina with illumination, or cohlea with sound, paralleled the increased interest in reflexes in general (Muller, 1844; Darwin, 1872; Exner, 1874; Forbes & Sherrington, 1914). Furthermore, research showed that reflex blinks were elicited by mechanical or electrical stimulation of the wide forehead area, zygomatic arch, nose, cornea and conjunctiva of the eye, palate, throat, etc. (Exner, 1874; Overend, 1896; Mayhew, 1897; Garten, 1898; McCarty, 1901; Zwaardemaker & Lans, 1900; Behterev, 1901, 1905; Moro, [1901; Wartenberg, 1944], Dittler & Garten, 1918, Simchowitz, 1922, Galant, 1924, 1925; Aronovitch, 1927; Imperatori, 1930; Weis, 1942; Wartenberg, 1930/1944, Kugelberg, 1952; Rushworth, 1962; Shahani, 1970).

Historically, Exner's research on reflex reaction time of various parts of nervous system are particularly interesting (Exner, 1874). He determined reflex reaction time of the eyelid following the stimulation by electrical flow. In his experiments, Exner used electrical stimulus generated by inductional apparatus. Exner applied the stimulus close to the eyelid of one eye, while at the same time specially construed mechanical device recorded eyelid movements of the other eye (Figure 2). He was thus stimulating nervus trigeminus and eliciting reflex reaction of the eyelid. Exner measured reaction time of the eyelid on weak and intense electrical stimuli. He reported the reaction time of 0.0662 sec on weak stimuli and reaction time of 0.0578 sec on intense stimuli.

Overend (1896) was the first who systematically investigated reflex blink evoked by a weak mechanical stroke on various parts of frontal region. He reported that weak stroke to the one side of the forehead evokes closure of the eyelid on the same side. More intense stroke to the area of middle forehead line evokes closure of the eyelids on both sides. As it was possible to evoke reflex closure of the eyelids by weak strokes on all parts of frontal area, Overend suggested that this reaction should be named "frontal reflex". Furthermore, he argued that this reaction was not elicited by the perception of movement or shadow of an object used for the stimulation of the forehead area, but just by mechanical stimulation itself. According to Overend, the fact that "frontal reflex" could be elicited even in completely blind subjects is the crucial evidence for this claim.

Using more precise devices, Mayhew (1897) measured reaction time of the reflex blink elicited by mechanical stimuli applied two centimeters above the outer edge of the eye (Figure 3). A piece of platinum wire, fixed on subject's eyelid, closed the electrical circle when the eye was opened. Closure of the eye moved the platinum wire, which caused opening of the electrical circle, or the interruption of electrical flow. Stimuli were generated by a weak stroke of the rubber hammer on a spring. The device was adjusted so that the application of a stimulus interrupted electrical circle for a moment, and the time of this interruption was registered on the revolving drum. Ten milliseconds later, rubber hammer moved back into its initial position and

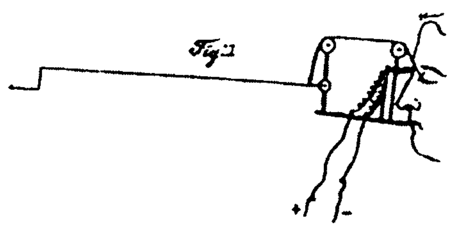


Figure 2. A simple mechanical device for the registration of eyelid movements. Subject holds one end of the light lever with his teeth. One end of the thread is fixed on the eyelid by a piece of plaster, while the other end is fixed at the end of the lever. Eyelid movements are transmitted to the lever, which in turn registers these movements on the revolving drum. Stimulus used to elicit reflex eyelid response is the electric shock applied to the opposite eyelid (Exner, 1874, pp. 527).

closed the electrical circle. After a period of latency following the application of a stimulus, reflex contraction of the eyelid interrupted the electrical circle again. This interruption was registered on the revolving drum. Time interval between first and second interruption of the circle was a latency time of the eye blink to mechanical stimulation. Mayhew reported the average latency time of 0,042 sec based on 450 measurements, a value lower than the one reported by Exner (1874). Mayhew's particular contribution to the field was his research of the influence of "psychic factors" on reaction time of palpebra ("The influence of mind on the reflex time had to be considered", pp. 45). He investigated the influence of various aspects of "mental activity" on the latency time of reflex contraction in three experimental situations. In the first situation subject had to perform arithmetic operations of addition and multiplication, while in second experimental situation subject was required to press the rubber bulb of the manometer to keep the level of the mercury at the certain height. During the third experimental situation, subject received a warning few seconds prior to the stimulus. Mayhew found that the rank order of latency times in three situations varied among subjects, while the rank order of latency times in each of the experimental situations was stable in repeated experiments.

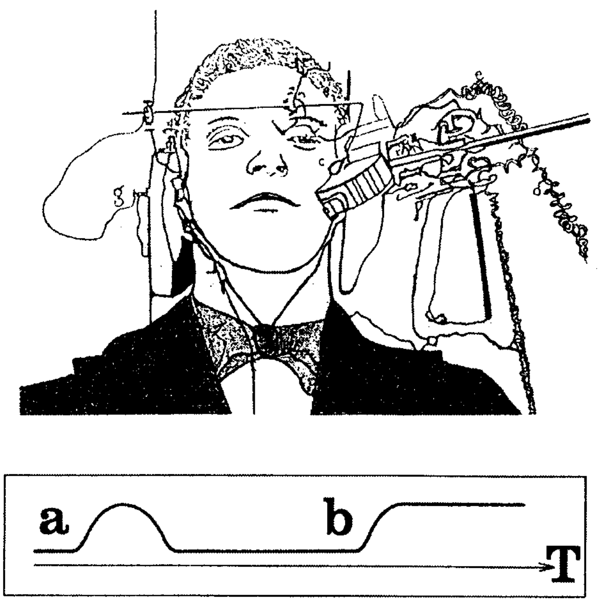


Figure 3. Apparatus for the registration of reflex contractions of musculus orbicularis oculi (according to Mayhew, 1897, p. 47). A curve beyond the picture shows the traces of time changes registered on the revolving drum. These changes are produced by the shifts of a hammer (a), and the eyelid movements (b). (Modified after Mayhew, 1897, p. 38).

Garten (1898) measured latency time of reflex eyelid contractions as a function of optical and mechanical stimuli. Garten was registering eyelid movements photographically, by a photopaper fixed on kymograph. Stimulation of trigeminus by strong electrical flow elicited reflex closure of the eyelid with reaction time of 0,04 sec. Garten also elicited reflex eyelid contraction following optical stimulation by strong electrical spark 10 cm away from a subject's eye. Reaction times to optical stimuli ranged from 0,061 to 0,132 sec. for one subject. Zwaardemaker and Lans (1900) examined reflex blinks following photic stimulation (spark light), using Exner-Mayhew's apparatus for the registration of reflex contraction. Their contribution to the research of refractory time in orbicularis oculi reflex with "two sparks" is particularly interesting. They tried to establish the shortest period between two sparks, allowing for an independent reaction on each optical stimulus. According to their research, an interstimulus interval of four seconds allowed separate eyelid blinks in 100% of the cases.

McCarthy (1901) was the first to examine reflex eyelid closure following the stimulation of nervus supraorbitalis. His research proved that mechanical stimulation of supraorbital nerve was associated with the reactions of musculus orbicularis oculi. His research introduced the term "supraorbital reflex" into the literature. According to McCarthy, reflex arc of this reaction involved nervus supraorbitalis, nervus trigeminus, nervus facialis and musculus orbicularis oculi.

Behterev (1901) used the term "eye reflex" (glazni refleks) for identical contraction of the palpebra. He disagreed with McCarthy's idea that reflex contraction is elicited exclusively by the stimulation of supraorbital region. Behterev claimed that wide frontal and temporal regions represent a reflexogenic zone. Using hammer and small metal box close to the subject's ear, Behterev (1905) produced strong acoustic stimulation and elicited reflex contraction of the palpebra. He named this reaction "acousticopalpebral" or "acousticofacial reflex". According to Behterev, this procedure may serve to discover the "simulation" of deafness, and for a long time this reflex was considered very important in clinical examinations. Behterev also tried to establish a connection between certain anatomical structures in brain stem participating in reflex contractions of musculus orbicularis folowing acoustic stimulation.

Dittler and Garten (1918) report their very complex research of reflex reactions in pigeons and humans. They used photokymograph for the registration of eyelid movements in their research of reflex reaction time on eye stimulation by air puff and by touching of the cornea by a hair fixed on electromagnet. They found approximately equal reaction times of the eyelid following both of the stimuli, but this reaction time was significantly longer in humans than in pigeons. Reaction time of the eyelid on air puff stimulation was 0.038 sec. in humans, compared to the time of 0.089 in pigeons, or to the time of 0.0099 sec. in pigeons when stimulated by a hair.

Subsequent authors tried to discover all areas whose stimulation leads to reflex contraction of musculus orbicularis oculi. Shimchowitz (1922) offered the term "nasenaugenreflex" for the reflex contraction elicited by the percussion of the top or root of the nose, while Galant (1924) used the terms "corneal" or "conjunctival" reflex for the reflex contraction of musculus orbicularis following the stimulation of cornea or conjunctiva. Reflex blinking elicited by a flash of light Galant (1925) labeled "optical blink reflex" (optische blinzelreflex). Imperatori (1930) finds the stimulation of palate and various parts of the throat to be accompanied by reflex contractions of palpebra. According to Imperatori, afferent part of the reflex arch of this reflex reaction involves V, VII, IX and XI cranial nerve. Wartenberg (1930/1945) reports that mechanical stimulation (weak strokes), applied in the area of glabella, is the most effective source of blinking reflex. He used the term "glabellar reflex" for the reflex eyelid blinking following mechanical stimulation in this region. He also described the possibility of eliciting the reflex contractions by weak strokes on the muscle itself. In his opinion, stroke applied on the muscle itself causes the extention of muscular fibers, which in turn leads to the reflex contraction. He termed thus elicited contraction "muscle-muscle reflex".

The established line of "eyelid reflexes", having in common the final act of the contraction of musculus orbicularis oculi regardless of the type and the area of stimulation, inspired various authors to attempt the systematization of palpebral reflexes. Various authors were giving new labels to the already known reflexes, so the literature suffered from conceptual confusion for a long time. For example, Galant (1925) listed 16 reflexes in his paper, since identical contraction could be produced in 16 different ways. To resolve this chaotic situation in literature, Wartenberg (1945) suggested unitary, physiologically correct term "reflex orbicularis oculi". His term emphasized the essential feature of a number of "facial" reflexes, namely, the orbicular muscle underlying all of them. This term replaced a long list of facial reflexes, mostly termed according to the unique concepts of their "discoverers".

The researh of reflex orbicular contraction included various animal sorts as well. Aronowitch (1927) demonstrates the reflex contractions of orbicularis in monkeys. Observing reflex palpebral contractions in various ZOO animals, Borowsky (1929) considers this reflex to be vital and widespread in animal world. He labeled this reflex "defense blinking reflex" (blinzelabwehrreflex). The existence of a sort of corneal reflex, reflected in the withdrawal of the eyeball in orbit upon the stimulation of cornea, was demonstrated even in Californian salamander (triturus torosus), evolutionary very primitive animal (Weiss, 1942).

It is reasonable to hypothesize that reflex closure of palpebra, with underlying reflex contraction of musculus orbicularis oculi elicited by stimulation of various sensory receptors, has a great biotic value. As shown later, this reflex reaction represents a segment of complex nociceptive functions protecting the organism from intensive environmental stimulation. Reflex contractions of musculus orbicularis oculi protect the eye from intensive, unexpected environmental stimuli that could destroy its peripheral parts. This muscle enables rapid and firm eye closure.

Spontaneous blinking can be caused by drying of cornea as already mentioned. Such an action can be understood as a kind of reflex activity which helps in maintaining of thin tear film over cornea. There is a hypothesis that "the eyelid would appear to be an adaptation to keep the cornea moist in species spending a significant amount of time out the water" (Evinger, 1995).

It should be emphasized here that tonic elevation and control of vertical position of the upper eyelid is subserved by the musculus levator superioris palpebrae. This muscle is situated in the roof of the orbit, just above musculus rectus superior. It has fibers which are suitable for fatigue resistent tonic activity such as tonically holding up the eyelid. It is innervated by the superior division of the oculomotor nerve. The motoneurons of the two levator palpebrae superioris are located in a single, unpaired central caudal nucleus of the ocullomotor complex in the midbrain (Averbuch-Heller, 1997). The motoneurons of both levator palpebrae superioris within the central caudal nucleus are completely intermingled and any modulation in activity of the nucleus is bound to influence both levators.This muscle is not present in birds and reptiles but only in mammals. When musculus orbicularis oculi contracts itself (motoneurons produce high-frequeny burst of bioelectric activity), musculus levator palpebrae is synchronously inhibited (motoneurons cease discharging). It means that during any kind of a blink (contraction of the musculus orbicularis oculi) the activity of musculus levator palpebrae is abruptly inhibited. In that way eyelid system is a hybrid of the tonic eye movement control system and phasic protective reflex (Evinger, 1995). Fatigue and decrease of general activation during sleep (even in REM phases) makes activity of motor units of musculus levator palpebrae inhibited. An increase of general activity in the central nervous system elicits activity in this muscle and elevation of the upper eyelid (Kennard & Smyth, 1963).

As we know there are two phases during blinking. The first phase is the closing, the other is opening . The velocity of these phases is not equal. The closing phase or downward movement of palpebrae is more rapid then phase of moving up which is much slower. All neural mechanisms which underlie the complex interactions above mentioned, are not completely known as yet (Schmidtke & Buttner-Ennever, 1992).

Investigating reflex reactions on intense acoustic stimuli (a revolver shot close to the subject's ear), Strauss

(1929) described "a startle pattern", referring to the reflex contraction of complete skeletal musculature (zusammenschrecken), evoked by intense stimulation of cochlea. Main features of this reflex are its speed (it appears in half a second), symmetry, and presence in subjects of all ages. Reflex reaction of orbicularis oculi is only a small segment of general motorical reaction (diffuse cochleo-muscular reaction) (Strauss, Landis & Hunt, 1938). According to Landis, Hunt, and Strauss (1939), reaction of musculus orbicularis oculi is the most rapid and the most sensitive segment in surprise reaction. Landis, Hunt and Strauss registered reflex contractions of the muscles following acoustic stimuli using ultra fast film cameras whose exposition range varied from 64 or 300 up to 3000 expositions in one second.

The analysis of these film shots offered an estimation of the speed of muscular reactions to certain stimuli. However, the development of electronics and electromyographical registration of muscular contractions opened a new era in the research on reflex reactions to sensory stimulation. Bitterman (1945) was the first to develop an adequate procedure for the registration of bioelectrical potentials evoked in musculus orbicularis oculi. He placed an active electrode below the eyebrow, centered between the outer and the inner edge of the eye. The other electrode was fixed on earlobe. Registered bioelectrical potentials were channeled into an amplyfier connected to the ink printer. Spontaneus eyelid blinks were thus registered on the moving paper tape. Davis (1948) published the first experiment on motoric responses of skeletal musculature to intensive acoustic stimulation using electromyographical registration of bioelectric muscular potentials. Davis presented stimuli ranging in intensity from 100 to 103 db, with duration of 2 and 4 seconds, and frequency of 500 Hz. He fixed active electrodes above the extensor muscles on the dorsal part of the forearm. Amplified bioelectrical muscular potentials were channelled to the cathode oscilloscope. Cathode ray, i.e. its shifts on the screen of the oscilloscope, were registered by film camera.

Subsequently, technological advancements enabled numerous experiments using more sophisticated procedures for the registration of bioelectrical muscular potentials. The research of Kugelberg (1952), Cobb and Sears (1957), Rushworth (1962), Shahani (1970), and Gogan (1970) is particularly worth mentioning.

Kugelberg (1952) investigated electromyographic responses of musculus orbicularis oculi to mechanical stimulation of periorbital region, as well as electrical stimulation of supraorbital nerve. The existence of the double response upon stimulation by means of one stimulus was demonstrated (Figure 4). The first response was unilateral, well-synchronised volley and with a stabile latency time of 12 msec after the stimulus. The reaction had a duration in a range of 5 to 10 msec. It was shown that this reaction was transmitted through a simple monosynaptic reflex arc. The afferent branch of this arc is the trigeminal nerve. The second response is bilateral. It is a long lasting asynchronous discharge (approximately 30 msec) with a variable latency time in a range of 21 to 40 msec. The second response has a multisynaptic reflex arc. This reflex arc comprises the trigeminal nerv and part of the spinal marrow with a great number of intermediate neurons. An increase in stimulus intensity causes bilateral reactions with both components of the reactions. The results of the stimulation were essentially the same whether the glabella, the frontal bone, the nose or the zygomatic had been stimulated. But the stimulation of the conjuctival area produces the second response only. Kugelberg demonstrated that the blink reflex produced by mechanical stimulation, rapidly decreases to extinction under anaesthesia.

After Kugelberg's research, interest for the research on blink reflex following mechanical stimulation decreased. At the same time researchers started to investigate palpebral reflex following photic and auditory stimulation. For example, research of Cobb and Sears (1957), applying photic (flash), auditory (click) stimuli and electromyographical registration of reflex responses, demonstrated stable differences in latency times of reflex responses of mus-

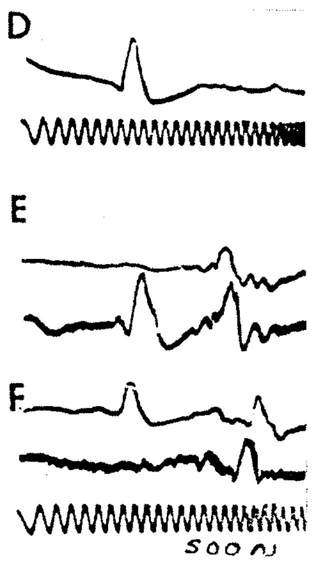


Figure 4. Recordings of the EMG response of musculus orbicularis oculi on mechanical stimulation of the supraorbital zone. D – only the first response (unilateral). E and F – first (unilateral) and second (bilateral) response (upper trace in each figure represents a response of the left eyelid, while lower trace represents a response of the right eyelid). Fig. E – reactions of the musculus orbicularis oculi to the stimulation of the right facial side. Fig. F – reactions of the musculus orbicularis oculi to the stimulation of the left facial side. (Modified after Kugelberg, 1952, p. 389).

culus orbicularis oculi for the two types of stimulation. The average latency time of reflex response to sound was significantly shorter (*M*s = 35 msec) than the average latency time of reflex response to the very intense photic stimulus producing minimal delay in electroretinogram (*M*p = 45 msec).

Rusworth (1962) was the first to systematically apply modern electronical devices in his research. Rushworth investigated latency times, duration and amplitudes of the reflex contraction of musculus orbicularis oculi in healthy and sick subjects, presenting mechanical, electrical, acoustic and photic stimuli. He registered electromyogram using precise coaxial needle-shaped electrodes placed in right and left orbicularis oculi muscle. The comparative results he obtained were considered a sort of parameters or standards for the practical evaluation of the level of damage of acoustic, optical, facial and trigeminal nerve. The stimulation of supraorbitalis produced double answer on homolateral side and single response (second component) on the opposite side. The comparison of latency times of first and second component, when glabella and supraorbitalis were stimulated, showed shorter latency times following the stimulation of nervus supraorbitalis. Rushworth also demonstrated the second answer to be influenced by habituation processes. He found that the stimulation of cornea resulted in bilateral contraction of orbicularis. The range of latency times of reflex contraction was between 25 and 40 msec, that is comparable to the range of latency times for the second component of glabellar reflex.

As was already mentioned, Rushworth (1962) first observed the phenomenon of habituation of the late components of the blink reflex when mechanical stimulation was applied to glabella. Struppler and Dobbelstein (1963) confirmed the phenomenon using electrically and mechanically evoked blink reflexes. Later, Sanna, and Mesina (1967) discovered the habituation threshold frequency. They showed that progressive decrease of the late component of the blink reflex appears when the blink reflexes are repeatedly elicited by the rate at which intervals between stimuli were below 4 seconds.

Gandiglio and Fra (1967) investigated facial and extrafacial areas on which electrical stimulation produces reflex contractions of orbicularis. Surprisingly, the reflex contractions of palpebra were also elicited by the stimulation of lateral cervical region as well as of wrist and elbow areas of nervus medianus.

Shahani (1970) used precise procedures to investigate latency times of first and second component following the stimulation of the left supraorbital nerve. His findings could not prove the first component to be proprioceptive or myotatic reflex, and the second to be nociceptive reflex. He claimed that reflexes with longer central delay develop more rapid habituation compared to the reflexes with shorter central delay.

In his research, Gogan (1970) attempted to make a synthesis of previous research with his own research on the impact of unexpected auditory stimuli (impulses of the white noise) on motorical, vegetative and, EEG activation, and to incorporate this research into existing neurophysiological theories. Within this framework he also analyzed reflex contractions of musculus orbicularis oculi following the stimulation of various intensity levels, the development of habituation, as well as the impact of these variables on primary and secondary component of the reflex answer.

First research of bioelectrical potentials of skeletal musculature elicited by acoustic stimulation of cochlea and photic stimulation of retina was published in Croatia by Ljubin and his collabolators (Ljubin & Licul, 1970, and Ljubin, Ljubin, and Fatur, 1973). These authors suggested the term "audiomotor reflex" for the muscular reaction on auditory stimulation of defined intensity, duration, and frequency. Similarly, the term "videomotor reflex" was suggested for those muscular reactions evoked by stimulation of the retina with photic stimuli of defined intensity, duration, and chromatic quality. In line with these suggestions, reflex contraction of the palpebra could be termed audiomotor reflex of the musculus orbicularis oculi when an auditory stimulus was presented, or videomotor reflex of the musculus orbicularis oculi when a photic stimulus was presented.

Studies of electromyograms of the reflex responses of musculus orbicularis oculi to auditory and photic stimuli supported the existence of two responses originally observed and termed primary and secondary reaction by Landis, Hunt and Strauss (1939). According to Strauss, primary reaction is influenced by a "state of consciousness", while secondary reaction is under the influence of "mental states" and personality traits. Davis (1948) used terms "a" and "b" answer for the similar reactions to auditory stimulation. The latency time of an "a" answer was approximately 100 msec, while the latency time of a "b" answer was difficult to determine. This answer reached the maximal amplitude one second after the presentation of auditory stimulus. Gogan (1970) claims the existence of a primary (startle) component with latency time of 20 – 40 msec and secondary (orienting) component with the latency time of 300 msec. According to Gogan (1970), primary component is an immediate reflex answer inaffected by acquired behavioral pattern. Late or secondary component would be similar to "what is this?" reflex described by Pavlov (1927/1960), or to "orienting reaction" of Voronin and Sokolov (1960). The increase in intensity of an auditory stimulus leads to the increase of the amplitude of the primary answer, while the amplitude od the secondary answer remains unchanged. Repeated stimulation leads to rapid

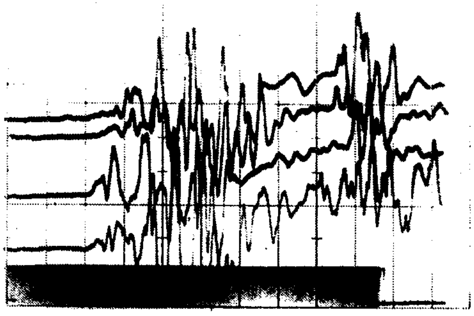


Figure 5. Electromyograms of the reflex contractions of musculus orbicularis oculi for the subject F.T.I., elicited by acoustic stimuli (110 dB, 1 kHz, 100 ms). Electromyograms show primary and secondary components of reflex contraction. Lower trace marks the duration of acoustic stimuli of 100 ms. Recording conditions: time of analysis (horizontal shift) 40 ms/DIV, sensitivity (vertical shift) 200 μV/DIV

habituation and extinction of secondary component, while the habituation and extinction of the primary component is much slower (Figure 5).

It is important to point out that primary and secondary components of the reflex contraction of musculus orbicularis oculi elicited by photic or auditory stimulation should not be identified with first and second response of musculus orbicularis oculi to electrical stimulation demonstrated by Kugelberg (1952). Afferent parts of reflex arches and reflex centres of these two reactions are different. Contemporary investigators tend to use the term "blink reflex" only for reflex contractions of musculus orbicularis oculi elicited by mechanical or electrical stimulation of certain reflexogenic zones. Frequently used term for the first, early, or ipsilateral component of reflex arch is R1 response, while second, later, or bilateral component is named R2 response of blink reflex. Reflex contraction of musculus orbicularis oculi elicited by auditory and photic stimuli is frequently termed startle reflex, in order to differentiate this reaction from blink reflex. However, regardless of the preferences for the terms blink or startle, each researcher should precisely specify the area of the stimulus application, as well as the type of stimulation used to elicit reflex reaction of the musculus orbicularis oculi. Specification of the type of stimulation reveals the potential reflex arch underlying reflex contraction of musculus orbicularis oculi.

The accumulation of general knowledge about the reflex contractions of the palpebra was paralelled by research investigating anatomical substrate underlying these reactions. Johannes Muller (1844) provided first interpretations of the possible anatomical substrate of reflex contractions of musculus orbicularis oculi. According to Muller, intense sound and bright illumination can elicit reflex excitation of facial nerve, which in turn leads to the contraction of facial musculature. This contraction is reflected mostly in eyelid blinks. Forbes and Sherrington (1914) significantly contributed to the initial explanation of the anatomical basis of reflex muscular contractions elicited by auditory stimulation of the cochlea. Bender (1943) and Weinstein and Bender (1943) provided explanations for the reflex contractions of musculus orbicularis oculi evo- ked by mechanical stimulation of the facial skin. Forbes and Sherrington (1914), experimenting on decerebrated cat, investigated reflex reactions to auditory stimulation. These authors performed the resection on the level of colliculus anterior. They concluded that neural impulses were transmitted from auditory nerve to posterior colliculus (colliculus inferioris), then from its tegmental region by descendent pathway to spinal cord. Electrical stimulation of this pathway also elicits flexions of certain muscles. Using the procedure of stimulation and destruction of neural tissue, Bender (1943) and Weinstein and Bender (1943) tried to identify the nerves involved in reflex contractions of the musculus orbicularis oculi. Neural impulses from various sensory nerves reach the core of facial nerve that inervates musculus orbicularis oculi. These impulses converge in brain stem. According to Bender and Weinstein (1943), this convergence is responsible for the appearance of identical reflex contractions of musculus orbicularis oculi following various stimuli applied on various areas of the head.

Reflex contractions of musculus orbicularis oculi following auditory stimulation are probably due to neural excitation spreading from cochlea through acoustic nerve (VIII cranial nerve) and through nucleus cochlearis to colliculus inferioris (colliculus caudalis) structure in mesencephalon, being the primary acoustic reflex centre (Ascher, 1966, Buser et al. 1966, and Gogan, 1970). Neural excitation further spreads from colliculus inferioris through reticular nuclei of midbrain (nucleus giganto reticularis) and tectobulbar pathway to the nuclei of cranial nerves. Neural excitation from the motoric nucleus (nucleus originis nervi facialis) passes through facial nerve to musculus orbicularis oculi, eliciting contraction of this muscle reflected in eyelid blinks. Neural excitation elicited in retina by photic stimulation passes through optical nerve. A branch of this nerve reaches colliculus superioris (colliculus rostralis), primary reflex centre for visual stimulation. Neural excitation is being transmitted from sensory neurons of colliculus superioris to motor neurons of tectobulbar pathway. It should be noted that neural excitation from both colliculi spreads through nearly identical pathways to musculus orbicularis oculi, while different sensory pathways transmit neural impulses from receptors to colliculus superioris and colliculus inferioris.(Figure 6).Reticular nucleus gigantocelularis is, according to Gogan (1970), probably the centre

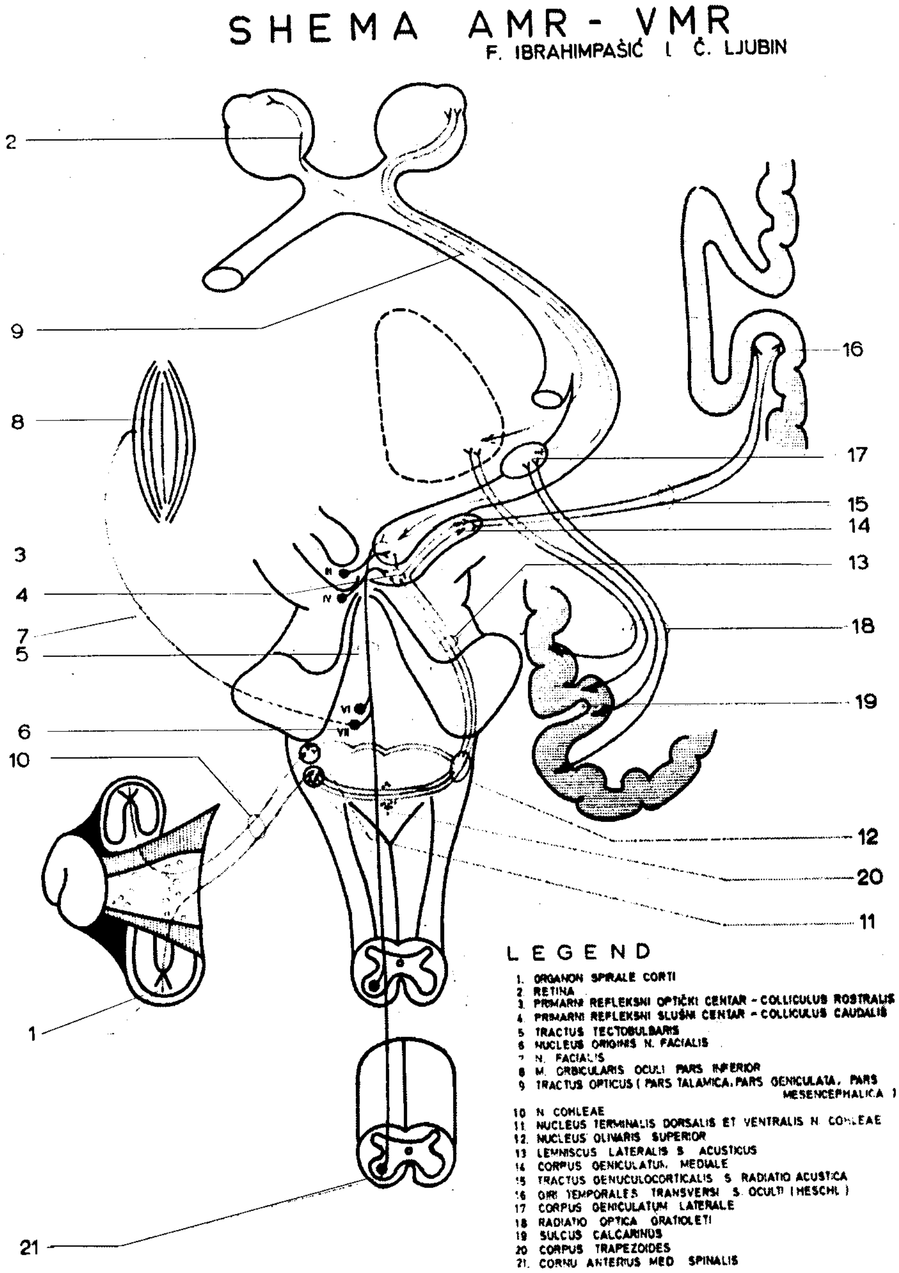


Figure 6. A scheme of the presumable anatomical substrate underlying reflex contractions of the musculus orbicularis oculi on light and acoustic stimuli.

of convergence of neural impulses from both colliculus superioris and colliculus inferioris. Furthermore, Gogan (1970) concludes that both startle and orienting reactions are likely to be influenced by common structures in reticular formation. A controlled observation of the clinically dead subjects revealed that blink reflex to sudden illumination appears even in anoxic neocortical death, which implies the absence od evoked cerebral potentials on illumination and "bioelectrical silence" on EEG (Keane, 1979). This finding supports the observation that reflex arch underlying reflex response photic stimulation does not exceed the level of brain stem.

Verifications

Research on this topic from seventies to date can be divided into few clusters. First cluster consists of research applying new technologies and new procedures for the registration of reflex responses. These experiments attempted to verify previous research findings obtained on both animals and humans, as well as their underlying hypotheses. Trontelj and Trontelj (1978) thus demonstrate that the early component of blink reflex is probably due to oligosynaptic reflex arch, which is shorter than reflex arch involved in second, late component. Berardelli (1985) compares the features of reflex contractions of orbicularis oculi elicited by mechanical stimulation of the cornea with those elicited by electrical stimulation of the nervus supraorbitalis. Latency time as well as the duration of reflex response of corneal reflex was longer than correspondent parameters of the late bilateral component of blink reflex.

Response of musculus orbicularis oculi elicited by stimulation of the cornea remains unchanged at the moment of extinction of the second component of blink reflex during habituation. This is additional physiological evidence for different neuronal connections of the two reflex reactions. Hammond et al. (1996) offered an explanation for the functional role of the early (R1) component of the blink reflex. According to their interpretation, early component initiates eyelid movements, while late, R2 component facilitates eyelid closure. Electrical stimulation of the infra- or supraorbital nerve, eliciting R1 component of the reaction, generates electrical fields dominantly on the contralateral side of the head. These electrical fields can be registered by EEG techniques on the scalp (Leandri et al. 1994). Jaaskelainen (1995) demonstrated that electric stimulation of nervus mentalis elicits reflex closure of the eyelids. It is known that nervus mentalis innervates the skin on the chin and mucous part of the lower lip. The stimulation of nervus mentalis elicits bilateral contraction of the eyelids with long latency times. Miwa el al. (1998) stimulated supraorbitalis and nervus medianus, and demonstrated that blink reflex elicited by electrical stimulation is due to the convergence of neural excitation in the brain stem. A complex interaction of the excitatory and inhibitory neural mechanisms occurs prior to the redirection to the final pathway leading to facial nucleus.

Ljubin et al (1986) offered a matematical and a graphic model which links functions of the amplitude of the reflex contraction of the musculus orbicularis oculi with the strenght and duration of stimuli according to the law of energy.

Experiences from previous experiments and the application of new computer systems in the past twenty years led to the breakthrough in research on the effects of various variables on the reflex contractions of musculus orbicularis oculi. Elaborated procedures now enable even the less experienced investigators to precisely register reflex responses of the musculus orbicularis oculi elicited by various stimuli (Van Bokstel et al., 1998).

Facilitation and inhibition

A distinct group of studies investigated facilitatory and inhibitory effects on the parameters of the reflex contractions of musculus orbicularis oculi. Krauter et al. (1973) demonstrated that previous stimulation, although not eliciting a reflex reaction itself, can have an inhibitory effect on corneal reflex. Graham et al. (1975, 1977) investigated this phenomenon in detail. They presented a prestimulus at 70 db intensity, 1kHz frequency, and 20 msec of duration, and an auditory stimulus, white noise at 105 dB intensity and 50 msec of duration, eliciting reflex contraction of eyelids. They varied the time interval between the presentations of prestimulus and stimulus and demonstrated that time interval of 120 msec from prestimulus to the onset of intense stimulus had the strongest inhibitory effects on the amplitude of reflex response. Short intervals of 30 msec to 60 msec produced the strongest facilitation effect on latency time of the reflex response. Marsh, Hoffman, and Stitt (1976) investigated the effects of the monaural and binaural prestimulation on the reflex blink elicited by an airpuff directed into eye.

Prior to the presentation of the air flow stimulus, they presented an auditory stimulus of 70 db intensity, 1 kHz frequency and 20 msec of duration. Auditory stimulus was presented 100 msec prior to the airpuff eliciting reflex contraction. Prior auditory stimulation reduced the amplitude of the reflex contraction elicited by an airpuff. Monaural prestimulation had stronger inhibitory effect on the amplitude of reflex contraction compared to the binaural prestimulation.

Reiter & Ison (1977) investigated the effects of photic and auditory prestimuli on the amplitude of reflex contraction of the eyelid. Reflex contraction was elicited by an "impulse" of airpuff on the cornea. They varied the time interval between prestimulation and stimulation eliciting reflex contraction. Fixed time interval of 100 msec had the largest inhibitory effect on the amplitude of reflex response. Furthermore, they found that, with fixed time interval of 100 msec, the increase in prestimulus intensity had direct effects on the amplitude of reflex contraction of orbicularis oculi evoked by an airpuff. These results were verified in research using mechanical stimulation applied on subject's forehead in order to evoke glabellar reflex (Ison & Pinckney, 1980; Stitt, Hoffman & DeVido, 1980).

Hammond and his collabolators investigated time integration of the asynchronous stimuli eliciting blink reflex

(Plant & Hammond, 1989; Hammond & Dennon, 1991). They tried to investigate the effects of auditory stimulation, following cutaneous stimulation, on the parameters of reflex response elicited by cutaneous stimulation. Electrical stimulation of the supraorbital branch of nervus trigeminus was followed by an interval of 1 to 8 msec prior to the presentation of an auditory stimulus. The effect of subsequent auditory stimulation on latency time and amplitude of R1 and R2 component of the reflex response was investigated. Auditory stimulus, presented up to 6 milliseconds after the cutaneous stimulus, had no effect on R1 component, while at the same time the amplitude of R2 component increased and its latency time decreased. This phenomenon was independent of the interval of stimulus asynchrony, but depended on the intensity of auditory stimulus. The presentation of second electrical stimulus following previous electrical stimulus eliciting reflex contraction, given the same conditions as in the stituation with auditory stimulation, had an effect on R1 component, while R2 component remained unaffected. Presenting asynchronous cutaneous stimulation, Hammond and Plant (1993) verified the effect of second electrical stimulus only on R1 component. The effect of an auditory stimulus, following electrical stimulus and facilitating only R2 component, is due to the summation of stimulus energy occuring in complex pathways in brain stem. Auditory stimulus had no effect on R1 component due to the fact that these pathways do not participate in the processes creating R1 component.

Flaten and Blumenthal (1996) examined the effects of auditory stimulation presented prior to, simultaneously with, and after an airpuff stimulus eliciting reflex eyelid reaction. Auditory stimulus presented simultaneously with an airpuff evoked the facilitation of reflex answer. Auditory stimulus of 95 dB elicited stronger facilitation of the reflex answer compared to the weaker stimulus of 55 dB. Flaten and Blumenthal (1998) investigate the effect of airpuff stimulation applied on the temple area. They comment that the application of an airpuff as tactile stimulus demands reduction of the auditory component of airpuff. Auditory component can produce methodological error in determination of the true facilitating factor in reflex response to the air "impulse".

The research cited above demonstrates the influence of preceding or subsequent stimulation on some parameters of reflex contraction of musculus orbicularis oculi that are due to complex neurophysiological interactions in brain stem.

Attention

Studies of the role of attention in reflex response represent an important contribution to the research on the facilitation and inhibition of reflex reaction. Instruction to the subjects to direct their attention to stimulus eliciting reflex response leads to the facilitation of reflex response and to the deceleration of heart rate (Bohlin & Graham, 1977). Selective attention on weak tactile stimulus presented prior to or simultaneously with acoustic stimulus eliciting blink reflex produces different outcomes. Simultaneous presentation of tactile preparatory stimulus and acoustic stimulus without instruction for selective attention does not reduce the amplitude of reflex response. However, simultaneous presentation of stimuli, accompanied by instruction evoking selective attention to the tactile stimulus, reduces the response amplitude (Silverstein et al., 1981). Hackley and Graham (1983) systematically studied the effect of selective attention on blink reflex. They displayed acoustic and tactile stimuli (airpuffs), and compared amplitudes and latency times of the reflex response in two situations. One experimental situation demanded selective attention to the stimulus evoking reflex reaction, while in the other situation subjects responded to the stimulus evoking reflex response of the musculus orbicularis oculi without selective attention. Selective attention was evoked by instruction to estimate duration of the stimulus eliciting reflex response as well as duration of the stimulus not eliciting reflex response. These two types of stimuli were introduced in pairs. Situation with selective attention to the auditory stimulus evoked reponses of greater amplitude and shorter latency time compared to the situation without selective attention. Although much weaker, this effect was also observed in situations with cutaneous stimulus eliciting reflex response. Hackley and Graham concluded that selective modulation of the parameters of reflex response probably occurred in the sensory branch of reflex arch.

Simons and Zelson (1985) presented visual material with interesting (male and female nude models) and with uninteresting content (home appliances). During the period of visual fixation, subject received an auditory stimulus eliciting reflex response of the eyelids. Subjects' orientation to visual stimulation led to the significant inhibition and to the prolonged latency time of the reflex response. Bruno and Putnam (1985) studied the effects of attention directed toward and away from the acoustic stimulus in children and adults. Subjects were instructed to turn their attention to vibrotactile stimulus during measurement of reaction time, and thus were redirected from the acoustic stimulus. These two groups of subjects showed somewhat different types of attenuation of blink reflex. Adults showed a decrease in the amplitude of reflex response to the stimulus demanding motor reaction, while children showed an increase in the amplitude of the same reflex response. Anthony and Graham (1985) investigated the effect of attention on sensory –perceptual analysis in infants and adults. Subjects were selectively directed to the certain perceptual contents in visual and acoustic sensory modality. Selective attention to the perceptual content of the sensory modality identical to the modality of the presented stimulus facilitated the reflex response. Facilitation was indicated by the increase of the amplitude of reflex contractions in infants and by decreased latency times of the reflex response in adults. Furthermore, facilitaion was more pronounced with the presentation of more interesting stimuli. Situations with the incompatible modalities of the perceptual content and subsequent sensory stimulation did not elicit facilitation effect.

Haerich (1994) studied the effect of attention with emotional engagement on blink reflex. Attention focused on aversive stimulation produced facilitation of reflex response. Lipp et al. (1998) investigated the effects of attention on the reflex eyelid response in conditional processes. Facilitation of reflex answer was more pronounced when conditioned stimuli precipitating aversive unconditioned stimuli were presented compared to the situation when unconditioned stimuli was presented alone, regardless of the modality used.

The research on the attentional effects on reflex characteristics of the musculus orbicularis oculi demonstrates the influence of superior neural structures on some fractions of reflex arches participating in reflex reactions evoked by various types of stimulation.

Habituation of reflex responses

Desmedt and Godaux (1976) published some of the basic findings on habituation kinectics in various polysinaptic pathways of brain stem. They report that stimulus presentation in intervals shorter that 8 seconds evokes habituation of the second component of blink reflex. Rimpel et al. (1982) compared processes of the habituation of blink reflex on acoustic, electrical, and photic stimuli presented separately or in pairs. They find the habituation to increase when paired stimuli were presented with time separation of 250 msec, and to decrease when this interval was 30 msec, as compared to single stimuli of the same repetition rate. Boelhouwer et al. (1982) studied the process of habituation of R1 and R2 responses of blink reflexes elicited by electrical stimuli in the situation of mental task (binaural choice task with one hand signalizing high-frequency tones and the other hand signalizing low-frequency tones) and in the rest phase. The same course of habituation was observed for R1 component for both situations, while for R2 more rapid habituation was observed in the situation of mental task, with increased arousal. Bolino et al. (1993) compared decrements in the amplitude of reflex contraction following acoustic and electrical stimuli. Habituation was shown to be independent of the type of stimulation and of the experimental design. Electrical prestimulus decreases the amplitude and the latency time of R2 response. These results support the hypothesized effects of central mechanisms in sensory pathways.

A distinct group of studies pertains to the phenomenon of habituation in pathological states of the organism. Gregorić (1973) reports the evidence showing habituation of R2 response of blink reflex being influenced by superior brain structures. Subjects with dementia, diffuse cerebral injuries, and Parkinson's disease thus don't show the habituation of reflex response. Studying habituation of blink reflex elicited by mechanical, electrical, and visual stimuli, Gandhavadi (1982, 1985, 1989) reports similar results for mentally retarded. Geyer and Braff (1982) investigated the habituation of reflex eyelid reponse (amplitude and latency time) elicited by acoustic stimuli at 116 db in schizophrenic patients and in normal subjects, and found the habituation process to be different in two groups. Habituation of reflex responses in schizophrenic patients was delayed and incomplete. Geyer and Braff thus concluded that schizophrenic patients had an abnormal habituation of defensive responses to exteroceptive stimuli. They did not offer any final explanations of these results.

Habituation processes emphasize the role of "central" inhibition of neurophysiological processes in reflex arches underlying reflex responses to the stimuli or, in other words, the role of superior anatomical structures in reflex activity on the brain stem level.

Arousal, emotions, and personality

Research conducted by 1970. provided basic information on the eyelid reflex. Subsequent research thus concentrated on the relations between parameters of the reflex eyelid blinks and complex states of the complete neural system. For example, Ferrari and Messina (1972) studied blink reflex during sleep and wakefulness. They utilized needle-shaped coaxial electrodes for the registration of reflex activity of the musculus orbicularis oculi elicited by electrical stimulation of the supraorbital nerve. Muscular activity was registered in three situations: 1) during wakefulness; 2) during deep, synchronized or delta sleep, and 3) during desynchronized or REM sleep. Characteristics of the early or ipsilateral component as well as the late bilateral component were depressed during synchronized sleep - more marked for early component. Also, a threshold of stimuli of early response was strongly increased during synchronized sleep. During the REM phase all the parameters of the reflex response to stimuli approached wakefulness values.

Ljubin and Reichherzer (1976) examined the latency time of the reflex contraction of the musculus orbicularis oculi provoked by acoustic stimuli in a state of rest and in a state of fatigue in a group of shift workers after a sleep deprivation. It was shown that the latency time was shortened in the state of fatigue in comparison with the same variable in the state of rest.

Boelhouwer and Brunia (1977) studied the effect of the activation level on blink reflex. They registered electromyogram of reflex contractions with surface electrodes. Reflex contractions were elicited by electrical stimulus applied on the supraorbital branch of nervus trigeminus.

Reflex reactions of the orbicularis were recorded during rest phase and during experimental task requiring lower and higher levels of nervous system activation. Amplitude of the early monosynaptic reflex response increased in the situation of increased vigilance, while the amplitude of later response showed a continuous decline as a function of experiment duration. Latency time of the early response was not modified by the experimental task, while the latency time of late response increased as a function of experiment duration. According to Boelhouwer and Brunia (1977), facilitation of the early answer is due to the increased activity of reticular formation. Boelhouwer (1982) studied blink reflex in the three-second period between the presentations of a warning signal and the reaction signal. One group of subjects had to react by pressing the button with both hands, while the other group had to react with voluntary eyelid blinks. Increase in the amplitude of the early component of reflex response in first 300 ms and the simultaneous inhibition of the second, polysinaptic component was observed in both groups. Similar results were reported by Boelhouwer et al. (1987). Presentation of the warning signal increased the ipsilateral component, while later, bilateral component was inhibited. Boelhouwer et al. (1991) studied R1 and R2 components elicited by electrical stimuli presented shortly before, simultaneously with, and shortly after the onset of weak warning acoustic stimulus, and prior to the acoustic stimulus demanding motor reaction. Warning stimulus and reaction stimulus were presented with an interstimulus interval of 3 seconds. The amplitude of R1 component increased when R1 was elicited by electrical stimulus 50 ms after the warning stimulus onset. Bilateral component R2 was enhanced only when electrical stimulus was presented before the warning acoustic stimulus. Similar results were obtained in a somewhat different experimental situation, demanding voluntary blink reaction to acoustic stimulus (Boelhouwer et al, 1996).

Jancke et al. (1994) studied the effects of various levels of previous, voluntarily maintained tonic preinnervation of the musculus orbicularis oculi (i.e., 25%, 50%, 75%, and 100% of maximum voluntary activation) on R1 and R2 components of electrically evoked blink reflex. Subjects had to contract their orbicularis oculi muscle, according to instruction, with certain effort while the EMG responses were elicited by electrical stimulation. Amplitude of R1 component was increasing until the preinnervation level reached 50% of the maximum value. Further increase of the preinnervation level had no effects on the R1 component. The amplitude of R2 component increased as a function of preinnervation level longer than R1 component. Latency times decreased only to the preinnervation level of 25% for both components .

Rossi et al. (1995) investigated the influence of combined visual and acoustic prestimuli on blink reflex elicited by subsequent stimulation of the supraorbital nerve. First group of subjects received information about the onset of a visual or acoustic warning prestimulus, while the second group was not informed about prestimulus (i.e., prestimulus had no warning value for this group). R1 component was enhanced at an interstimulus interval of 0.1 sec in the warned group with visual prestimulus preceding electrical stimulus. At the same time, R2 component was reduced, while R3 component was blocked. The increase in R1 component with the simultaneous decrement in R2 and R3 was observed for both types of prestimuli in the unwarned group.

A number of researchers investigated the influence of anxiety, fear, or expected stimulation of positive and negative emotional valence on the parameters of reflex response. Hamm et al. (1991) studied the effects of preceding visual stimulation on the contraction of orbicularis oculi which were produced by acoustic stimuli. Visual stimuli which varied in emotional valence and arousal, were previously conditioned by electro-shock stimulation. The amplitude of reflex response, compared to the baseline amplitude prior to the conditioning process, increased with the pleasantness level of visual stimulus that was reinforced by an electric chock. Bradley et al. (1991) investigated the influence of the prior exposure to the pleasant, neutral, and aversive visual material on blink reflex elicited by auditory stimulation of left or right ear. When auditory stimulus was presented to the left ear, the amplitude of reflex contractions on aversive stimuli increased compared to the situations with pleasant and neutral stimuli. On the other hand, the amplitude of reflex contraction was not modulated by presentation of the same visual material to the right ear. According to Bradley et al. (1991), these results suggest that emotional processes can modulate blink reflex due to the lateralization of brain hemispheres. Grillon et al. (1991) studied the effects of previously induced anxiety on blink reflex elicited by auditory stimulation. In the first experimental situation subjects did not receive an electrical shock (a safe period), while in the second situation they anticipated an electric shock. Reflex blink on auditory stimulation in the situation of anticipated shock was of greater amplitude and shorter latency time compared to the situation without an electric shock. Authors thus consider the utility of this procedure as an objective measure of anxiety elicited by anticipated discomfort.

Findings of Hamm et al. (1997) provide further support to the relation of the amplitude of reflex reaction elicited by acoustic stimulus and state of fear evoked by fear-eliciting objects. Cuthbert et al. (1996) report the increase in reflex response to acoustic stimuli following the presentation of pictures with aversive content. On the other hand, subject's reflex answer was reduced in the situation with pictures of pleasant content. In the same study, Cuthbert et al. (1996) paired contents evoking three arousal levels (low, moderate, and high) with reflex eliciting stimulation. Blink reflex of largest amplitude was observed in the situation with aversive content eliciting high arousal. The greatest reduction of the reflex response amplitude was observed in the situation with pleasant content evoking high arousal. Similar results were reported by Schupp et al. (1997). They compared parameters of blink reflex elicited by acoustic stimuli and P3 component of evoked brain potentials. Variability of the amplitude of reflex response depended on pleasantness of the stimuli, while the variability of P3 component of evoked brain potentials depended on arousal.

Some researchers studied the effects of endogenous arousal, as reflected in personality type, on the amplitude of reflex response of musculus orbicularis oculi. Zelson and Simon (1986) investigate relations of the personality type, measered by Jenkins Activity Questionnaire, with the amplitude of reflex response elicited by acoustic stimuli during vigilance tasks. Reflex reactions of type A subjects were more attenuated compared to reactions of type B subjects. Degree of this attenuation was proportional to the duration of vigilance task. Ljubin and Ljubin (1990) report relations between extraversion, as measured by Eysenck's Personality Inventory, and the amplitude of reflex blink on acoustic stimuli (audiomotor reflex). The average amplitude of reflex response in introverts was larger than the one in extraverts. According to the authors, these results are due to the higher level of endogenous arousal in introverts.

Research cited above clearly demonstrates the effects of general arousal on parameters of reflex contractions elicited by various stimuli.

Pharmacology

Some researchers tried to determine the utility of reflex contraction parameters as the indicators of the effects of various substances on neurophysiological processes. For example, Dupreyon (1973) studied the utility of reflex blink in rabbitts as the possible index of the activity of a certain substance in nervous system. The increase in reserpine level, at certain point leads to the inhibition of reflex reaction to the light stimulus. The intake of substances having the opposite effect (antireserpine) can recover previously inhibited reflex activity. Ljubin et al. (1979) studied the effects of somatostatin, substance isolated from sheep's hypotalamus and known for its inhibitory effects on the secretion of growth hormone, on reflex response of musculus orbicularis oculi to acoustic stimuli. Somatostatin had inhibitory effects on reflex reaction, reducing the maximal amplitude and duration, and at the same time increasing the latency time of reflex response. Ferracuti et al. (1994) determined parameters of corneal and blink reflex (R1 and R2) prior to and after the intake of piroxicam and lysin acetylsalycilate - substances having analgetic and antiinflammatory effects. Both substances reduced the amplitude of corneal reflex for 20-30%, but had no effect on R1 and R2 components of blink reflex. The application of naloxane did not restore the initial values of the blink reflex parameters.

Research dealing with the effects of pharmacologically active substances on the parameters of the reaction of musculus orbicularis oculi is still sparse. However, in our opinion, future research of the reflex blink parameters will provide information on the dynamics of psychoactive substances in nervous system.

Pathology

A distinct group of research pertains to the study of blink reflex in pathological states of the nervous system. These studies primarily have clinical relevance, as thus collected data are useful source of information for clinical and therapeutic purposes. However, the pathological organic states are a sort of "natural experiment". Data collected for diagnostic purposes can, therefore, enhance our knowledge about reflex reactions of musculus orbicularis oculi. Dehen et al. (1976) experimented with patients having central facial paralysis caused by unilateral hemispheric injury, and found bilateral changes of the blink reflex. Responses on hemiphlegic side of the head were reduced, while responses on the "normal" side were facilitated. Latency times of the R2 component on both sides remained unchanged. Studying patients with senile dementia, Tavy et al. (1986) find significantly longer latency times of blink reflex elicited by light stimuli. Ljubin (1989) stimulated the cochlea of hemiparetic children with acoustic stimulation, and registered the motor responses of musculus tibialis anterior. He found the increase in excitability of motor neurons in those children. Furthermore, he reported the increase in latency times and reduction of the amplitude of reflex response to acoustic stimuli in patients with paresis of the nervus facialis. Cassachia et al. (1990) demonstrated significant increase in latency time of R2 component of the blink reflex in schizophrenics compared to the control sample of normal subjects. They stated that inhibitory reflexes were the result of the increased dopaminergic-striatal impact on reflex activity. Hackley and Johnson (1996) presented flash stimulation to the subjects with scotoma of the visual field caused by occipital lobe damages. Both early and late component were unchanged following the stimulation of the scotoma regions as well as the intact regions. These results support the role of subcortical structures in the components of reflex contraction. Furthermore, later component was greater following the stimulation of temporal region of the visual field compared to the same component elicited by stimulation of the nasal region of the visual field.

The analysis of the characteristics of reflex responses is particularly interesting in

the cases of posttraumatic stress disorder (PTSD). Ornitz and Pynoos (1989) compared startle responses elicited by bursts of white noise in group of 8-13 years old children with PTSD and in control group of children without traumatic experiences. They investigated inhibitory and facilitatory effects of the acoustic prestimulation on the modulation of blink reflex characteristics. Children with PTSD demonstrated a significant loss of the inhibitory modulation of reflex response to the acoustic stimuli. According to Ornitz and Pynoos (1989), long-lasting effects of the PTSD cause certain dysfunctions in the brain stem.

The influence of PTSD was investigated in combat veterans with traumatic experience from the Vietnam (Morgan et al., 1995a, 1995b) and Gulf War (Morgan et al., 1996). The average age of their subjects was 43 years. They found evidence of greater amplitude of reflex response elicited by acoustic stimulus in the groups of subjects with PTSD than in control groups. Increase of the amplitude of reflex response was observed both in situations with and without electric shock.

Morgan et al. (1995b) investigated the effects of yohimbine and saline placebo on the amplitude of startle response in the group of veterans with PTSD and in control group of combat veterans without PTSD. Yohimbine increased the amplitude of acoustic startle reflex in the group of combat veterans with PTSD, but had no effect on the amplitude of the analogous response in control group of combat veterans. These results imply a relationship between the symptomatology of PTSD, as expressed in the characteristics of startle response, and noradrenergic functions (Morgan et al., 1995b).

Morgan et al. (1996) compared reflex responses to the acoustic stimuli in Gulf War veterans with PTSD, in group of veterans without PTSD, and in normal subjects whose average age was 24 years. Amplitude of the first startle response was found to be greater in veterans with PTSD compared to the other two groups. Authors conclude that the sensitisation of the fear/alarm response, influenced by combat stress, increases the level of startle response.

This brief overview illustrates the interest of researchers in the application of reflexological methods for diagnostic purposes. A more detailed overview of numerous studies in the field of pathology is beyond the scope of this article. However, it should br noterd that, beyond their clinical importance, the results of these studies substantially contribute to the understanding of complex processes and features of the reflex contractions of the musculus orbicularis oculi.

RESEARCH ON THE REFLEX CONTRACTIONS OF THE MUSCULUS ORBICULARIS OCULI IN CROATIA

Our research on reflex contractions of the musculus orbicularis oculi following light and acoustic stimuli was conducted using the apparatus assembled by Ljubin (1970, 1973). The apparatus provided recordings of the electromyogram of reflex contractions of musculus orbicularis oculi to acoustic and light stimuli at the most appropriate moment. Scheme of the apparatus for the registration of the EMG of reflex contractions to acoustic stimuli is presented in Figure 7. Three most important instruments are visible on this figure: oscilloscope, electromyograph with memory and tone generator. First, surface silver electrodes were fixed on subject's skin above the inferior right musculus orbicularis oculi (pars inferior dextra) with an interspace of 2 cm. Ground electrode was fixed on subject's right hand. Subjects also received earphones for binaural stimulation. Subject had to lay still in bed visually fixing a point in the silent experimental room with weak light. Spontaneous, continuous bioelectrical activity or, in other words, tension of the musculus orbicularis was observed on the oscilloscope. At the moment of muscular relaxation following the spontaneous blink (the moment of "bioelectrical silence" on the oscilloscope), subjects received acoustic stimulation. At the same time, automatically activated electromyograph started registering a segment of continuous bioelectrical activity containing complete information about the reflex blink: latency time, duration, and amplitude of reflex contraction. Parameters of the electromyogram recording were previously established and adjusted on the electromyograph. EMG of the muscular response was "frozen" on the screen of the electromyograph as a static picture. Polaroid camera, a part of the electromyograph, photographed the electromyograph screen. The quality of the photograph was controlled few minutes later. The content of the screen was then erased, and the apparatus was ready for new recordings of the electromyogram of the blink reflex on acoustic stimuli. Electromyograph allowed the saving of several electromyograms on various screen levels and thus comparing the several consecutive responses.

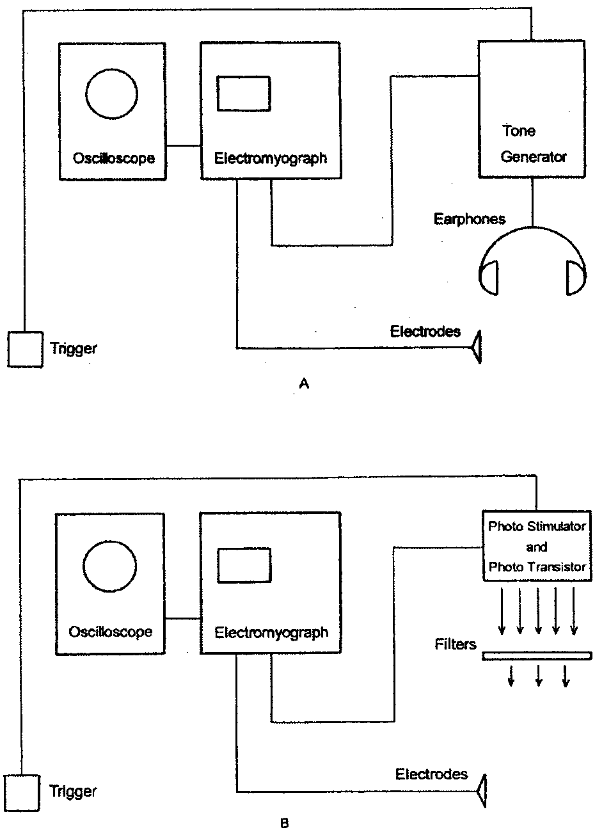


Figure 7. Block scheme of device for the registration of electromyographic reflex responses of musculus orbicularis oculi to (A) acoustic and (B) light stimuli.

Tone generator allowed the variations in tone intensity, duration, and frequency. Repeated stimuli were presented with the 5 minutes interval. This procedure inhibited the effects of habituation and fatigue on some parameters of the reflex response.

Similar procedure was used for the recording of the electromyogram of reflex responses elicited by the stimulation of retina with light. The source of intense light was connected to the electromyograph via ultra fast phototransistor. Tension of the musculus orbicularis and spontaneous blinks were followed on the oscilloscope screen. Presentation of light stimulus, illuminating the subject, initiated registration of the electromyographic response of musculus orbicularis oculi. Previous observation of the activity of musculus orbicularis allowed the registration of the electromyogram of reflex response at the most appropriate moment. Varying sensitivity of the retina to light was the possible source of errors in the registration of the electromyogram of reflex muscular contractions. Experiment has to be done in acoustically and visually isolated room, with the preceding 30 minutes of eye recuperation in darkness. Furthermore, sensitivity of retina has to remain constant during the course of the experiment. Intervals between consecutive stimuli in our experiment were 5 minutes. During experimental period, subjects were constantly in a dark room.

We tried to carry out a series of investigations using the apparatus developed by Ljubin (1970, 1973). The aim of this research was to establish the possible "signs" or correspondences in parameters of the reflex reactions of musculus orbicularis for various independent variables. We had in mind the following Sherrington's thought: "But we have first to remember that in dealing with reflexes even experimentally we very usually deal with them as reactions for which reflex-arc as a whole and without any separation into constituent parts is laid under contribution. The reflex-arc thus taken includes the receptor. It is assuredly as truly a functional part of the arc as any other. But, for the analysis of the arc's conduction, it is obvious that by including the receptor we are including a structure which, as its name implies, adaptation has specialized for excitation of a kind different from that obtaining for all the rest of arc. It is therefore advantageous, as we have to include the receptor in the reflex arc, to consider what characters its inclusion probably grafts upon the functioning of the arc." (Sherrington, 1906/1948, pp. 9).

Ljubin and Ibrahimpašić (1977) tried to elicit reflex contraction of musculus orbicularis oculi presenting a flash of monochromatic light to the subjects with normal color vision (Figure 8) and to the subjects with color vision anomalies. In addition, authors tried to determine possible effects chromatic quality of the stimuli on latency time of reflex response. They presented chromatic stimuli of characteristic wavelengths for blue (425 nm), green (522 nm), red (685 nm), and white light (Figure 8). Both monochro-

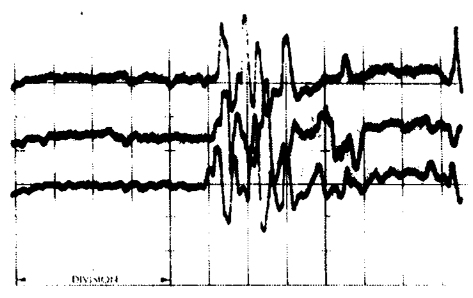


Figure 8. Electromyograms of the reflex contractions of musculus orbicularis oculi for the subject F.T.I. to the stimulation of retina with an intense flash of red light lasting 3 ms (50 cm from subjest's eyes). Recording condition: time of analysis 40 ms/DIV, sensitivity 200 μV/DIV.

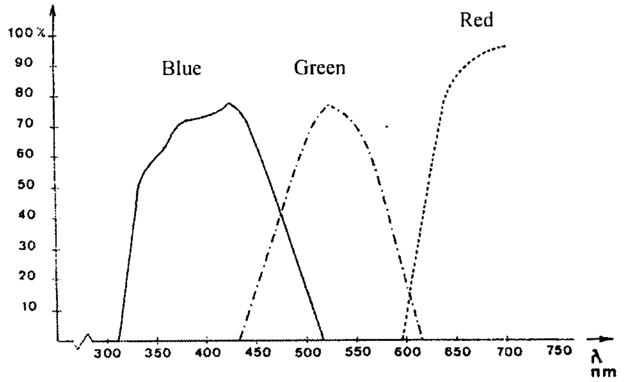


Figure 9. Permeability characteristics of the filters for blue, green, and red light. Abscisse – wavelenghts in nm. Ordinate – percentage of the permeated monochromatic light for each wavelength.

matic and polichromatic light elicit reflex contraction of the musculus orbicularis oculi both in subjects with normal color vision and in those with color vision anomaly. Small, statistically nonsignificant differences in latency times can be attributed to the small differences in intensity of the presented light stimuli (Figure 9). The shortest latency time was observed for white light, followed by red light, and then for the blue and green light having approximately equal latency times.

The comparative analysis of latency times of reflex contraction of musculus orbicularis oculi elicited by tone and light tried to determine whether there are stable differences in these latency times, as well as the possible determinants of these differences (Ibrahimpašić & Ljubin, 1977). Authors presented acoustic stimuli whose intensity was 110 dB, duration 100 ms and frequency 1 kHz. White light from the photographic flash served as a visual stimulus, with intensity of approximately 6000 lx and duration of approximately 3 ms. The average latency times in our subjects (*N*=14) were 46,1 ms for the reflex contractions elicited by light, and 30,6 ms for the reflex contractions elicited by acoustic stimulation. The obtained difference was 15,5 ms. This difference is almost identical to the difference in estimated duration of refractory period for the ear and eye (the shortest interval between two stimuli allowing the occurrence of different sensations), determined by Exner (Schaffer, 1900, str. 614). According to Exner, refractory period is 0.017 sec for the eye, and 0.002 for the ear, so the difference between the two values is 15 ms.

As it is known, the time needed for transduction of physical energy into energy of neural impulses in cochlea and the time needed for the same process in retina are not equal. These two time periods are significantly different when two measures of the duration of receptory processes for two sensory modalities are latency time of B-wave of retinogram for the light stimulation (Johnson,1958), and latency time of N1 component of action potential of auditory nerve for acoustic stimulation (Yoshie et al. 1967). We thus suggested a hypothesis that differences in latency times of reflex contractions of musculus orbicularis oculi elicited by the light and acoustic stimuli are almost entirely due to the differences in duration of the processes of transduction of physical energy into neural impulses energy in retina and cochlea. This hypothesis is supported by the findings about the difference between recyprocal values of fusion frequency for the light and tone. The difference between reciprocal values of critical frequency of fusion for the intense light (1/60 = 16.7 ms; Bartley, 1958), and intense tone (1/1000 = 1 ms; Symmes et al., 1955) was found to be equal to the differences between average values of the latency times of reflex contractions of orbicularis on light and sound.

In our investigation, we applied stimulus intensities close to the upper limit of sensory continuum, providing minimal latency times of reflex contractions. Ibrahimpašić and Ljubin (1979) examined the relations between reflex and cortically controlled (voluntary) contractions of musculus orbicularis oculi using light and acoustic stimuli balanced according to the absolute limen for light and sound. Light intensity of 95 dB of light (Stevens, 1955) and tone stimulus of 95 dB of sound were used to elicit reflex contractions. Cortically controlled contractions were elicited by stimulus intensities of 40 dB of light, or 40 dB of tone. The stimulus intensities of 40 dB did not elicit reflex contractions (Figure 10). The differences in latency times of reflex contractions of musculus orbicularis oculi are similar to the differences in latency times obtained in previously cited study (Ibrahimpašić & Ljubin, 1977), although the stimulus intensities were of lower values compared to the previous study. The average values of latency times of reflex responses in our group of subjects (*N* = 8) are somewhat longer due to the fact that subjects received weaker stimuli. The processes of transduction in receptors are slower for weaker stimulation (*M* = 53,2 ms for light stimuli; *M* = 35,8 ms for acoustic stimuli; D = 17,4). The obtained results for both reflex and cortically controlled contractions can be interpreted within the hypothesis about the differences in duration of the processes of transduction of physical energy in neural impulses energy to retina and cochlea. They are not attributable to the differences in stimulus conduction processes in pathways involved in neural transmission between receptors and effectors.

The role of receptory functions in latency time of reflex responses to acoustic stimuli was further examined in the study of the effects of acoustic stimulus duration on parameters of the reflex and cortically controlled contractions of the musculus orbicularis oculi (Ibrahimpašić & Ljubin, 1979). The intensity of tone stimulus was 95 dB, while the

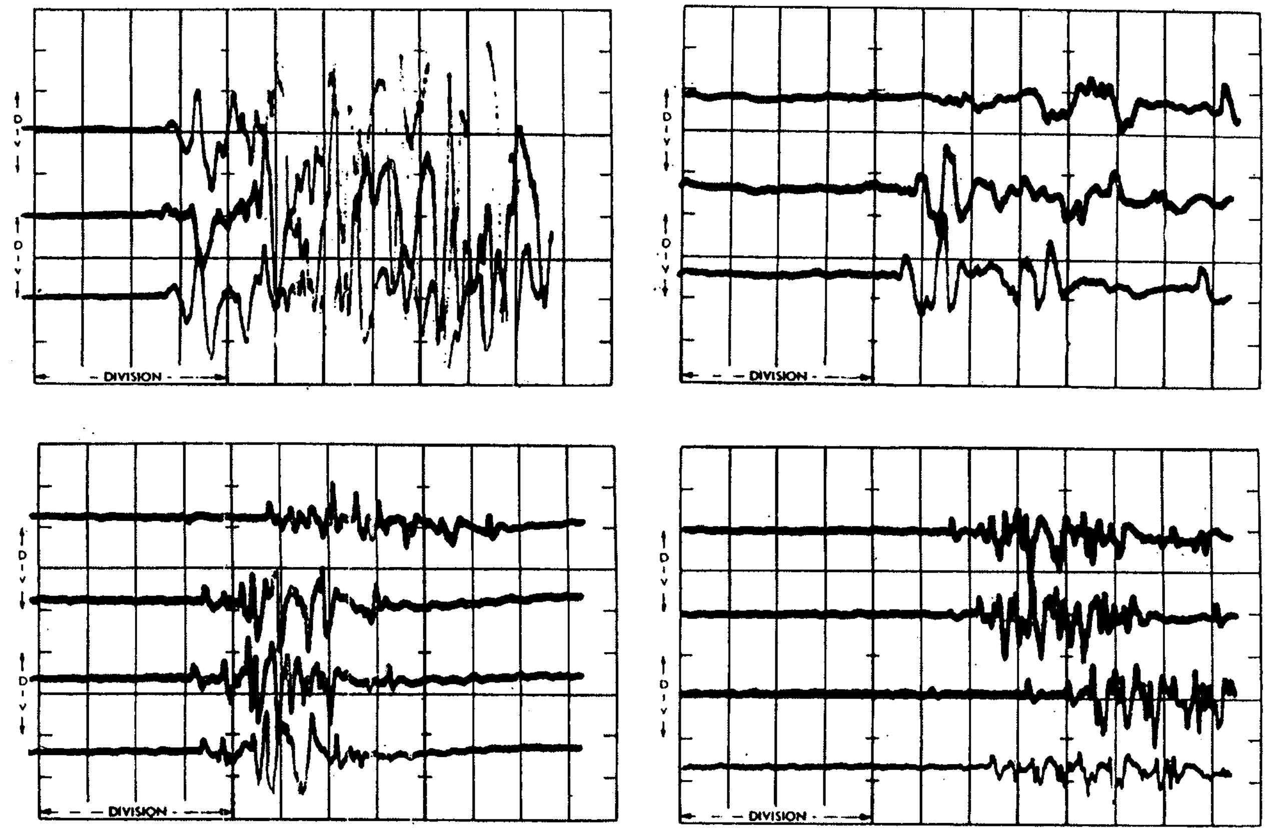


Figure 10. Electromyograms of the reflex (upper pictures) and cortically controlled contractions (lower pictures) of the musculus orbicularis oculi on acoustic (left pictures) and light (right pictures) stimuli for the subject Ž.K. Recording conditions: for reflex contractions – time of analysis 40 ms/DIV, sensitivity 100 mV; for cortically controlled contractions - time of analysis 100 ms/DIV, sensitivity 200 μV/DIV.

frequency was 1kHz. The tone duration was 1 ms, 3 ms, 10 ms, 30 ms, 60 ms, and 100 ms. Latency times for both reflex and cortically controlled contractions varied in a similar way, depending on the duration of acoustic stimulus (Figure 11). The obtained equations of hyperbolic functions had similar exponents. The exponent for latency times of reflex contractions was $n = -0.043$. The exponent value for latency time of cortically controlled contraction was $n = -0.035$. Reduction in latency times of reflex and cortically controlled reaction as a function of increased duration of acoustic stimulus could be interpreted by the well known law about the excitability of receptory organs. According to this law, the product of intensity and duration of physical stimulus is constant (I x T = C). The increase in duration of acoustic stimulus of constant intensity thus leads to the increase in the amount or, in other words, in intensity of physical stimulation innervating the receptors.

Ibrahimpašić and Ljubin (1982) examined the relations between physical stimulus intensity and the intensity of

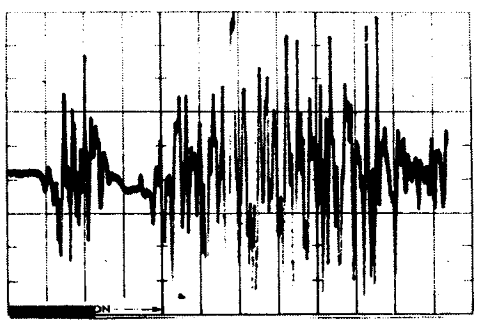


Figure 11. Electromyogram of the reflex and cortically controlled contraction of the musculus orbicularis oculi for the subject F.T.I. elicited by acoustic stimulus (95 dB, 1 kHz, 60 ms). Subject had to display the quickest possible "voluntarily" blink after presentation of the acoustic stimulus. Recording conditions: time of analysis 100 ms/DIV, sensitivity 200 μV/DIV.

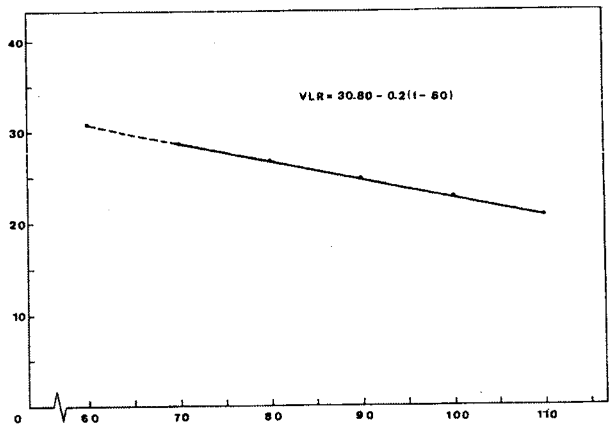


Figure 12. Relation between the latency time of the reflex contraction of musculus orbicularis oculi (VLR) and the intensity of acoustic stimulus (I) – (lin-log system). Ordinate – latency time of reflex response in milliseconds. Abscisse – the intensity of acoustic stimulus in decibels (SPL). Measured and approximated values completely converge.

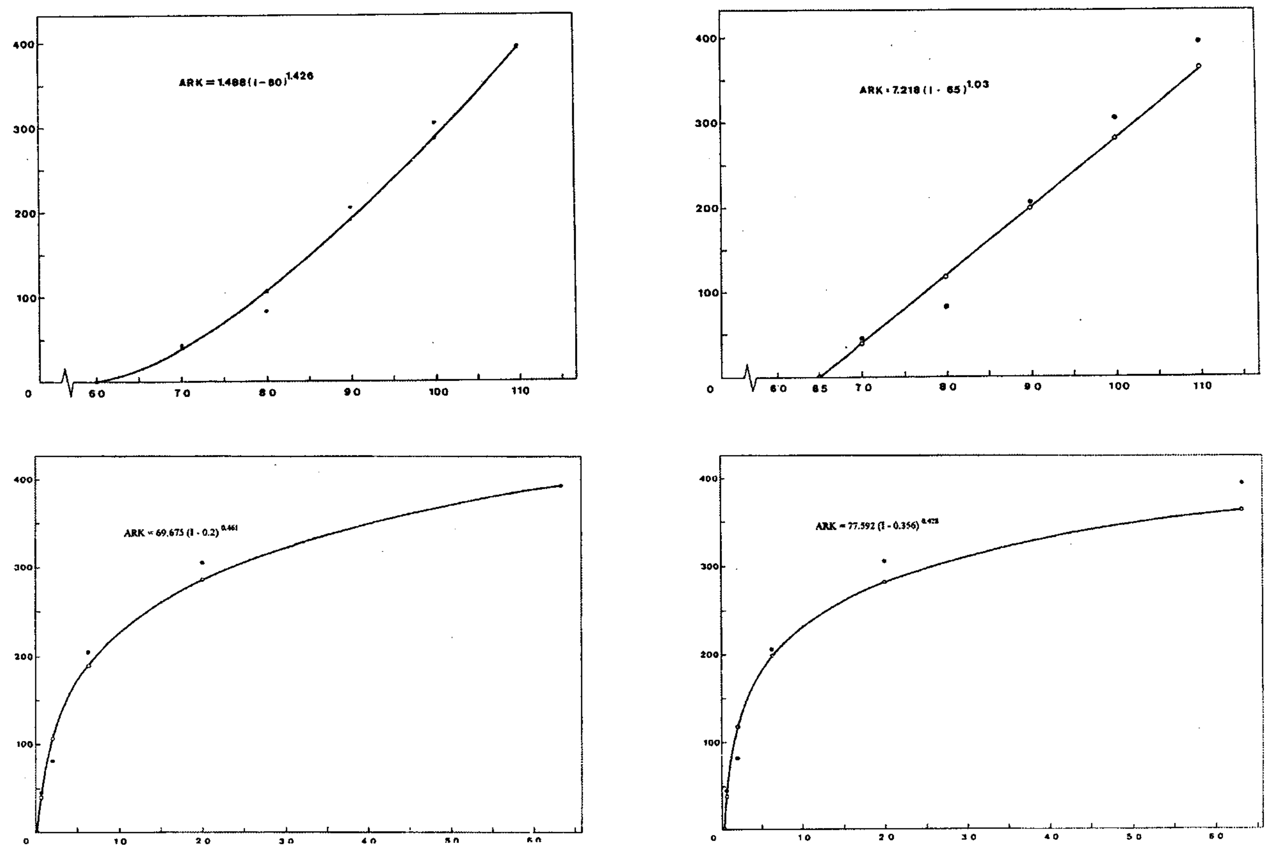


Figure 13. Relation between peak to peak amplitude of the reflex contraction of musculus orbicularis oculi (ARK) and the intensity of acoustic stimulus (I) for the two modes of determining the zero point of reflex response. Top pictures: ordinate – amplitude in microvolts. Abscissa – intensity of the acoustic stimulus in decibels (SPL) (lin-log system). Bottom pictures: ordinate – amplitude of the reflex contraction in microvolts. Abscissa – the intensity of air pressure in microbars (lin-lin system). Full circles represent the measured values of dependent variable. Empty circles are the approximated values of dependent variable obtained by the least squares method.

neurophysiological reaction elicited by the stimulus. In other words, authors tried to determine the shape of neurophysical function. The acoustic stimulus intensities were 60 db, 70 db, 80 db, 90 db, 100 db, and 110 db. Neurophysiological reaction was indicated by the maximal amplitude of reflex contraction of the musculus orbicularis oc

Data were analyzed by a least squares method (Lewis, 1960). Results of the study reveal a relation between intensity of acoustic stimuli and maximal amplitude of electromyogram of reflex contraction of musculus orbicularis oculi. This relation is adequately represented by a power function with an exponent of n = 1,426 and with 60 db taken as a zero point of the abscissa. However, if 65 db are taken as a zero value, least square method yields a function with an exponent of n = 1,03. With an exponent of n = 1, relation between the amplitude of reflex response and the intensity of acoustic stimulus expressed in decibels (logarithmic scale) can be approximated by a straight line.

The conversion of logarithmic values on the abscissa into linear (ordinal) scale values of micro bar units produces functions of parabolic type. These functions have an exponent of n = 0,461 with a zero point of 0.2 microbars (60 db), or n = 0,428 with a zero point of 0,356 microbars (65 db) (Figure 13).

Ibrahimpašić (1981) studied the effects of selective attention on the amplitude of reflex contraction of musculus orbicularis oculi. Starting from the theory of centrifugal inhibition (Hernandes-Peon, 1956, 1961), Ibrahimpašić tried to investigate the effects of selective attention to acoustic and tactile stimuli on the maximal amplitude of reflex contraction of musculus orbicularis oculi elicited by a short acoustic stimulus of defined intensity, duration and frequency. Results showed that qualitatively different selective attention could have an impact on the maximal amplitude of reflex contraction of musculus orbicularis oculi. The level of excitation or inhibition, as reflected in the increase or decrease of the maximal amplitude of reflex contraction, can be modulated by experimental design and instructions. Generally, maximal amplitude of the EMG of reflex contraction is greater in the situation of selective attention to acoustic stimuli compared to the situation of selective attention to tactile stimuli (Figure 14). This finding

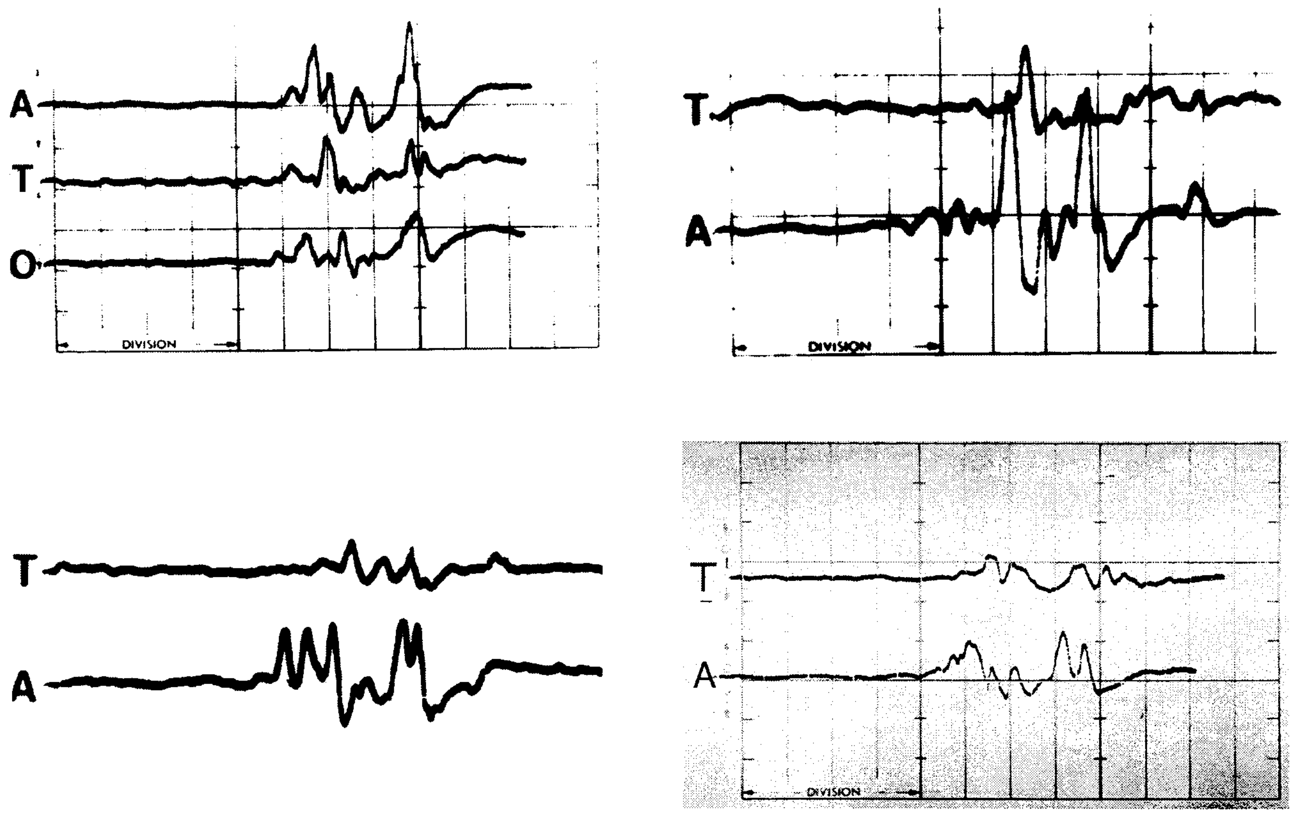
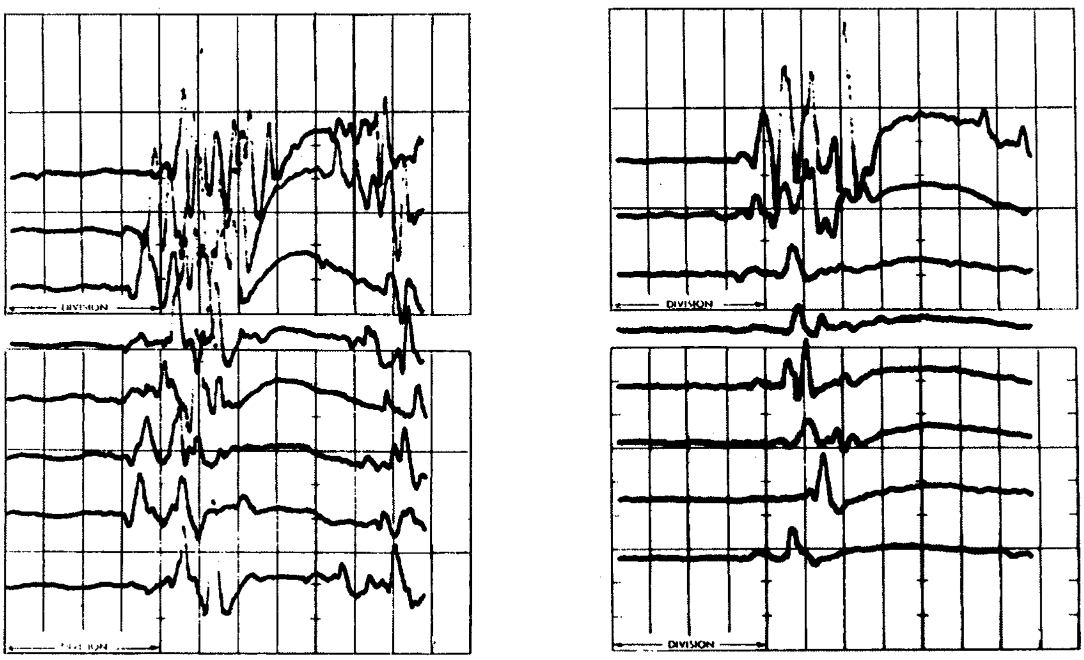


Figure 14. EMG's of reflex contractions of musculus orbicularis oculi on acoustic stimuli for the various situations of selective attention (for the subjects K.M. and F.T.I.): A – situation of selective attention to acoustic stimuli, T – situation of selective attention to tactile stimuli, O – situation without selective attention. Recording conditions: time of analysis 40 ms/DIV, sensitivity 200 μV/DIV.

is probably due to the control of neurophysiological processes in the sensory branch of the reflex arch involved in reflex contractions on acoustic stimuli. This process of control in the sensory branch is probably a result of central descendent processes. Results of this experiment were subsequently verified by several researchers.

A number of studies investigated the influence of pharmacologically active substances on parameters of reflex contraction of orbicularis oculi. Ibrahimpašić, Ljubin, and Marković (1990) studied the effects of nootropic substance of piracetam (2-oxo-pyrolidin acetamid) on electromyographic parameters of the reflex contractions of musculus orbicularis oculi. Piracetam is a cyclic derivate of GABA - neurotransmitter with inhibitory effect on CNS. In a double blind experiment, authors tried to investigate the impact of a nontoxic, nonadditive nootropic substance on reflex action of musculus orbicularis oculi. Piracetam is known to have a preferential action in bioenergetic metabolism of brain cells, particularly those in cerebral cortex, facilitating conversion of the energy ADP in ATP of brain cells. In the first experimental situation, electromyographic responses of musculus orbicularis were registered during an interval between the peak concentration of the substance in blood (70 minutes after oral intake) and the peak concentration in brain cortex (120 minutes after oral intake). This experimental situation had to demonstrate the effects of piracetam on simple transmission of neural excitation in reflex arch. Acoustic stimuli (105 dB of intensity, 100 ms of duration, and 1kHz of frequency) were presented with an interstimulus interval of 5 minutes. Second experimental situation was designed to investigate effects of the habituation of reflex response 120 minutes after the intake of piracetam. During this situation, acoustic stimuli were presented with an interstimulus interval of 10 seconds.

Piracetam did not demonstrate measurable effects on several parameters (latency time, maximal amplitude, and duration of reflex response) of the electromyogram of musculus orbicularis oculi on acoustic stimuli when frequency of stimulus presentation did not facilitate the process of reflex response habituation. However, when the frequency of presented stimuli favored habituation process, piracetam had facilitatory effects on habituation process. The facilitatory effect was reflected in decrease of the maximal amplitude of reflex response following repeated stimulus presentation. The process of habituation of the maximal amplitude of reflex response was shown to be more rapid with the intake of priracetam compared to the placebo intake situation (Figures 15 and 16). A hypothesis about the development of "neural model" (Sokolov, 1960) during successive presentation of acoustic stimuli could



PLACEBO PIRACETAM

Figure 15. Consecutive electromyograms of the reflex contractions of musculus orbicularis oculi (subject G.B.) to the acoustic stimuli during development of the habituation process. Frequency of the acoustic stimuli: one stimulus in 10 seconds. Recording conditions: time of analysis 40 ms/DIV, sensitivity 200 μV/DIV.

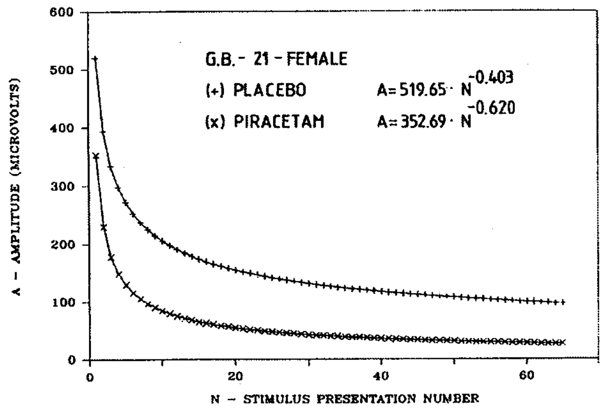


Figure 16. Curves of the development of habituation process of the maximal amplitude of reflex response (subject G.B.) to the acoustic stimuli in two situations: in situation with the intake of placebo (++++) and in the situation with piracetam (xxxx). Curves are based on the calculated values of the amplitude of reflex response using the least squares method. Minimum squares method was applied on the measured values of maximal amplitude of the reflex contraction. Ordinate – amplitude of the reflex contraction in V (A). Abscissa – ordinal number of the stimulus (N).

serve as a possible explanation of the findings. In addition, these findings could be mediated by the extinction of orienting response due to the inhibitory processes from the central structures. These influences are probably manifested at the reticulary formation level.

Piracetam elevates the energetic level in cortical cells and thus probably speeds up the processes of the development of "neural model". Piracetam also speeds up the central descendent inhibitory effects on reticular formation. Changes in the activity of reticular formation then lead to the decrease in the amplitude of reflex response. In other words, the presence of piracetam in metabolysm of the cortical brain cells speeds up the elementary learning processes in neural system, reflected in the more powerful habituation of the reflex response on consecutive acoustic stimuli. However, this hypothesis has still to be verified by future research.

CONCLUSION

Studies of the reflex response of musculus orbicularis oculi clearly demonstrate that the evidence about the activity of a small muscle can provide a valuable information about the activity of nervous system underlying functions of the sensory organs and the complex psychological processes such as attention and learning. Even with the scant laboratory equipment, research following strict methodological rules can provide valuable results. For all of us adhering to the well-known saying of Johannes Muller: "Nemo psychologus nisi physiologus" (Brown, 1976, pp. 87), research of simple reflexes can provide an opportunity to study and test various theories about complex psychological processes.

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Received October 1999
Accepted November 1999