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Nociceptive Quality of the Laser-Evoked Blink Reflex in Humans

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Romaniello, A., J. Valls-Solé, G. D. Iannetti, A. Truini, M. Manfredi, and G. Cruccu. Nociceptive quality of the laser-evoked blink reflex in humans. *J Neurophysiol* 87: 1386–1394, 2002; 10.1152/jn.00041.2001. Laser radiant-heat pulses selectively excite the free nerve endings in the superficial layers of the skin and activate mechano-thermal nociceptive afferents; when directed to the perioral or supraorbital skin, high-intensity laser pulses evoke a blink-like response in the orbicularis oculi muscle (the laser blink reflex, LBR). We investigated the functional properties (startle or nociceptive origin) of the LBR and sought to characterize its central pathways. Using high-intensity CO_2-laser stimulation of the perioral or supraorbital regions and electromyographic (EMG) recordings from the orbicularis oculi muscles, we did five experiments in 20 healthy volunteers. First, to investigate whether the LBR is a startle response, we studied its habituation to expected rhythmic stimuli and to unexpected arrhythmic stimuli. To assess its possible nociceptive quality, we studied changes in the LBR and the R2 component of the electrical blink reflex after a lidocaine-induced supraorbital nerve block and after intramuscular injection of the opiate fentanyl and the opiate-antagonist naloxone. To characterize the central pathways for the LBR, we investigated the interaction between the LBR and the three components of the blink reflex (R1, R2, and R3) by delivering laser pulses to the perioral or supraorbital regions before or after electrical stimulation of the supraorbital nerve at various interstimulus intervals. Finally, to gain further information on the central LBR pathways, using two identical CO_2-laser stimulators, we studied the LBR recovery curves with paired laser pulses delivered to adjacent forehead points at interstimulus intervals from 250 ms to 1.5 s. The LBR withstood relatively high-frequency rhythmic stimulations, and unexpected laser pulses failed to evoke larger responses. When lidocaine began to induce hypoalgesia (about 5 min after the injection), the LBR was abolished, whereas R2 was only partly suppressed 10 min after the injection. Fentanyl injection induced strong, naloxone-reversible, LBR suppression (the response decreased to 25.3% of predrug values at 10 min and to 4% at 20 min), whereas R2 remained appreciably unchanged. Whether directed to the perioral or supraorbital regions, preceding laser pulses strongly suppressed R2 and R3 though not R1. Conversely, preceding electrical stimuli to the supraorbital nerve suppressed the LBR. In response to paired stimuli, the LBR recovered significantly faster than R2. These findings indicate that the LBR is a nociceptive reflex, which shares part of the interneuron chain mediating the nonnociceptive R2 blink reflex, probably in the medullary reticular formation. The LBR may prove useful for studying the pathophysiology of orofacial pain syndromes.

INTRODUCTION

Current information on the functional and anatomical characteristics of the blink reflex comes mainly from studies on the electrically elicited blink reflex (Cruccu et al. 1991; Ellrich and Treede 1998; Ongerboer de Visser and Cruccu 1993; Pellegrini et al. 1995; Rimpel et al. 1982; Valls-Solé et al. 1999). In humans, this reflex consists of three components: R1, R2, and R3. R1 is relayed through an oligosynaptic arc probably located close to the main sensory nucleus of the trigeminal nerve (Ongerboer de Visser and Cruccu 1993). R2 is mediated by a polysynaptic chain of interneurons belonging to the lateral reticular formation in the lower medulla (Ongerboer de Visser and Cruccu 1993). R1 and R2 are both nonnociceptive in origin and mediated by Aβ fibers (Ongerboer de Visser and Cruccu 1993; Pellegrini et al. 1995). R3 is mediated by a polysynaptic circuit in the medulla (Ellrich and Hopf 1996) or in the rostral segments of the cervical spinal cord (Rossi et al. 1989); its afferents are still unclear (Cruccu et al. 1991; Ellrich and Hopf 1996; Rossi et al. 1989). The organization of the R1 and R2 blink components differs quantitatively in humans and other mammals. In a few tested species, reflex blinking results from the activity of various neural control systems not always or exclusively involved in this motor response (Gruart et al. 1995). The R1 and R2 neural circuits are weighted differently in primate and nonprimate species. The fact that R1 contributes substantially to lid closure in rodents but very little in humans depends on the stronger synaptic weighting of R1 circuits in nonprimate species. Frontal-eyed species, such as humans and primates, might depend on crossing R2 blink reflex circuits to keep the blink consensual (Porter et al. 1993; Shahani 1970), whereas lateral-eyed animals such as rodents, cats, and guinea pigs rely heavily on the R1 component because blinking is frequently unilateral (Basso et al. 1993; Pellegrini et al. 1995; Tamai et al. 1986).

Besides having a protective function, blinks appear to be associated with changes in visual information during the attentive process (Kennard and Glaser 1964). The sequence of blink responses represents an attempt to optimize eye closure thus guaranteeing reflexive protection of the eyes without obstructing the flow of visual information (Evinger et al. 1984; Rossi et al. 1995). In the Sherringtonian sense, all blink reflexes are nociceptive responses because their main function is to evoke a protective response of the eyelids. But the only response mediated by nociceptive afferents is the eye-blinking evoked by corneal stimulation (corneal reflex).

The laser blink reflex (LBR)—probably also mediated by

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nociceptive afferents—is elicited by delivering high-intensity laser pulses to the facial skin (Ellrich et al. 1997). Insofar as laser pulses selectively excite the free nerve endings in the superficial layers of the skin and activate mechano-thermal nociceptive afferents (Bromm and Treede 1984; Magerl et al. 1999; Treede et al. 1999), the LBR may be the nociceptive counterpart of the R2 component of the blink reflex. If so, as a nociceptive reflex, it might be useful for investigating the trigeminal nociceptive pathways in clinical orofacial pain syndromes.

The purpose of this study was to characterize the functional properties (startle or nociceptive origin) and the central pathways of the LBR in humans. In healthy volunteers, to investigate whether the LBR is a part of a startle response, we compared LBR responses to expected (repetitive) and unexpected (arrhythmic) laser stimuli. To confirm the nociceptive origin of the LBR, we studied changes in the LBR and in R2 of the electrically elicited blink reflex**,** induced by an anesthetic block of peripheral afferents and by the opiate analgesic fentanyl. We then investigated the central pathways mediating the LBR, and possible interactions between the LBR and the electrically elicited blink reflex (R1, R2, and R3), by studying conditioning–test responses to homotopic and heterotopic stimuli and by assessing the recovery curves for the two types of blink reflex.

METHODS

Subjects

Twenty healthy volunteers (13 men and 7 women) aged 25–32 yr participated into the study. The subjects were PhD students, residents of the School of Neurology, and some of the authors. All participants gave their informed consent. The study, including the administration of lidocaine, fentanyl, and naloxone, was approved by the local ethics committee.

Stimulation and recording technique

The subject sat in a dentist's chair with a headrest and wore protective goggles. To avoid possible changes in pain perception due to ambient temperature (Strigo et al. 2000), the experiments took place in a temperature-controlled room, kept at 25°C, and the skin temperature also was measured to keep across subjects the skin temperature as similar as possible (Arendt-Nielsen and Bjerring 1988).

The electrically elicited blink reflex was evoked by electrical stimulation (0.1 ms, 10–70 mA) of the supraorbital nerve through surface electrodes.

Using a CO_2-laser stimulator (Neurolas, El. En., Florence, Italy), we delivered laser pulses (wavelength, 10.6 μM; intensity, 1.5–15 W; duration, 10–15 ms; beam diameter, 2.5 mm; irradiated area, approximately 5 mm^2) to the skin of the supraorbital (V1) or perioral region (V2–V3). The mean perceptive threshold of laser pulses was 6.5 ± 1.5 (SD) mJ/mm^2. In all experiments, the stimulus intensity was adjusted to a level eliciting a well-defined and stable LBR (39.5 ± 6.4 mJ/mm^2). To avoid skin damage, adaptation or sensitization of nociceptors, and central habituation, we slightly shifted the spot of stimulation after each stimulus and delivered stimuli 15–20 s apart.

Electromyographic (EMG) signals were recorded from the orbicularis oculi muscles by surface electrodes with the active electrode over the mid lower eyelid and the reference 2–3 cm lateral. Signals were amplified, filtered, (bandwidth 20–2000 Hz), full-wave rectified, and stored for off-line analysis.

In all experiments we measured the latency, duration, and the mean area of responses (computer arbitrary units of the area under the curve) over 10 trials. The area of test responses was expressed as a percentage of control responses.

Expected and unexpected laser stimulation

In experiments to assess whether the LBR is a part of a startle response (4 subjects), laser pulses to the supraorbital region were delivered rhythmically (expected) and arrhythmically (unexpected). Expected stimulation consisted of eight pulses delivered rhythmically, at 20-s intervals; unexpected stimulation consisted of eight pulses delivered arrhythmically, at very low frequency (pseudo-random intervals from 10 to 20 min). After each stimulus, the spot was slightly moved in an area of 4 cm^2 so that the same spot was never stimulated twice. To avoid prealerting, white noise was given through earphones. The latency and duration of the rectified responses were measured in single trials. Participants rested for 1 h between the two trials.

Lidocaine-induced anesthetic block of peripheral afferents

In four subjects, we investigated the changes induced by a lidocaine block of peripheral afferents on the LBR and R2. Lidocaine (1%) was injected subcutaneously just above the supraorbital foramen; LBR and R2 were recorded before, and at 5, 10, 15, and 30 min after the injection. Immediately before LBR and R2 recordings (at 5, 10, 15, and 30 min after the injection), to monitor the block of peripheral afferents, touch and pinprick sensations were tested by asking the subject to describe the sensations evoked by a cotton wool and a sterile pin applied to facial skin.

Opiate-induced modulation

In six subjects we studied the effect of opiates (fentanyl) on the LBR and R2. The intensity of the electrical supraorbital stimuli was adjusted to evoke an R2 response matching the LBR in size, and this level (23 ± 4.5 mA) was maintained throughout the session. Subjects underwent five recording series of 10 trials each: two predrug baseline series 20 and 10 min before drug administration; two postdrug series 10 and 20 min after an intramuscular injection of fentanyl (0.1 mg), and one antagonist series 5 min after intravenous injection of naloxone (0.8 mg). Series with electrical and laser stimuli were alternated. The mean area of the EMG responses obtained in the two predrug series was taken as control value.

Reflex interaction (conditioning–test experiments)

In six subjects, the interactions between the LBR and the three components of the blink reflex (R1–R2–R3) were studied with homotopic (supraorbital-supraorbital: V1–V1) and heterotopic (perioral-supraorbital: V3–V1) stimuli. In the first session, conditioning laser pulses applied to the right supraorbital region or to the perioral region preceded electrical stimulation of the left supraorbital nerve. To allow for the long peripheral times of the laser-elicited afferent volley (receptor activation and conduction), we set the interstimulus interval to 150 ms. In the second session, conditioning electrical stimulation of the left supraorbital nerve was delivered before the laser pulses (to the right supraorbital or perioral region) at the 150-ms interstimulus interval. The intensity of electrical supraorbital stimulation was adjusted (approximately 6 times sensory threshold, 45 ± 10 mA) to evoke a stable R3 component. Control series were obtained at the beginning and at the end of each session. We also studied the time course of the interaction between the supraorbital-LBR and R2 at the conditioning–test intervals of 250 and 500 ms and 1 and 1.5 s.

Four subjects, in whom laser stimulation failed to evoke the LBR (see next section), were tested only for the effect of conditioning laser stimulation on the electrically elicited blink reflex.

LBR and R2 recovery curves

In six subjects, we studied LBR recovery curves after double laser pulses applied to the right supraorbital region. To prevent skin damage and to avoid possible perturbation of receptor responsiveness caused by delivering two short-interval radiant heat stimuli to the same spot, we used two identical CO_2-laser stimulators so that we could stimulate two adjacent spots (1 cm apart) simultaneously. After delivering each pair of laser pulses, we redirected the two laser beams so that they irradiated a slightly different spots. For comparison, in the same subjects we studied the recovery curve of R2 with the double-shock technique (Kimura 1973). In both recordings, paired stimuli were delivered at interstimulus intervals of 250 and 500 ms and 1 and 1.5 s. Control series were run immediately after testing each interval.

Statistics

Group differences were evaluated by ANOVA, intraindividual differences by paired *t*-test or repeated-measures ANOVA, and goodness of fit of the recovery curves by the r^2 correlation coefficient. All data are given as means ± SD.

RESULTS

At an intensity of about six times the perceptive threshold, we evoked well-defined and stable LBR responses in 16 of 20 subjects. LBR after supraorbital stimulation had similar latencies in ipsilateral and contralateral muscles (ipsilateral: 73.2 ± 10.5 ms; contralateral: 74.3 ± 11 ms; $P > 0.20$) and duration (ipsilateral: 51.7 ± 5.6 ms; contralateral: 52.9 ± 5.3 ms; $P > 0.20$) and closely matched values found in an earlier study using a CO_2 laser (Cruccu et al. 1999). Laser stimulation of the perioral region elicited LBR as well with latencies (ispilateral: 71.3 ± 7.3 ms; contralateral: 72.8 ± 8.4 ms; $P > 0.50$) and duration (ipsilateral: 51.1 ± 6.2 ms; contralateral: 50.8 ± 7.8 ms; $P > 0.50$) similar to the LBR after supraorbital stimulation. The latency and duration of the LBR after supraorbital and perioral region were not significantly different ($P > 0.50$). In the same 16 subjects, the latency of the electrically elicited R2 blink reflex was 31.9 ± 5.6 ms in the ipsilateral and 33.1 ± 7 ms in the contralateral muscle and was similar to commonly found values in normal subjects (Kimura 1983; Ongerboer de Visser and Cruccu 1993).

In no subject was the LBR followed by a later (R3-like) response. The subjects described the laser-evoked sensation as a distinct pinprick that was always painful and sometimes followed by a burning sensation. In four subjects, all of whom had normal perceptive thresholds and reported the same sensation as the others, supraorbital and perioral stimulation, even at a high-intensity (50 mJ/mm^2), invariably failed to elicit the LBR.

Expected and unexpected laser stimulation

LBR responses showed none of the characteristics of a startle response (Fig. 1). Repeated rhythmic stimulation (1/20 s) failed to induce progressive suppression (LBR duration of the first trial: 51.2 ± 3.4 ms; LBR duration of the last trial: 50.3 ± 4.7 ms). LBR responses to unexpected stimuli (arrhythmic pulses delivered at very-low-frequency) matched responses to standard (expected) laser stimulation (LBR duration of the first trial: 50.8 ± 1.7 ms; LBR duration of the last trial: 51.3 ± 3.2 ms).

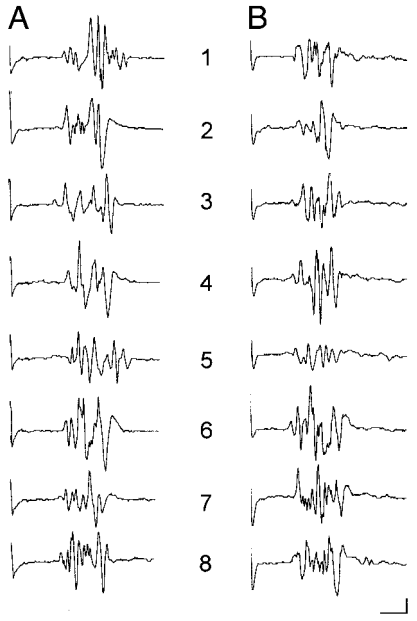

FIG. 1. The laser-blink reflex (LBR) to expected (*A*) and unexpected (*B*) laser stimulation of the right supraorbital region in a representative subject. Expected stimulation consisted of 8 pulses delivered rhythmically (at 20 s intervals); unexpected stimulation consisted of 8 pulses delivered arrhythmically (pseudo-random intervals: 10–20 min). Expected stimuli failed to induce progressive suppression of the LBR. Unexpected and expected stimuli evoked similar responses. In *A* and *B*, the 8 single reflex responses are unrectified. Calibration: 30 ms; 200 μV.

Lidocaine-induced anesthetic block of peripheral afferents

As soon as the subjects described the pinprick sensation as "tactile," rather than sharp, about 5 min after lidocaine injection, the LBR was abolished. Concomitantly, all subjects reported that they no longer perceived the laser stimuli. R2 behaved differently: only 10 min after the injection it was affected and only partly suppressed (by about 30%). Both responses recovered almost completely within 30 min (Fig. 2).

Opiate-induced modulation

In the 10-min postdrug series, fentanyl strongly suppressed the LBR (25.3 ± 4.5% of the predrug values) and in the 20-min postdrug series, abolished it (4 ± 9.5%). Naloxone almost completely restored the response (75.7 ± 27.8%). Fentanyl left R1 practically unchanged and only minimally depressed R2 (97.3 ± 2.1% and 94.1 ± 4.4% in the 2 postdrug series; Fig. 3). The opiate-induced changes in the LBR and R2 differed significantly in the two postdrug series ($P < 0.0001$; paired *t*-test).

Reflex interaction (conditioning–test experiments)

Conditioning laser pulses applied 150 ms before electrical stimulation abolished R3 and strongly suppressed R2 regardless of the homotopic or heterotopic site of stimulation (test R2: 5.2 ± 9.3% of the control after supraorbital and 4.5 ± 7.5% after perioral laser stimulation; Fig. 4), while R1 remained unchanged (107.2 ± 24.3% of the control after supraorbital and 105.8 ± 27.4% after perioral laser stimulation). In turn, conditioning electrical supraorbital stimulation 150 ms before the laser stimulation abolished the test supraorbital and

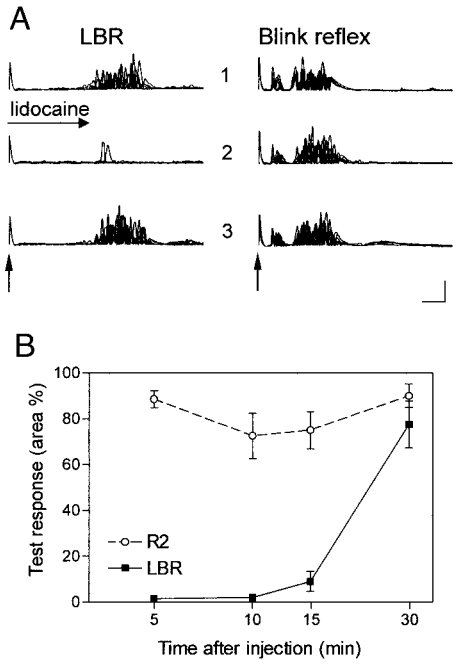

FIG. 2. Modulation of the LBR and the electrically elicited blink reflex by lidocaine-induced supraorbital nerve block. *A*: reflex responses in 1 subject. About 5 min after the lidocaine injection, as soon as the subject no longer reported perceiving the laser stimuli, the control LBR (*1*) was abolished (*2*), whereas the R2 of the blink reflex was practically unchanged. Both responses recovered in 30 min (*3*). Ten trials for each block are superimposed and full-wave rectified. Calibration: 20 ms; 200 μV. ↑, the stimulus onset. *B*: graph of the time course of the LBR and R2 responses in 4 subjects. *x* axis; time (min) after lidocaine injection. *y* axis: area of the test responses expressed as a percentage of the control response. Bars are standard errors. Whereas LBR was suppressed from 5 to 15 min after the lidocaine injection, R2 was only partially reduced. Both responses recovered almost completely at 30 min.

perioral LBR. As the conditioning–test interval increased the test LBR progressively recovered, with a slow time course, similar to that of the test R2 conditioned by laser stimulation. At the 500-ms interval, the test LBR after electrical conditioning was more suppressed than the test R2 after laser conditioning, but the difference was not statistically significant (Fig. 4).

In the four subjects with no LBR responses, conditioning laser pulses delivered 150 ms before supraorbital electrical stimulation left R1 unchanged and still abolished R3 as they did in the subjects who had LBR responses. The test R2 was only partially reduced (75 ± 5.6% of the control after supraorbital and 83 ± 1.3% after perioral laser stimulation). This conditioning effect on R2 was still significant ($P < 0.001$; paired *t*- test); but it was weaker in these subjects than in the subjects in whom laser stimulation evoked an LBR ($P < 0.001$; ANOVA).

LBR and R2 recovery curves

The R2 recovery curve was similar to that commonly found in normal subjects (Kimura 1973; Ongerboer de Visser and Cruccu 1993). LBR recovered faster than R2 (Fig. 5). Both functions had an excellent fit ($r^2 > 0.99$). Although the slopes were similar, the LBR curve was shifted higher than the R2 curve. Standard curve calculations indicate that the test LBR would recover to 50% of control values at an interval of 347 ms and the test R2 at an interval of 570 ms. The LBR and R2 test responses differed significantly at the intervals of 250, 500, and 1,000 ms ($P < 0.02$, *t*-test); the whole curves differed significantly ($P < 0.01$; repeated-measures ANOVA).

DISCUSSION

This experimental study in healthy volunteers suggests that rather than being part of a startle reaction, the human LBR is a purely nociceptive reflex. Like other nociceptive reflexes, the LBR is mediated by A-δ afferents and is completely and quickly suppressed by anesthetic block of peripheral afferents and by opiates. Our data on reflex interactions and recovery curves showed that LBR is relayed through a polysynaptic circuit and shares part of the interneurons with the nonnociceptive R2 blink reflex.

General characteristics of LBR and comparison with blink reflexes elicited by different inputs

CO_2-laser stimuli exclusively excite free nerve endings in the most superficial skin layers (Bromm and Treede 1984, 1991) and concomitantly induce pinprick and burning sensations, secondary to activation of A-δ and C nociceptive afferents, respectively (Magerl et al. 1999; Treede et al. 1999). The conduction velocity of unmyelinated afferents in the human

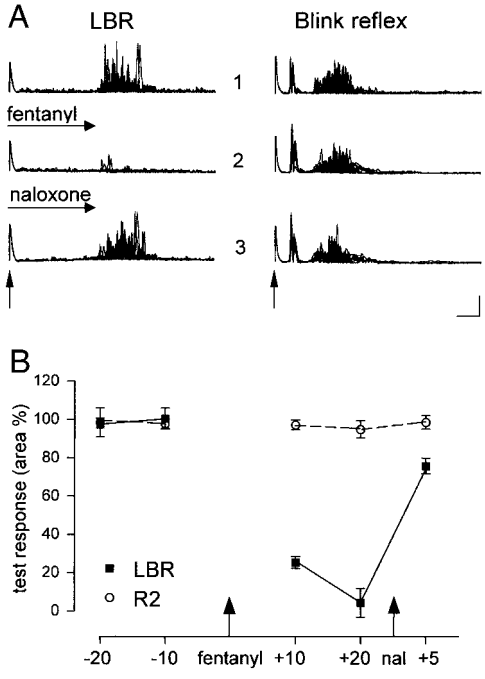

FIG. 3. Modulation of the LBR and the electrically elicited blink reflex by opiate injection. *A*: reflex responses in 1 subject. Twenty minutes after an intramuscular injection of fentanyl, the control LBR (*1*) was suppressed (*2*) and the fentanyl induced-effect was reversed by the naloxone injection (*3*). The electrically induced blink reflex remained unchanged. Ten trials for each block are superimposed and full-wave rectified. Calibration: 20 ms; 200 μV. ↑, the stimulus onset. *B*: graph of the drug-induced modulation of the LBR and R2 responses in 6 subjects. *x* axis: time (min) before (−20 and −10) and after (10 and 20) i.m. injection of fentanyl and after (5 min) IV injection of naloxone. *y* axis: area of the test responses expressed as a percentage of the predrug baseline response. Bars are standard errors. LBR was suppressed 10 min after the fentanyl injection and abolished 20 min after fentanyl injection. The opiate-induced effect was almost completely reversed by naloxone. R2 remained unchanged. The difference between LBR and R2 in the 2 postdrug series was significant ($P < 0.0001$).

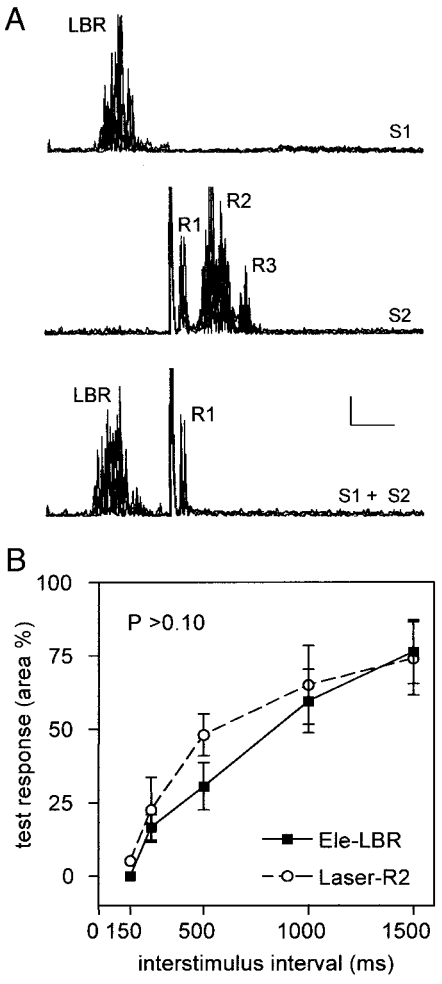

FIG. 4. *A*: interaction of the LBR evoked by laser stimulation of the right supraorbital region with the 3 components of blink reflex (R1, R2, and R3) evoked by electrical stimulation of the left supraorbital nerve. The conditioning LBR abolished R2 and R3 and left R1 unchanged. *B*: graph of the time course of the interaction between the supraorbital-LBR and R2, in 6 subjects, at the conditioning–test intervals of 250 and 500 ms and 1 and 1.5 s (*x* axis). The axis is the area of the test responses expressed as a percentage of the unconditioned responses. Bars are standard errors. The 2 curves had a similar time course. Ele-LBR, the time course of the LBR conditioned by electrically elicited blink reflex; Laser-R2, the time course of R2 conditioned by LBR.

supraorbital nerve is 0.6–1.4 m/s (Nordin 1990). From measurements on adult skulls and stereotactic atlases (Shaltenbrand and Wahem 1977), the route from the supraorbital region (just above the eyebrow) to the lower medulla (where the primary afferents terminate) amounts to 145 mm. The resulting afferent conduction times (always longer than 100 ms) are too long for the LBR latency (about 70 ms). Furthermore, the reflex latency also includes receptor times, central delay, and efferent conduction time. We therefore conclude, in agreement with earlier studies (Cruccu et al. 1999; Ellrich et al. 1997), that the afferents for the LBR belong to the small-diameter A-δ fiber group, probably the A-δ mechano-heat fibers (AMH) type II that mediate pinprick sensation in the hairy skin.

The LBR had a similar latency and duration after stimulation of the supraorbital and perioral territories. The electrically elicited R2 blink reflex is also similar after supraorbital and infraorbital stimulations (Kimura 1983; Ongerboer de Visser and Cruccu 1993). Consistently, blink reflexes elicited by air puffs directed to the forehead and the cheek have similar latency, EMG amplitude, lid movement and peak velocity in cats (Gruart et al. 1995). The early (R1) component alone is strongly dependent on the site of stimulation, being consistently elicited only by mechanical or electrical stimuli close to the eyelids, in animals as in humans (Gruart et al. 1995; Kimura 1983).

Reflex activity in the orbicularis oculi muscle can be elicited by various stimulus modalities in humans and animals: tone, light, mechanical, and electrical stimuli (Domingo et al. 1997;

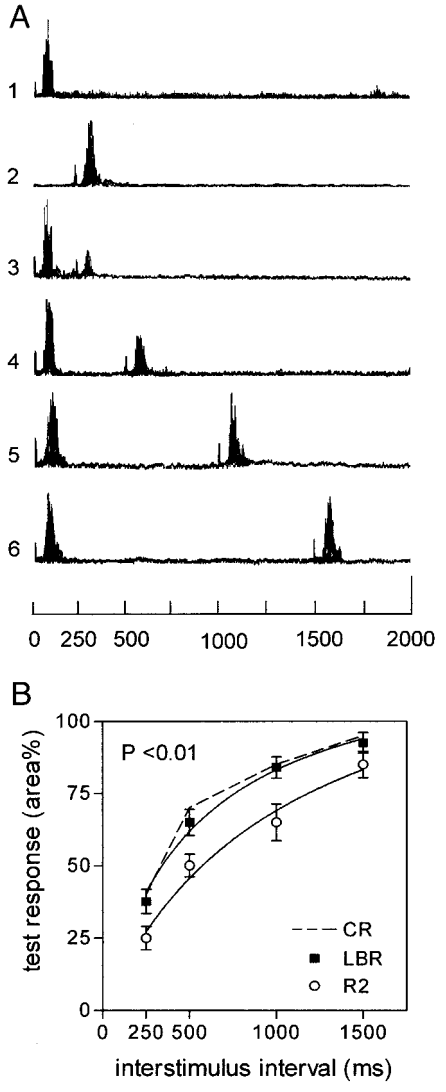

FIG. 5. Recovery curve of the LBR. *A*: rectified and superimposed signals from the right orbicularis oculi muscle in 1 subject. Paired laser pulses of equal intensity were delivered to the skin of the right supraorbital region. *1*: conditioning LBR; *2*: unconditioned LBR; *3–6*: the conditioning–test interaction at 250, 500, 1,000, and 1,500 ms. Note the strong suppression of the test LBR at the 250-ms interstimulus interval. As the interstimulus interval increases, the test LBR progressively recovers. Time base: 250 ms per division; vertical calibration: 200 μV. *B*: graphs of the recovery function of LBR and R2 in 6 subjects. For comparison, the graph also shows the recovery function of the corneal reflex (CR) determined from the mean data for normal subjects. The *x* axis is the interstimulus interval. The *y* axis is the area of the test responses expressed as a percentage of the unconditioned responses. Bars are standard errors. The LBR, like the CR, recovered faster than R2. LBR and R2 responses had similar slopes but the LBR recovery curve was shifted higher than the R2 curve. LBR and R2 curves differed significantly ($P < 0.01$).

Gruart et al. 1995, 2000; Kugelberg 1952; Rimpel et al. 1982; Tackmann et al. 1982). The neural circuits mediating blink reflexes evoked by these various sensory modalities remain unclear. Blink reflexes evoked by extratrigeminal stimuli (tone and light) have longer latency and quicker habituation than the blink response evoked by trigeminal stimuli (Gruart et al. 1995; Rimpel et al. 1982; Tackmann et al. 1982). These findings suggested that blink responses to tone and light stimuli have more polysynaptic and more complex pathways that may involve several relay centers before projecting to the facial motoneurons (Tackmann et al. 1982). However, impulses generated by various sensory inputs exert some mutual influence before they reach the facial motoneurons, which themselves are not involved in conditioning effects (Fox 1978).

Expected and unexpected laser stimulation

Because the brain stem interneurons that mediate spontaneous and reflex blinking are extremely sensitive to all sorts of sensory inputs, in some circumstances, the blink reflex may be also part of a somatosensory startle reaction. Indeed, some consider it the most representative and consistent component of a startle response (Brown et al. 1991; Valls-Solé et al. 1999). Blink reflexes in response to startle and standard blink reflex responses have distinctly different EMG patterns. In startle responses, the burst of EMG activity in the orbicularis oculi not only habituates to repeated rhythmic stimulation but also lasts longer than the activity recorded during a nonstartle blink reflex elicited by other stimuli (Brown et al. 1991). In this study, the LBR did not habituate to rhythmic stimuli at 20-s interval and unexpected laser pulses elicited standard LBR responses. Hence our findings do not support a startle origin for the LBR.

Lidocaine- and opiate-induced effects

That the LBR is mediated by small-diameter fibers receives support from the findings we obtained in this study after blocking the peripheral afferents by local anesthesia. It also accords with the opiate-induced changes in the LBR and R2.

Local anesthetic drugs such as lidocaine block the formation and the transmission of action potentials in thin unmyelinated fibers before the thicker myelinated fibers. In accordance with a previous report, all our subjects no longer perceived laser stimuli about 5 min after the injection (Arendt-Nielsen and Bjerring 1988), and concomitantly the LBR was abolished. Laser perception and the LBR returned to control values at about 30 min. During the same postinjection period (5–10 min), R2 was practically unchanged, in agreement with Shahani's report (Shahani 1970) that R2 is scarcely influenced by the anesthetic block of small-diameter fibers.

In this study, intramuscular injection of the opiate fentanyl left R1 and R2 practically unchanged, in line with previous reports (Cruccu et al. 1991; Dauthier et al. 1981). Although the reflex pathways for R2 involve the trigeminal spinal complex pars caudalis, the structure considered the integral brain stem relay of trigeminal nociceptive information, the afferents for R2 are unlikely to project to the nociceptive-specific (NS) neurons. They mainly project to the wide dynamic range (WDR) neurons located between the border of the magnocellularis layer and the superficial border of the adjacent subnucleus reticularis dorsalis and probably to the low-threshold mechanosensitive (LTM) neurons (Price et al. 1976; Yokota et al. 1979). In the monkey, the activity in WDR and LTM neurons evoked by innocuous stimuli is relatively unaffected by morphine or antinociceptive brain stimulation (Hayes et al. 1979).

In our study, the LBR, unlike R2, underwent potent (75%) fentanyl-induced, naloxone-reversible suppression. The probable reason why fentanyl differentially modulated the two responses is that their inputs differ in quality: nonnociceptive for R2 and nociceptive for the LBR. Small facial afferents (both Aδ and C fibers) project to NS and WDR neurons in the superficial layers and deep in the medullary equivalent of laminae V–VI (Hu 1990; Sessle 2000). Animal studies have demonstrated that activity in NS and WDR medullary neurons, evoked by nociceptive inputs, is reduced by morphine (Hayes et al. 1979; Price et al. 1976; Yokota et al. 1979). This inhibitory effect may be mediated by descending projections from the midbrain periaqueductal gray and medullary nucleus raphe magnus to nucleus caudalis or directly exerted by opiates on the presynaptic endings of A-delta and C afferents in the medullary, as in the spinal dorsal horn (Fields et al. 1980; Hayes et al. 1979).

In a previous study, fentanyl also suppressed R3 (Cruccu et al. 1991). Whether this is a nociceptive reflex is still debated. On the basis of its high activation threshold and its susceptibility to the anesthetic block of peripheral afferents, some investigators have suggested that R3 is mediated by nociceptive fibers (Rossi et al. 1989, 1995). On the other hand, the selective activation of small afferent fibers by laser pulses only sporadically induced an R3-blink-like response and after the stimulus was announced, the response disappeared (Ellrich et al. 1997). In our experiments, even when we delivered laser stimuli at maximum intensity, we never elicited the R3-blink-like response. R3 is therefore probably unsuitable for investigating the trigeminal nociceptive pathways (Ellrich 2000; Ellrich and Hopf 1996), whereas the LBR holds promise. The LBR is probably the brain stem equivalent to the RIII limb flexor reflex at spinal level (Willer 1977, 1983; Willer et al. 1984). For clinical application, the LBR has some advantages over the corneal reflex evoked by mechanical or electrical stimulation of the corneal mucosa. For example, it can be easily evoked by delivering stimuli to the skin of any trigeminal division and does not require the active collaboration of the subject.

Reflex interaction (conditioning–test experiments)

In this study, conditioning electrically-elicited blink reflex suppressed the supraorbital and perioral test LBR. In turn, conditioning LBR (perioral or supraorbital) abolished the test R2 and R3 but left R1 unchanged. Conditioning laser pulses that failed to evoke the LBR only slightly suppressed the test R2 but still abolished R3.

In humans and animals, prestimuli of various modalities, trigeminal and extra-trigeminal, modulate the blink reflex (Boulu et al. 1981; Gomez-Wong and Valls-Solé 1996; Pellegrini and Evinger 1995; Powers et al. 1997; Rossi et al. 1995; Sanes and Ison 1979; Valls-Solé et al. 1999). Depending on the intensity and modality of the prestimulus and also on input synchronization and timing of arrival, prestimuli initiate facili-

tatory and inhibitory processes on the subsequent blink test (Valls-Solé et al. 1999). The facilitatory effects predominantly involve R1, the inhibitory effects R2. A likely site for the facilitation of R1 is the facial motoneuron, whereas the inhibition of R2 may originate in the reticular formation. Accordingly, animal studies showed that conditioning stimuli that suppress R2 diminish the responses of reticular neurons (Tamai et al. 1986). Alternatively, the response decrements in reticular neurons could merely reflect sensory suppression mediated by primary afferent depolarization (PAD) in the trigeminal nucleus. Numerous studies demonstrated PAD within the trigeminal nuclear complex after conditioning trigeminal stimuli (Darian-Smith 1965; Hu and Sessle 1988; Young and King 1972). PAD seems to be an afferent-specific phenomenon: Hu and Sessle (1988) showed that nonnociceptive conditioning stimuli in cats were particularly effective on the low-threshold mechanosensitive afferents, nociceptive conditioning stimuli on the cutaneous nociceptive afferents. Furthermore, even though PAD might have a role in modulating the blink response, the mechanism of presynaptic inhibition is probably insufficient to abolish a test response (Darian-Smith 1965; Lindquist 1972).

More than one finding indicates that the inhibition of the test response, disclosed by our conditioning–test experiments investigating interactions between LBR and R2, is postsynaptic. Postsynaptic inhibition may result from afferent conditioning via an inhibitory interneuron projecting onto any central neuron in the test response circuit ("external conditioning"). Or, if the two reflexes share one or more interneurons in the circuit, the inhibition may result from refractoriness of the interneurons that had been invaded by the first volley of impulses ("internal refractoriness"). The conditioning exerted by extratrigeminal stimuli on R2 is probably mediated by external conditioning and may take place in the premotor area in the pons (Holstege et al. 1986; Rimpel et al. 1982). Yet anatomical-functional studies in patients with focal brain stem lesions indicate that the last interneuron of the R2 circuit is far more caudal, possibly below the obex in the lower or mid-medulla (Aramideh et al. 1997; Ongerboer de Visser and Kuypers 1978; Vila et al. 1997). The strong LBR–R2 interaction found in our study therefore probably originates from the refractoriness of interneurons that the two responses share. Common interneurons might explain why—regardless of the conditioning input (nociceptive or nonnociceptive) and site of stimulation (homotopic or heterotopic)—we obtained equally suppressed test responses. On the other hand, stimulus pairs of different modalities, not sharing the same central pathways, do not induce the same reciprocal effect on the test response (Powers et al. 1997). Hence, the LBR and R2 could therefore share some of the interneurons in their central pathways.

Shared interneurons for the LBR and R2 circuits receives further support from the findings in the four subjects without LBR responses. Our four subjects all had normal laser thresholds. Like the other subjects who had normal LBR responses, they also described the laser stimulus as a sharp pinprick sensation. This finding suggests that although the laser input neither reached nor excited the facial motoneurons, it did excite the Aδ-nociceptors. The afferent volley therefore reached the trigemino-thalamic projection neurons and the cortex (Agostino et al. 2000; Arendt-Nielsen 1994; Torebjork and Ochoa 1980). Yet the laser input only weakly suppressed the test R2. Hence we conclude that in these subjects the laser pulses probably only partially engaged the reflex interneurons, suggesting that the degree of suppression increases as a function of interneuronal circuit engagement.

An essential step toward understanding the mechanisms underlying blink reflex conditioning is to identify the interneurons responsible for coordinating the blink reflex. Some evidence in humans suggests that the reflex interneurons of the R2 evoked by innocuous stimuli are the medullary WDR neurons (Ellrich and Treede 1998). WDR neurons, also called convergent neurons, receive nociceptive and nonnociceptive inputs from the ophthalmic, maxillary, and mandibular territories (Amano et al. 1986; Sessle 2000; Sessle and Greenwood 1976; Sessle et al. 1986). We suggest that WDR neurons mediate R2 and the LBR.

Even in subjects with no LBR, a preceding laser pulse invariably abolished R3. This is not surprising because R3 is a notably unstable response and highly susceptible to all sorts of extra-segmental modulations (Ellrich and Hopf 1996; Rossi et al. 1989, 1993, 1995). None of our reflex interaction experiments disclosed appreciable conditioning-induced changes in R1. In humans, conditioning stimuli usually facilitate R1 (Boulu et al. 1981; Rossi et al. 1995; Valls-Solé et al. 1994, 1999). Some reports have nonetheless described R1 insensitivity (Ellrich and Treede 1998; Valls-Solé et al. 2000). The lack of modulation in our experiments may depend either on the relatively long interstimulus-interval (150 ms) or on the fact that nociceptive afferents do not directly influence the excitability of the facial motoneurons (Valls-Solé et al. 2000). Alternatively, the high-intensity electrical stimuli used in this experiment could have induced a ceiling effect on the conditioning R1 response so that the test stimulus was unable to facilitate the test R1 response further.

Recovery curves and central delay

The recovery curves to paired stimuli measure the excitability of a reflex circuit engaged by two identical stimuli at different intervals; the inhibition of the second response (test) reflects the refractoriness of the same reflex pathway after the activation induced by the first response. Most investigators consider the differences in the time courses of the trigeminal reflexes mainly to reflect differences in the number of synapses in the circuit (Cruccu et al. 1984, 1986, 2001; Esteban 1999; Kimura 1973; Kimura et al. 1994; Rimpel et al. 1982). Oligosynaptic reflexes such as the R1 component of the blink reflex and the early silent period of the masseter inhibitory reflex (SP1) are always less inhibited in the paired stimuli paradigm than the corresponding polysynaptic components (R2 and SP2) (Cruccu et al. 1984; Kimura 1973). The corneal reflex, a purely nociceptive reflex mediated by fewer interneurons than R2, recovers earlier than R2 (Cruccu et al. 1986). In our experiment, LBR recovered significantly faster than R2: its time course closely matched that of the corneal reflex (Fig. 4). Hence our results would suggest that the LBR, like other nociceptive reflexes (Cruccu et al. 1986; Inghilleri et al. 1997), has a multisynaptic circuit relayed through fewer synapses than the polysynaptic R2.

The time for receptor activation of type II AMH units after CO_2-laser stimulation on the back of the hand is about 40 ms (Bromm and Treede 1984). The conduction velocity of type II

AMH afferents has been estimated in primates and humans (14–15 m/s) (Bromm and Treede 1991; Treede et al. 1995). The afferent conduction time from the supraorbital region to the lower medulla (145 mm, see preceding text) is about 10 ms. The afferent delay (40-ms receptor time plus 10-ms conduction time) is 50 ms. The efferent conduction time from the facial motoneurons to the muscle is 5 ms (Møller and Jannetta 1985; Schriefer et al. 1988). Given the 73-ms latency of the LBR and the 55 ms for afferent and efferent times, the resulting central delay for synaptic times and conduction from the lower medulla to the facial motor nucleus in the caudal pons is 18 ms. The electrically elicited blink reflex has a far shorter afferent delay. The conduction velocity of trigeminal Aβ fibers and that of blink reflex afferents, measured intraoperatively in man, is about 44–51 m/s (Cruccu and Bowsher 1986; Cruccu et al. 1987). On the same afferent pathway as that for the LBR, the afferent conduction time for R2 would be 3 ms. Given the 32-ms latency of the R2 blink reflex, the 3 ms for afferent and the 5 ms for efferent times, the resulting central delay is 24 ms. The slightly shorter central delay of the LBR (about 6 ms less) may be due to a slightly smaller number of interneurons in the LBR circuit than in the R2 circuit.

Were the LBR relayed through fewer synapses than R2, the LBR primary afferents should, rather than projecting directly onto the same second-order neurons that mediate R2, converge onto more proximal stations in the interneuronal chain of the lateral reticular formation that mediates reflex eye-blinking.

Nevertheless, neither the recovery curves nor the estimation of the central delay provide sure evidence that LBR is mediated through fewer synapses than R2. The difference in recovery times of LBR and R2 may result from a different strength of the inhibitory processes that act on innocuous and nociceptive-evoked blinks. The LBR central delay may be under-estimated because the times for receptor activation by CO_2-laser pulses may be longer on the hand than the facial skin, which is thinner and probably has a higher receptor density (Agostino et al. 2000; Whitton and Everall 1973).

In conclusion, the LBR is anatomically and functionally a purely nociceptive reflex. Its afferents belong to the Aδ fiber group. Although the LBR is nociceptive, it shares part of its multisynaptic circuit with the nonnociceptive R2 reflex in the medullary region of the lateral reticular formation. The LBR may prove a useful tool for studying the pathophysiology of orofacial pain syndromes.

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