Somatosensory Evoked Response and Jaw Opening Reflex Elicited by Tooth Pulp Stimulation in awake freely moving rats

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Abstract

OBJECTIVE: Investigation of pain and nociception refers to different models. Depending upon the intensity of stimulation, unmyelinated pulpal fibers or periodontal A-fibers can be stimulated producing a short or a long latency jaw opening reflex of the digastric muscle. This paper investigates the different components of the jaw opening reflex in addition to the correlation between afferent fibers involved in the cortical evoked response.

DESIGN AND SETTING: Fifteen awake male rats were implanted with tooth pulp stimulation electrodes, digastric and cortical recording electrodes. Ten rats were submitted to recordings after a single tooth pulp stimulation, while five rats were using conditioning and test stimulation. Tooth pulp evoked potentials and digastric EMG were simultaneously recorded. A multiresolution denoising method was used for signal processing.

RESULTS: Following tooth pulp stimulation, a cortical response was produced including the following peaks: P6.5 ± 1.1, N11 ± 1.2, P17 ± 1.2, P27 ± 2.9, N53 ± 7.5, P69 ± 5.8, P88 ± 13, N160 ± 9.7, P204 ± 14.2. The distribution and amplitude of these peaks are correlated to the stimulation intensity ($r=0.96$, $p<0.01$). An interaction between the different components of the jaw opening reflex was identified on EMG, following a conditioning shock, where a cortical evoked response showed a P30 ± 2.7 peak which was observed concurrently with the jaw opening long latency reflex.

CONCLUSION: Our results identify the interaction between the different components of the jaw opening reflex and the correlation to the cortical evoked response.

ORIGINAL ARTICLE

ABBREVIATIONS:

TPS	- tooth pulp stimulation
EP	- evoked potentials
TPEPs	- tooth pulp evoked potentials
JOR	- jaw opening reflex
LL JOR	- long latency component of the jaw opening reflex
SL JOR	- short latency component of the jaw opening reflex
ISI	- inter stimulus interval

INTRODUCTION

Different animal models were identified in order to determine the relationship between pain, nociception and motor control. In this respect, electrical tooth pulp stimulation allowed the activation of high threshold receptors, mainly connected to A-delta and C-fibers. This reflex, considered as a relevant orofacial protective reflex, mediated at the brain stem level can also be elicited by the stimulation of low threshold mechanoreceptors, such as those located in the periodontal ligament and oral mucosa. Previous studies showed an interaction between pain and behavioral activity [33]. In humans, the study of cortical responses following tooth pulp stimulation (TPS) has been used for pain investigation [9,27].

In rats, tooth pulp stimulation of incisors produces a jaw opening reflex (JOR) usually recorded on the digastric muscle, this usually being a way to analyze the mechanisms of pain [36].Two different JOR components are recorded at different latencies, depending upon the TPS intensity. It is commonly admitted that moderate TPS evokes a long latency (LL) reflex (10–25 ms) corresponding to the stimulation of pulpal unmyelinated fibers [8,9,26]. With the increasing stimulus intensity, the long latency reflex amplitude increases, then decreases and disappear; concurrently, a short latency (SL) reflex (≈6 ms) appear. This short latency reflex is due to the electrical stimulus spreading to the periodontal myelinated nerve fibers [3]. The LL response loss is due to an inhibitory effect produced by the periodontal afferents on the response of the motoneurones of the digastric muscle [8]. Different authors investigated the JOR following TPS. In rats, the LL and SL JOR has been well identified and few papers demonstrated that LL JOR could be recorded without SL JOR, following TPS [7,8].

Following TPS, a response can be evoked in the somatosensory cortex concurrently with the JOR. The afferent volley is triggered by the TPS and is conducted through sensory relays to the somatosensory cortex. The study of tooth pulp evoked potentials (TPEPs) allows to analyze the projection of primary sensory afferent neurons to the somatosensory cortex. In this respect, different investigations were performed on rats [15,25,33,36,38], on cats [1,20], on monkeys [17] and on human [9,10].

In rat, though the central projection of the pulp has already been identified with trans-ganglionic transport of HRP peroxidase [4,24], the central pathway of the JOR is still unknown. The incisors of the rats are continuously growing teeth, the pulp of which is almost entirely innervated by unmyelinated axons [6,23,28,34,35]. The conduction velocity of these fibers may vary between 0.6 and 2.9 m/s [38], although some of them may be myelinated outside of the pulp with a conduction velocity of 25m/s. Thus, the conductive period of time between the crown and root apex of the lower incisor is about 12–25 ms when stimulation was performed at the incisal part of the crown of the teeth. When high intensity TPS was performed on the lower incisor of the rat, it is possible that the stimulus spreads to periodontal tissues [8].

Neither the interaction of the different components of the JOR nor the cortical evoked responses following the TPS have been completely described. This paper aims at analyzing the interaction of the different components of the JOR in freely moving rats, the correlation between the different components of the JOR and the cortical potentials, and the interaction between the different components of the JOR and cortical response after paired shocks.

MATERIALS AND METHOD

Surgical procedures

The experiments were performed on 15 male rats of the Sprague-Dawley strain, weighing 300 to 350 g which were operated under anesthesia with Ketamine® (Imalgène 500, 100 mg/Kg i.m.). Peripheral bipolar stimulation silver electrodes were implanted in the lower incisor tooth pulp and sealed with dental cement (SuperBond C&B, Sun Medical Co.,LTD, Shiga, Japan). Silver EMG electrodes were wrapped over the ipsilateral anterior belly of the digastric muscle to record the JOR [7]. Then a peripheral reference electrode was inserted subcutaneously behind the auricular area. Epidural recording electrodes were placed in the contralateral somatosensory cortex over the tooth field (bregma-anterior +1 mm, lateral 3 mm below the crest of the skull) [33]. A cortical reference electrode was implanted in the left nasal bone and the wires were connected to a connector which was sealed on the cranial skull [23]. The animals were placed in a controlled animal house for six days in order to recover from surgery, all experiments were realized in accordance to ethical guidelines.

Recordings

Freely moving animals were divided into 2 groups, according to the stimulation paradigm.

In the first group (G1, n=10), the JOR and TPEPs were recorded during 250 ms following a single TPS. TPS intensity was adjusted to 3 different levels slightly above the threshold of the 3 components of the JOR (LL, LL and SL, SL alone) so as stimulating pulpal unmyelinated fibers and then periodontal myelinated fibers.

Regarding the second group (G2, n=5), the JOR and the TPEPs were recorded by means of a stimulation paradigms consisting of a conditioning stimulation followed at variable delay by a test stimulation of variable intensities. In this group, different intensities of stimulation were applied for conditioning and test stimulation in order to produce: a double LL reflex, a SL reflex followed by a LL reflex, double LL + SL and double SL reflex. Recordings were performed for an inter-stimulus interval (ISI) varying from 80 ms to 30 ms with steps of 10 ms and from 30 to 5 ms with steps of 5 ms.

Before each experiment a test was performed to ensure that animals presented clearly differentiated LL and SL jaw opening reflex. The animals were placed in a controlled environmental area to ensure that experimental conditions will prevent them from stress which inhibits the LL muscular responses [29,34].

TPS was produced by a stimulation unit (302-T, WPI, UK) at 2 V (continuous current), for 1 ms, every 2 s. Different TPS intensities was adjusted slightly, between 100 μA and 10 mA, above the level corresponding to the threshold of each components of the JOR, after testing individually each animal. TPS was delivered using dedicated software (Clampex 8.0, Axon instrument, USA) by means of an D/A converter (Digidata 1200 A, Axon instruments) and a linear stimulus isolator (A 395, WPI, UK). Responses were recorded using a preamplifier probe (AI 417 and AI 405, Axon instrument) and a programmable amplifier (Cyberamp 380, Axon instrument). A/D acquisition was realized through a converting card (Digidata 1200 A) at 2 kHz sampling rate. Then data were stored and assessed on a PC. In all cases, in accordance with respecting the rule of ethic committee, TPS amplitude were applied without nociceptive behavioral expression of pain or distress like vocalization, depression or other behavioral changes, abnormal appearance or abnormal posture or immobility.

Signal processing

100 sweeps of 250 ms were recorded for each run in order to perform an acceptable digital filtering. Following A/D converting, a signal processing procedure was used to standardize sweeps and to eliminate artifact responses. Then a multiresolution thresholding method was applied to TPEPs data to eliminate noise of the signals. Each EP was divided (transforming process) into a set of basic sinusoid functions called wavelets. Different processing tests demonstrated that the 8th order Symlet wavelet was the most efficient to eliminate noise. Following shrinkage of the wavelets coefficients, an inverse transforming process was applied to the signal to restore a de-noised signal, as previously demonstrated [5]. Signal processing was programmed by means of the Matlab® software language (Mathick, CA, USA) with the Wavelet toolbox®. Signal averaging was also performed on the same data in order to evaluate the performance of the multiresolution method in relation with the averaging method.

Statistical analysis

JOR or TPEPs recordings presented important inter individual variations of intensity between animals which limited the comparison of the results. So it was necessary to standardized data in order to allow a comparison across animals. In order to obtain results as ratios (percentage of variation) we compared each animal to himself. Then TPEPs were split into different classes of latency and tested using the ANOVA and complementary Tukey-test for an inter-group comparison. The low number of animals used in different portions of the studies did not affected statistical analysis and was compensated by the great number of datas which were recorded for each animal.

RESULTS

SIGNAL ANALYSIS OF EVOKED POTENTIALS

Signal analysis of the showed that the wavelet shrinkage method, for all the different components of the evoked response were preserved (Figure 1) though the noise was eliminated. The comparison between the averaging method and the multiresolution analysis significantly improved (71% ± 22) the signal-to-noise ratio measured out in relation to the averaging method ($t=4.95$, $p<0.001$).

SINGLE SHOCK STIMULATIONS

For all stimulation paradigms, TPEPs are described in relation to the JOR, which is used as a reference value to determine which type of fibers (pulpal and/or periodontal) was stimulated.

Jaw opening reflex

The latency of the JOR was recorded after TPS. For all animals, the two components of the JOR were recorded: between 5 and 8 ms for the SL JOR and between 12 and 20 ms for the LL JOR.

Cortical evoked responses

For low TPS situated under the threshold of the JOR, TPEPs did not appear on the somatosensory cortex.

For supraliminar TPS, the TPEPs recorded on the somatosensory cortex presented different patterns, in relation with the TPS intensity. The maximum TPEP amplitude and the intensity of the stimulation were significantly correlated ($r=0.96$, $p<0.01$), excepted when stimulus artifact obscuring recordings like when the TPEPs was saturated at high stimulus intensity. The maximum amplitude of the TPEPs varied among the rats, but was still recorded between 48 and 53 ms.

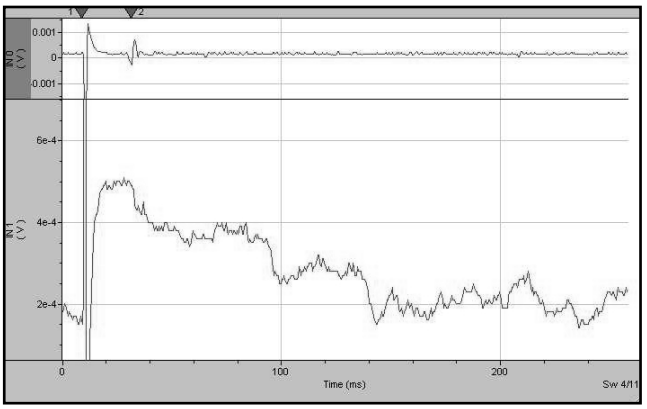
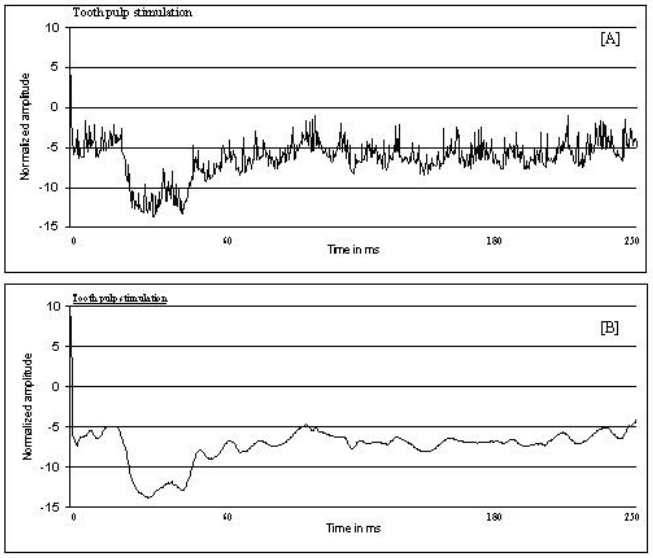


Figure 1a



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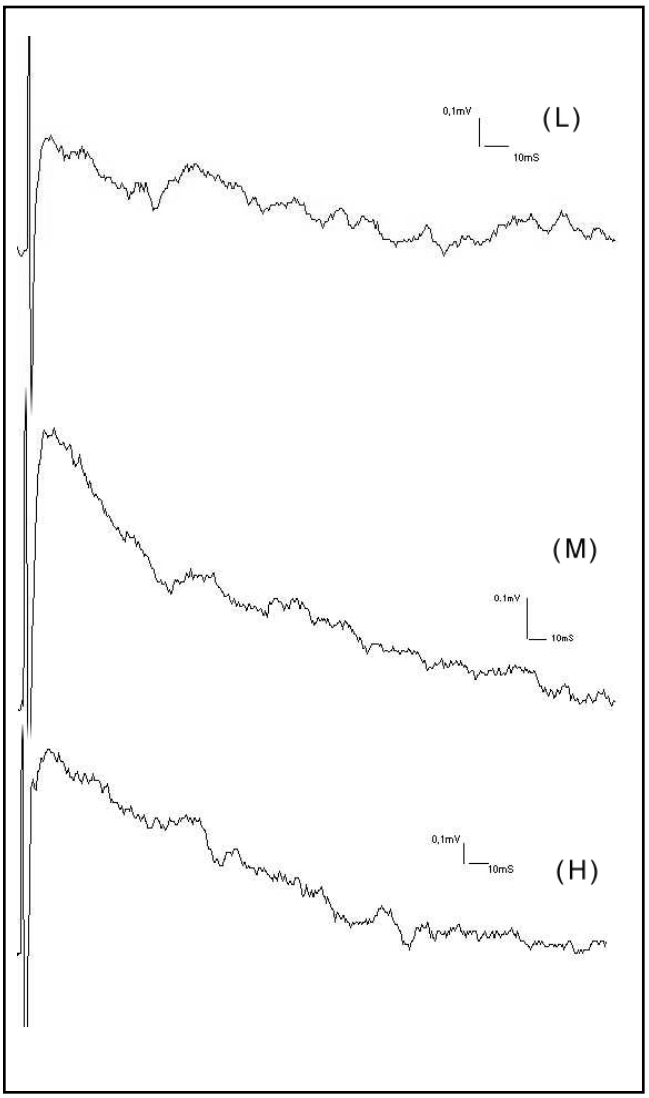

Figure 1d

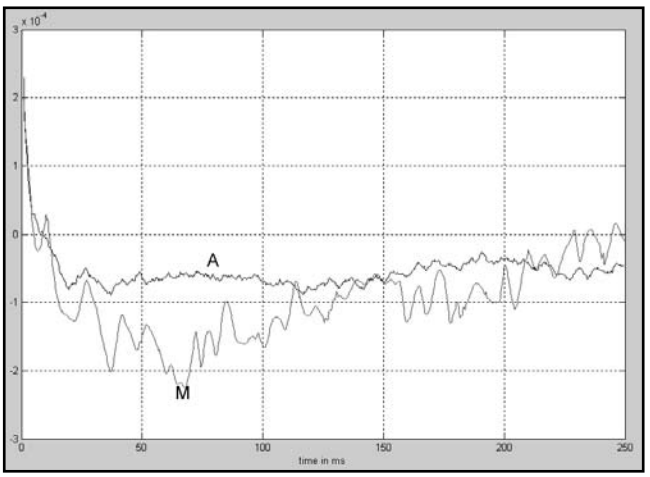

Figure 1c

Figure 1a. Time-locked EMG (IN0) and cortical activation (IN1) after moderate tooth pulp stimulation. At the same time of the EMG , the cortical activation was recorded.

Figure 1b. Effect of the multiresolution signal processing. Cortical tooth pulp evoked potential (TPEPs) of single sweep [**A**] transformed and denoised with a wavelet 'Symlet' of the 8th order then this signal was reconstituted without noise in [**B**]. The characteristics of the original signal remain present after wavelet signal processing.

Figure 1c. Denoising method: Comparison between averaging (A) and multiresolution method (M). In comparison to the averaging method; the mulstiresolution method gives a better reduction of the noise without attenuation of the output signal.

Figure 1d. When increasing the intensity of tooth pulp stimulation (low, moderate or high) different pattern of evoked potentials were recorded (L, M and H).

For low stimulation intensity, when TPS evoked a LL JOR, the TPEPs were characterized by 2 phases:

- An early component [0 ms–17 ms] (Table 1), consisting of a low amplitude (<32 μV ± 10) polyphasic wave P6.5 ± 1.1ms, N11 ± 1.2 ms and inconstant P17 ± 1.2ms.

- A late component [20 ms–240 ms] (Table 1), was characterized by a three-phase wave: P27 ± 2.9 ms, N53 ± 7.5 ms, P69 ± 5.8 ms, followed by a P88 ± 13 peak and a late multiphase response composed of a complex set of components of variable amplitude, P132 ± 4.7 ms, N160 ± 9.7 ms, P204 ± 14.2 ms . The [P27, N53, P69] components were statistically identified (ANOVA and Tukey test, $p<0.05$) and linked to the JOR LL component.

For medium stimulation intensities, when the SL and the LL JOR were recorded: TPEPs were characterized by an early, low amplitude (<32 μV ± 10) component [P7.6 ± 1.6, 11.2 ± 1.3, 17.1 ± 1] (Table 1) followed by a biphasic component [P23 ± 2.5, N48 ± 6.8] and a polyphasic component constituted by a complex set of components of variable amplitude [N87 ± 3.6, P125 ± 13.7, N155 ± 6.6, P220 ± 12.3] (Table 1).

For high stimulation intensities, when only the SL JOR persisted, TPEPs are characterized by an early, low amplitude (<32 μV ± 10) component [P6 ± 0.8, 10.7 ± 1.4, 16.5 ± 1.4] (Table 1) followed by a biphasic response [P22 ± 3.5, N51 ± 9.7] and a polyphasic component constituted by a complex set of components of variable amplitude [N73 ± 7.2, P124 ± 4.3, N161 ± 17.2, P216 ± 16.3] (Table 1).

The early component of the TPEPs [0–20 ms], was present in each case and no correlation could be found with any component of the JOR.

PAIRED SHOCKS EXPERIMENTS

Jaw opening reflex response

A.

When the conditioning stimulation evoked a SL JOR and the test stimulation intensity was adjusted to the amplitude that normally evokes a LL JOR, then a SL and a LL JOR reflex was present after the test stimulation.

B.

With a decreasing inter stimulus interval (ISI), in the same conditions of stimulus intensities as in (A.): After the test stimulation, the amplitude of the SL JOR raised when the ISI = 40 ± 3.2 ms, and then decreased to a threshold for ISI=10 ± 0.2 ms (Figure 2a), concurrently, the amplitude of the LL JOR after test shock increased by step (Figure 2b) until the ISI reached 25 ± 9.1 ms and then decreased and disappeared when the ISI was shorter than 10 ± 6.5 ms.

C.

When the conditioning stimulation evoked a SL JOR and the test stimulation intensity was adjusted to the amplitude that normally evokes a SL JOR, only the SL JOR was observed after the conditioning stimulus and the test stimulus. This response pattern changed when the ISI decrease below 45 ms (Figure 3 and 4). When the ISI was below 35 ms ± 7.4, an unexpected LL reflex appeared following the test TPS (Figure 3a and b). The amplitude of the LL reflex reached a maximum value (ISI = 9 ± 0.7 ms) (Figure 3c) and then faded away progressively

Table 1. Components of the tooth pulp evoked potentials recorded after tooth pulp stimulation.

Intensity of stimulation	TPEPs									
Low	P6.5	N11.1	P17.2	P27*	N53*	P69	N88	P132	N160	P204
sd	1.1	1.2	1.2	2.9	7.4	13	4.7	4.7	9.7	14.2
Moderate	P7.6	N11.2	P17.1	P23	N48	—	N87	P125	N155	P220
sd	1.6	1.3	1	2.4	6.8	—	3.6	13.7	6.6	12.3
High	P6.0	N10.7	P16.5	P22	N51	—	N73	P124	N161	P216
sd	0.8	1.4	1.4	3.5	9.7	—	7.2	4.3	17.2	16.3

Mean latency (in ms) and standard deviation (sd) of the tooth pulp evoked potentials. Low intensity tooth pulp stimulation corresponded on the EMG to the long latency component of the JOR, moderate intensity of stimulation corresponded to the short and the long latency component together and high intensity of tooth pulp stimulation corresponded to short latency JOR alone. For low intensity tooth pulp stimulation, when a long latency jaw opening reflex was recorded on the digastric muscle, the P27, N53 evoked potentials present significant differences; for medium and high intensity of stimulation; P69 could not be observed.

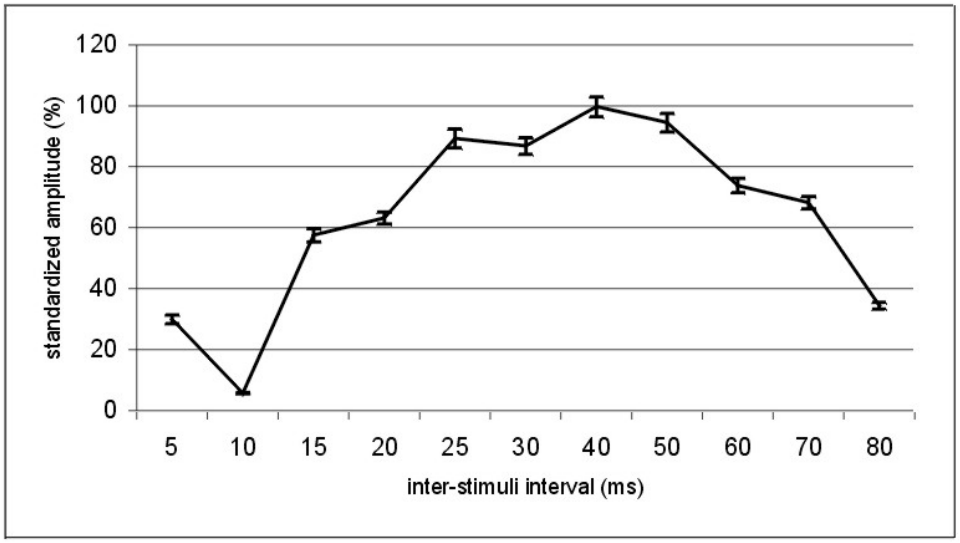

Figure 2a. Short latency jaw opening reflex after paired tooth pulp low intensity stimulations (mean values and standard deviation). During reduction of the inter stimuli interval, the standardized amplitude of the short latency jaw opening reflex after test stimulation increase and raised for a inter stimuli interval of 40 ± 3.2 ms, then the amplitude decreased till 10 ± 0.2 ms.

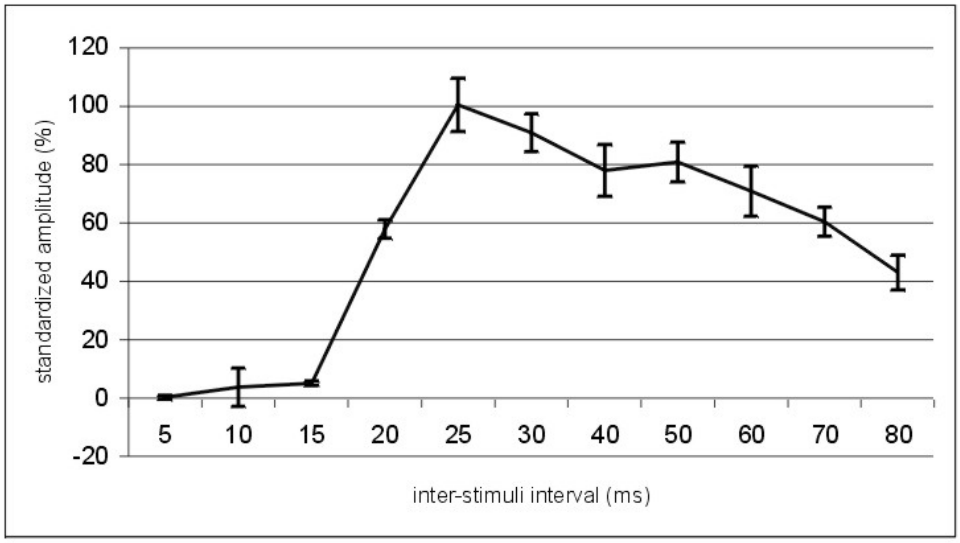

Figure 2b. Long latency jaw opening reflex after paired tooth pulp low intensity stimulations (mean values and standard deviation). During reduction of the inter stimuli interval, the standardized amplitude of the long latency jaw opening reflex after test stimulation increase and raised for a inter stimuli interval of 25 ± 9.1 ms, then the amplitude decreased till 10 ± 6.5 ms.

(Figure 3d). At the same time, the amplitude of the SL reflex decreased till a threshold for ISI = 5 ms (Figure 4).

Evoked potentials

The impact of paired TPS on responses evoked on the somatosensory cortex was investigated in group G2 (n=5) with freely moving rats. After low intensity TPS no change were observed in relation with the TPEPs after simple stimulation paradigms.

When the conditioning stimulation evoked a SL JOR and the test stimulation was adjusted to a high intensity that normally evokes a SL JOR a P30 component was observed after the second stimulation, when ISI was decreasing,. The amplitude of such a component increased till ISI = 26 ms ± 6.2 and then decreased and disappeared (ISI < 10 ms ± 4.1).

DISCUSSION

SIGNAL PROCESSING

The investigation of evoked cortical potentials with a low signal-to-noise ratio represents a serious challenge when the potentials are embedded in the ongoing EEG activity. Somatosensory evoked potentials are complex and transient non-stationary signals (i.e. not time locked to stimulus). Digital filtering of such signals, with time invariant descriptors such as Fourier's transform analysis, remains a difficult issue. The averaging of a high number of sweeps is generally used to obtain a satisfactory result. Nevertheless, due to the large analog bandwidth used during recordings, a high frequency noise still remains following averaging. It happens that this noise frequency is in the same frequency range as one of the evoked potentials (EP).

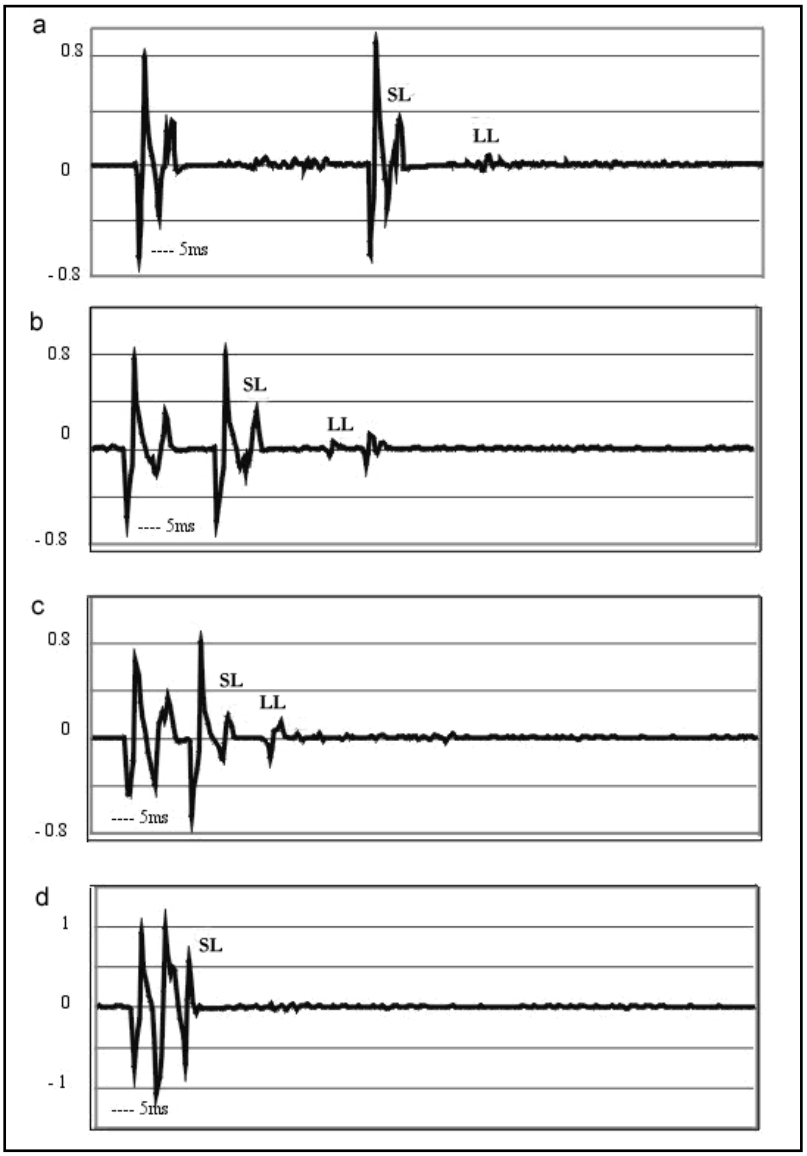

Figure 3. Interaction of the two component of the jaw-opening reflex after single sweep high intensity paired shocks. For decreasing inter stimuli interval of 30 ms (a), 15 ms (b), 10 ms (c) and 5 ms (d), the long latency (LL) component of the jaw opening reflex started to appear for a inter stimuli interval <32 ms. Then the amplitude of this component increase until the inter stimuli interval reach 9ms and then decreases to disappear for a inter stimuli interval <5 ms. Concurrently the short latency (SL) component of the jaw-opening reflex decreases and then increases again.

Several methods have been identified to improve the EP resolution such as Wiener filtering [12,13,21]. However time-varying filters or optimal time-varying filters [5,40] were more adapted to the characteristics of EP. In accordance with other studies [5, 31] the time-varying signal processing method used in this paper, improved significantly the signal-to-noise ratio in relation to the averaging method. The characteristics of the evoked signal are preserved after wavelet analysis (Figure 1). Usually the averaging method requires at least 800 sweeps for each run. Together with the rejection of overloaded responses the wavelet method allowed for the reduction of the number of sweeps necessary to obtain a satisfactory response with a ratio of 1 to 10.

JAW OPENING REFLEX

A two-component reflex was elicited in the ipsilateral digastric muscle after a single TPS [7]. A LL reflex component was produced at low stimulation intensity, by the activation of unmyelinated axons of the pulp. With increasing TPS intensity, a SL reflex component was elicited by the stimulation of periodontal myelinated fibers due to the current spread to periodontal tissues. Periodontal afferents caused an inhibitory period on digastric motoneurones canceling inputs from toothpulp, as demonstrated earlier [8]. Therefore, when the TPS intensity increased and reached the threshold of the SL JOR, the LL reflex was progressively inhibited.

Our results confirm these interactions between the JOR components. This inhibitory process may use different pathways from the pre-synaptic inhibition of

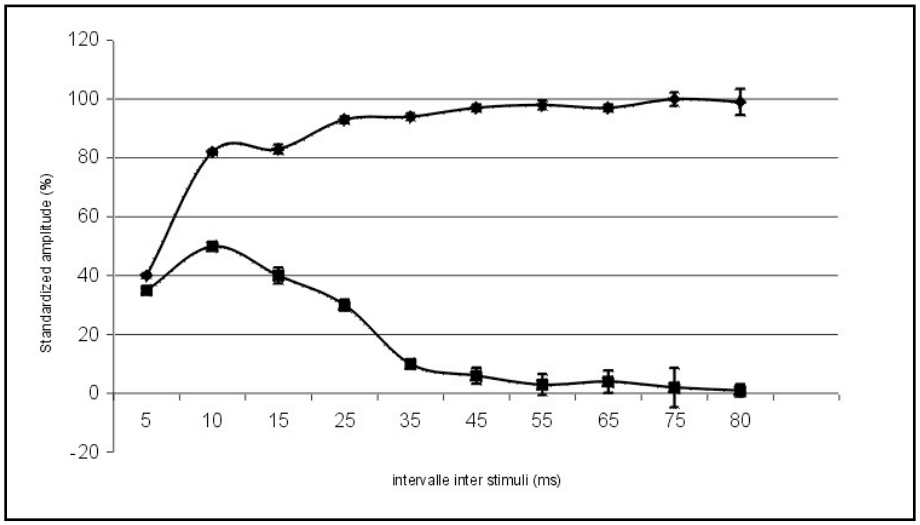

Figure 4. Interaction of the long latency (squares) and short latency (circles) component of the jaw-opening reflex after high intensity paired shocks (mean values and standard deviation). During reduction of the inter stimuli interval, while the amplitude of the short latency component decrease; the amplitude of the long latency component increases and then decreases gradually.

primary afferents to the activation of inhibitory interneurons controlling the excitatory neuronal chain. The interneurons involved in the early JOR are localized at all levels of the trigeminal sensory complex (TSC) and in the inter-trigeminal area [2].

In cats, previous experiments suggested that SL reflex interneurons could be localized in the rostral part of the TSC (subnuclei oralis and interpolaris). In rats, although controversial, interneurons could be localized in the nucleus principalis [7] and in the intertrigeminal area [22]. However the JOR pathway remains a speculative issue.

In our experiment we tried to apply the double stimulation paradigm to investigate the JOR and TPEPs. This method has not been used in the field of JOR studies in rats. The double stimulation enhanced a period of facilitation (ISI = 80 to 45 ms) after SL JOR component, followed by a phase of inhibition (ISI = 45 to 10 ms) (Figure 2a and 2b). The results show that the SL reflex decrease and the LL reflex increase are simultaneous (Figure 3b). This clarifies the relation between the two components of the JOR observed [7] and supports to the assumption of an interaction between C and A fibers.

The inhibition and facilitation process has been identified on other facial reflexes such as blink reflexes [14,30] which presents two components (early and late components) interacting together. In addition, double shock stimulation may be used to disclose component interactions and to elucidate the reflex pathways, produced according to different ways of stimulation.

In our experiment, a double high intensity stimulation eliciting a SL JOR allows the reappearance of a LL JOR component as soon as the second SL reflex component decreases (Figure 3 a,b,c and d). Those results confirm the importance of the SL JOR and the periodontal afferences in the facilitation/inhibitory process. Regarding our results it is possible to quantify the inhibitory effects of the periodontal afferents (Figure 4). The amplitude of the reappearing LL JOR did not reach the amplitude of the LL JOR produced at low intensity stimulation (–30%). This could indicate that the inhibitory process partially persists under these conditions.

SOMATOSENSORY EVOKED POTENTIALS

Previous results [33] showed that the TPS produced a cortical response in an area being one millimeter anterior to bregma in the TPS controlateral somatosensory cortex; it was confirmed in our results. The amplitude of the EP is in relation with the intensity of the TPS and follows a power law [37,39]. When the TPS intensity increases, this relation reaches a limit which could be attributed to fibers saturation. Thus we could consider that the EP is in relation with the recruitment of nervous fibers after TPS.

The number of unmyelinated axons of the mandibular incisor dental pulp of rats varies between 127 and 224 [6,16]. The low recruitment of fibers, in some experimental conditions, may be the reason why it was difficult to record the TPEPs after low intensity stimulation (LL evoking). This allows us to consider that there is a specific threshold of recruitment necessary to evoke a cortical response.

The lake of TPEPs for Low TPS, situated under the threshold of the LL JOR, showed that there is a link between JOR and TPEPs. This could be due, either to the threshold of nervous afferences or to an insufficient recruitment of fibers.

The question of a possible myogenic contamination of TPEPs was evoked by different authors [18]. This should be considered as a possible bias in our study for the [5–20 ms] interval in which the JOR was recorded. In our studies, the TPEPs elicited after a single stimulation show

an early component, at about [6 ± 0.8 to 17.2 ± 1.2 ms] (Table 1), in the range of a possible myogenic contamination. Selected rats presented a lower incisor length about 25 mm. Electrodes were implanted in their pulp at 12 mm from the incisal edge. If we consider the conductive time-period of the non-myelinated fibers into the pulp (0.5 m/s) and the conductive time-period of fibers outside the pulp (2.9 to 25 m/s) [32,35], consequently, the conductive period of time to the somatosensory cortex should not be less than 20 ms and the cortical evoked responses recorded for 6 to 20 ms of latency did not have a pulpal origin. Following TPS of the upper incisor, Rehnig [33] observed a P6 and N12 peak. In our study, P6 was concurrent with the SL JOR component, and it is possible to suppose that P6 corresponds to the periodontal afferents activation and the recording of motor reflex response with the TPEP recording electrodes.

The study of the thalamic projection of the primary sensory afferent neurons [36] confirms the lack of TPS evoked potentials prior to 8 ms. Furthermore the thalamic EPs were recorded for 8 ms, [20–30 ms] and [40–90 ms] following the TPS (4). In this respect, considering our results after single TPS, the [20–250 ms] EP components are acceptable as cortical TPEPs (Table 1).

When single TPS evoke cortical responses, our results showed different patterns depending on TPS intensity. Statistically identification of the [P23 ± 2.5, N48 ± 6.8] component after low intensity TPS, evoking LL JOR, could suggested that this TPEP component corresponds specifically to the pulpal unmyelinated fibers.

Concerning double stimulation experiments, when the second stimulation produced a LL JOR, no specific EP was evoked by the second stimulation. However, some potential were elicited by higher TPS intensities producing a SL reflex. This could be due to the weak afferent volleys reaching the cortex. As we observed for the JOR, a cortical evoked response could be expected following after conditioning shock, due to summation of responses. This effect was not observed at the cortical level, confirming the weakness of the input at this level. This assumption is confirmed by the effective correlation (r=0.96) between the integration of the EP and the TPS intensity.

In this experimental configuration, the results showed main peak values at P30 following the second stimulation. P30 ± 2.7 appeared only after the double SL TPS, namely when the current spread through the periodontal tissues and probably activated myelinated afferents. The window of appearance of P30 (ISI = 70 to 10 ms) corresponded to the window of appearance of the JOR LL component following the second stimulation (Figure 4). Accordingly we could assume that P30 was evoked by the stimulation of unmyelinated slow fibers.

As a conclusion our results allow to determine the TPEPs corresponding to the stimulation of the different afferences involved in the JOR. Results after paired shocks makes it possible to identify and to quantify the interaction between the different components of the JOR and to establish a correlation with the TPEPs.

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