CORTICAL TOOTH PULP EVOKED POTENTIALS IN FREELY MOVING RAT

Hans-Peter REHNIG, Jurij BRANKAČK and Fritz KLINGBERG

Institute for Brain Research, Department of Neurophysiology, Karl Marx University, 7010 Leipzig, GDR

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Abstract. The paper deals with the method of chronic implantation of electrodes for tooth pulp stimulation (TPS) in the upper incisor of rats, which enables investigations of tooth pulp evoked potentials (TPEP) under conditions of unrestrained behavior during several weeks. The most favorable point for recording TPEPs in the somatosensory cortex was 1 mm anterior of the bregma and lateral 3 mm below the crest of the skull. Here, TPEPs had amplitudes between 1000 and 2000 μV. TPEPs with amplitudes lower than 400 μV were recorded in other neocortical areas. The amplitudes of the primary components with peak times of 6 ms for positive and 12 ms for negative peaks were dependent on the behavioral state. The position of and distance between stimulation electrodes influence the TPEP. With increasing stimulus intensity TPEP amplitudes rised in an s-shaped curve. Current spread to alveolar nerve tested in acute experiments and was too small to evoke sufficiently large EPs of other origin.

INTRODUCTION

Pain interaction with behavioral activity and with information processing in sensory systems is an important problem. Experimental investigation of such a problem is dependent on the possibility to exactly apply and quantify pain stimulation. Electrical stimulation of different skin or body regions or of any afferent nerve results in mixed responses
of different sensory systems. Application of heat to certain points was used in several experiments to stimulate pain fibres selectively (16, 27), but it brings a lot of methodical difficulties evident in chronic experiments. Tooth pulp stimulation (TPS) was reported to selectively excite pain afferents (1, 11, 22, 23, 26, 27), if current spread to alveolar nerve is avoided (7).

Experimental investigations of different TPS effects were performed on humans (4, 5, 18, 19) monkeys (20), cats (1, 9, 12, 22), rabbits (23, 28) and rats (2, 6, 7, 10, 11, 17, 21, 24, 26). Tooth pulp evoked potentials (TPEP) were recorded in the somatosensory cortex of rats (11, 21, 25) rabbits (23, 28) and cats (12, 22). However, animal experiments with TPS were usually performed under acute or subacute conditions. This paper deals with chronical implantation of stimulation electrodes in the upper incisor of rats, with parameters of TPS and with recording the TPEP in the neocortex under conditions of unrestrained spontaneous behavior.

**METHOD**

The chronic experiments were performed on fifteen 6–8 months old male hooded rats of the Long-Evans strain. For acute testing of current spread to the alveolar nerve, 3 further rats were used. The rats were

![Diagram](http://example.com/scheme.jpg)

*Fig. 1. Scheme of chronical implantation of stimulating electrodes in the left upper incisor (a) of the rat; b, smooth wires, C, miniature plug-box, d, magneto-inductive device for recording animals' movements.*
operated under hexobarbital anesthesia (200 mg/kg body weight). Stimulation electrodes were implanted in the left upper incisor after opening the skin and preparing the dorsal surface of the skull and the maxilla and the proximal part of the tooth. A small piece of the alveolar bone was resected.

In 4 rats 6 holes of 0.8 mm diameter and 1.2 mm distance from center to center were bored in the enamel and dentin of the tooth without opening the pulp channel. In a preliminary examination we found that electrode implantation in the pulp channel as done by other authors (7, 10, 11, 25) is of no advantage for chronic experiments, as it may cause severe changes of threshold and excitability after a few days or some weeks. The six electrode tips (Nichrome wires) were embedded in silver amalgam. From there, the smooth wires leading to

![Cortex-TPEP](image)

Fig. 2. Samples of averaged TPEPs in different cortical areas of seven rats. Left side: TPEPs from the point with maximal activity on the border between areas 2 and 2a according to Krieg (15) from seven rats (c); right side: TPEPs from different points in areas 2, 2a, 41 (R 6) and 17 (R 7).
a miniature plug-box on the center of the dorsal skull surface, and the
tooth were covered with acrylate (Kalocryl CP-GM) as demonstrated
in Fig. 1. Current spread through the acrylate was impossible, so that
stimulation points and a part of the tooth were isolated. In these four
rats an epidural recording electrode was placed at the point of maximal
responses to TPS in the contralateral somatosensory cortex (compare
with Fig. 2), which was 1 mm anterior to bregma and lateral of the
midline 3 mm below the crest of the skull. Another electrode was im-
planted in the olfactory bulb (8 mm anterior to bregma, 1.5 mm lateral)
to control respiration rate (14) and a reference electrode in the left nasal

![Fig. 3. Representative samples of recordings of single TPEPs by 8 V 0.1 ms impulses from the contralateral somatosensory cortex of one and the same rat in the acute experiment. A, positions of stimulating electrodes on the upper incisor of the rat in a view from lateral; 1 and 2 corresponds to F and A in Fig. 5; point 3 is 2 mm more proximal, the position of pulpsae nerve destruction. B, when electrodes 1 and 2 are set on the enamel, C, when electrodes are inserted into the dentin as in the chronic experiment, D, after pulpsae nerve destruction in point 3, E, when the cathode is inserted in point 3 after the nerve destruction.](image)

bone. Other 11 rats had two tooth stimulation electrodes with
6 mm distance, three contralateral cortical recording electrodes in dif-
ferent areas (see Fig. 2) and three recording electrodes in the above
mentioned standard positions. Recording of the rat's movements was
performed by means of a magnetoinductive device on the animal's
head (Fig. 1). Two weeks after electrode implantation we started the
recording of EEG, evoked potentials, respiration rate and movement
patterns during unrestrained spontaneous behavior, which was perform-
ed in an electrically shielded box (30 × 25 × 40 cm). All data presented in this paper were taken from relaxed wakefulness (13), except those in Fig. 3 and 4 B–D. For TPS we used a square wave generator with an optoelectronic stimulus isolation unit. In all experiments, impulse duration was 0.1 ms and impulse interval 2.5 s. The intensity of the stimuli was voltage stabilized between 0–18 V. Single TPEPs were monitored and recorded by a DISA oscilloscope. TPEPs were averaged \( n = 10 \) by means of the multichannel analyzer NTA 512 B when polygraphic control, behavioral observation and the monitored single TPEPs indicated a rather homogeneous behavioral state (compare A with B–D in Fig. 4).

Acute experiments were performed on 3 rats under 200 mg/kg hexobarbital anesthesia. Using 8V 0.1 ms single impulses, TPEPs were compared before and after interruption of the pulpe nerve 2 mm proximal of the cathode (Fig. 3), two hours after an injection of hexobarbital, when the anesthesia became superficial.

Statistical differences of mean values were proved by use of the non-parametric Mann–Withney \( u \)-test.

RESULTS

We found TPEPs in different contralateral cortical areas with peak times of about 6 ms for the primary positive component and of 12 ms for the primary negative component (Fig. 2). Their amplitudes reached maximal values between 1000 and 2000 \( \mu \)V in a small field on the border between areas 2 and 2 a according to Krieg (15), which can be found 1 mm anterior to the bregma and lateral to the midline, 3 mm below the crest of the skull. Only 1 mm apart, amplitudes were in general smaller than 50\% of the maximal TPEP. In some rats we found TPEPs of 200 \( \mu \)V or smaller in the visual area 17 and of about 300–400 \( \mu \)V in the acoustic area 41. In the prefrontal area 10 TPEPs were still greater than 200 \( \mu \)V, so that we recommend to implant the reference electrode far rostrally on the nasal bone. Other localizations of the reference electrode or any kind of bipolar records were found to be less good for quantitative investigations.

The problem whether these TPEPs may be of other origin, as proposed by Engstrand et al. (7), was investigated in acute experiments. Figure 3 demonstrates the position of the stimulating electrodes (A, points 1 and 2) on the upper incisor in the lateral view. No primary response was evoked when the electrodes were set on the enamel (Fig. 3B). The maximal TPEP was regularly and constantly recorded from the same contralateral cortical point in the focus of maximal activity
as in chronic experiments in all three acute rats, when electrodes were implanted in the dentin as in the chronic experiments (Fig. 3C). After trepanation 2 mm proximally of the cathode (Fig. 3A, point 3) and destruction of the pulpe nerve at this position, the TPEP was strongly decreased (Fig. 3D). This small TPEP may be the response to current spread through the pulp channel to the proximal part rather than through the enamel, which has comparatively high resistance as revealed by the result in Fig. 3B. When thereafter the cathode was inserted into the pulp channel at the destruction point (Fig. 3A, point 3) we again recorded regularly TPEPs with maximal amplitudes as shown in

![SC-TPEP](image)

Fig. 4. Subsequent recordings of the single (1–10) and of the average (EP) TPEPs from the somatosensory cortex (sc.) of the same same rat during relaxed wakefulness (A), grooming (C) or non-homogeneous behavioral states (B and D).

Fig. 3E. Since these results could be reproduced in all three rats, we are sure that we obtained the response to TPS in the chronic experiments through the pulpe nerve.
Amplitudes of single TPEPs and even averaged TPEPs fluctuate strongly when the behavioral state changes (Fig. 4). In A of Fig. 4 the rat almost constantly remained in a relaxed state of wakefulness during 25 s, the time necessary to get an average of 10 single TPEPs. However, the third and the eighth TPEP are evidently smaller than the others. In B the behavior was more active in the first half, TPEPs 4–6 were recorded when the rat moved, and the rat was sitting relaxed in the second half of the stimulation. In C the rat was scratching intensively, but in D it was grooming in samples 4–9 mixed with locomotion. For this reason we recommend to classify TPEP according to the animals’ behavioral state (13, 21).

For TPEP recording during longer periods it was important how and where stimulation electrodes were implanted. When we inserted the electrodes into the pulp channel in previous experiments we recor-

Fig. 5. Amplitudes of averaged TPEPs related to stimulating electrode distance and polarity on TPEP amplitude: A, scheme of 6 electrode positions (A–F). Mean values and standard deviations from one rat (B) and interindividual mean values ± s.d. from four rats (C–E). Filled circles demonstrate amplitudes of TPEPs when the cathode was proximal and open circles when the anode was proximal, letters on the abscissae indicate electrode pairs.
ded smaller TPEPs which decreased after several days or a week. After implanting the electrodes in the dentin, embedded in silver amalgam, we observed no essential changes during several weeks, in so far as the wires were not broken by the tooth's growth. Some rats were investigated up to 3 months.

A small distance between the stimulating electrodes was less effective than a greater distance (Fig. 5). The amplitude of TPEPs increased exponentially as shown in one rat (Fig. 5B) or linearly as shown in four rats (Fig. 5D) with the stimulation electrode distance. The amplitudes were significantly greater when we stimulated with the same electrode distance, but the electrode position was more proximal, as compared in Fig. 5D and E. The TPEPs were much greater when the proximal electrode was always the cathode as demonstrated in Fig. 5B.

Fig. 6. Influence of stimulus intensity (s.i.) on TPEP amplitudes. Amplitudes (A) of single TPEP from one rat in μV, (upper part) and interindividual mean values of averaged TPEP amplitudes in % (responses to maximal stimulus intensity of 18 V = 100%) from four rats (lower part).
This also points to current spread rather inside the tooth than outside.

We investigated further the influence of stimulus intensity on TPEP amplitudes. Figure 6 demonstrates that threshold intensity was just below 3 V when the electrode distance was 6 mm and impulse duration was 0.1 ms and the impedance was about 5–6 kΩ. The responses increased exponentially with stimulus intensity up to 7–8 V and the amplitudes reached a plateau at 12 V. The individual variance increased during the averaging with the fluctuation of the behavioral state. The interindividual standard deviation was greatest in the exponential part of the mean curve (Fig. 6, lower diagram), which depends on rather small deviations of the individual curves. Individual amplitude differences were approximated by taking 18 V values as 100% and relating the other values to it. From this curve we concluded that stimulation with single impulses of 8 V and 0.1 ms is a rather practical parameter to investigate TPEP changes under different biological or behavioral conditions. TPEP amplitudes in relaxed wakefulness are then about 70% of the maximal response and this intensity is about the 3-fold threshold intensity.

DISCUSSION

TPS was used by many authors for different purposes, but in animals mostly under acute or subacute conditions. In rats TPS was applied to the lower incisor (2, 6, 7, 10, 11, 17, 24–26). In those experiments the pulp channel was opened and a bipolar electrode with a distance of 2 mm was inserted deep into the pulp channel (7, 11, 26). TPEPs which were recorded on the sensorimotor cortex of rats under such conditions were much smaller (11, 25) than we have demonstrated in this paper. It was generally accepted that TPS selectively excites pain receptors and fibres also in rats (2, 6, 11, 17, 24–26), but recently it was discussed that the lower incisor of rats is too thin to avoid current spread to extrapulpal afferent nerve fibres (7, 10) and it was found that afferents in the inferior alveolar nerve were also stimulated with higher intensities when the tooth was not isolated (7, 26).

We did not find data about the upper incisor, which is thicker than the lower one. Since in our experiments the alveolar bone was partly resected, the electrodes were inserted into the dentin, including the neighboring parts of the tooth, as they were covered with acrylate as a good insulator, the current spread to the surrounding tissue should have been small. These conditions were reproduced in the acute experiments, in which, after the destruction of the pulpa nerve proximal
to the stimulating electrodes, the TPEPs were reduced to less than 20\%. The remaining TPEP may originate from current spread to the proximal point of the interruption rather than from excitation outside the tooth, which becomes evident from the results demonstrated in Fig. 3. The stimulation current passes through the dentin channels which are about 2 \(\mu\)m wide and have a density of many thousand per \(\text{mm}^2\) (4, 8). Pain conducting nerve endings were found in these channels (3, 4, 18). A further argument for a rather selective stimulation of tooth pulp is the intensity curve (see Fig. 6) which can be interpreted in terms of Steven's function. The plateau of TPEP amplitudes which was reached at 10–12 V also speaks against essential involvement of extrapulpal afferents. Toda et al. (26) concluded that below 500 \(\mu\)A they selectively stimulated the intrapulpal nerve through electrodes in the pulp channel of the lower incisor. With stronger stimuli they got a further amplitude increase of the compound action potential. This comes from the so called \(f\) component which had a nerve conduction velocity of 42 m/s compared with 7 and 10 m/s of the slower pulpal components (\(s\) components). In our experiments the peak times of TPEP components did not change with stimulation parameters, neither with intensity nor with electrode distance. We suppose that in every case of our TPS experiments the responses of \(A\delta\)-fibers dominate, whereas the slower component originating in \(C\)-fibers is small and should be masked by the large fast component.

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