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A new paradigm for the reversible blockage of whisker sensory transmission

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ABSTRACT

The objective of this study was to explore a paradigm that would allow a temporary deprivation of whisker information lasting for a few hours. An additional requirement was to be non-invasive in order to be usable in awake chronically implanted rats without inducing stress. With that aim, electrophysiological recordings from the barrel cortex of anesthetized rats were obtained. The pressure of an air-puff (5–10 ms) delivered to the whiskers was adjusted to evoke a consistent response of around 100 μV (extracellular) or approximately 5 mV (intracellular) in the contralateral cortex. Lidocaine was then locally applied in different forms (cream, local injection, aerosol, drops) and concentrations (2–10%) to the base of the whiskers. The stimulus-induced response was monitored once every 5 s for several hours (3–6 h) in order to characterize its course of action. Local injection of lidocaine induced the fastest and most complete blockage, but was ruled out for being invasive. Out of the remaining forms of application, a lidocaine drop (0.4 ml, 10%) to the base of the whiskers was found to induce a reliable blockage (to an average 9% the original response). The maximum effect was reached after 150–200 min, and the response was totally recovered approximately 300 min after lidocaine application. This characterization should be useful to induce an efficient, short term and reversible blockage of whisker sensory transmission in both anesthetized and awake preparations, while not causing stress in an awake animal.

1. Introduction

Sensory deprivation has been a widely used manipulation to explore plastic changes occurring in sensory pathways either in short or long term. Whisker sensory deprivation has been most commonly achieved by whisker trimming (Diamond et al., 1993; Kossut, 1998; Lebedev et al., 2000; Lee et al., 2007; McRae et al., 2007). Other reversible nerve blocks such as procaine, a Ca^{2+}-induced Ca^{2+}-release (CICR) inhibitor of Merkel cells in the vibrissae in a dose dependent manner (Senok and Baumann, 1997), or nerve pressure have also been used (Decima et al., 2004). Trimming a whisker can induce changes in the corresponding barrel cortex within a few hours (Holtmaat et al., 2006). However, it takes 8–11 days for a new vibrissae to appear (Ibrahim and Wright, 1975), and several days to grow to their normal length at 1 mm/day. Here, we were interested in finding a method that would allow us to deprive rats of tactile information for a few hours, in a way that would cause no stress to an awake rat. This method should be easy to use, reliable, produce the least possible stress and be reversible in few hours. Lidocaine is a local anesthetic which is a potent blocker of sensory and sympathetic transmission, with a large effect on the nerve's refractory period (Scurlock et al., 1978) due to its blockage of sodium channels (Strichartz, 1973; Starmer et al., 1984). In this study we explored different forms of local application of lidocaine on the snout and the intensity and time course of its effect on the sensory-induced responses in barrel cortex.

2. Materials and methods

Eleven adult Wistar rats (250–300 g) were used for recordings in S1 cortex. Anesthesia was induced by intraperitoneal injection of ketamine (100 mg/kg) and xylacine (8–10 mg/kg). The animals were not paralyzed. Maintenance dose of ketamine was 75 mg/kg/h. Deep anesthesia levels were monitored by the recording of low frequency electroencephalogram (EEG) and the absence of reflexes. Rectal temperature was maintained at 37 °C by means of an electric blanket.

Once in the stereotaxic apparatus, a craniotomy (2 mm × 2 mm) was made at coordinates AP −1 to −3 mm from bregma, L 4.5–6.5 mm (Paxinos and Watson, 2005). After opening the dura, extracellular recordings were obtained by means of tungsten electrodes (FHC, Bowdoinham, ME, USA). For stability and to avoid desiccation, agar (4%) was used to cover the area. Sensory responses

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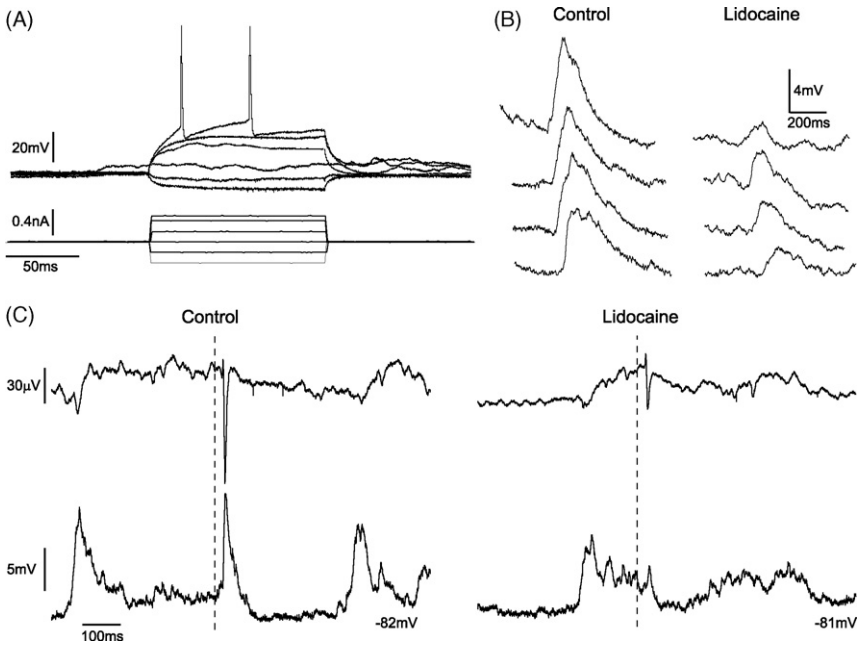

Fig. 1. Blockage of intracellularly recorded whisker-induced synaptic responses by topical application of lidocaine. (A) *I–V* protocol of the recorded neuron. Bottom, intracellularly injected current from −0.4 to 0.5 nA. Top, Vm responses to current injection. (B) Four raw traces of intracellular recordings in response to an air puff stimulation in control (left panel) and after application of topical lidocaine to the whiskers (right panel). Note the decrease of the amplitude to an 81%. (C) Traces of simultaneous LFP (top) and intracellular recording (bottom). The dash line represents the trigger of the air puff in both control (left panel) and in presence of lidocaine (right panel). The parameters of the stimulation were in both cases (control and lidocaine) 10 ms, 5 psi.

evoked in the local field potential (LFP) were used to adjust whisker stimulation (see below). Intracellular electrodes were made with borosilicate glass capillaries 1 mm O.D. × 0.5 I.D., filled with 2 M potassium acetate, and had final resistances of 50–80 mΩ. Intracellular recordings were obtained in close vicinity (<1 mm) from the extracellular recording electrode (Fig. 1A and B). Recordings were digitized, acquired and analyzed using a data acquisition interface and software from Cambridge Electronic Design (Cambridge, UK).

2.1. Whisker stimulation

A puff of air given through a 1 mm diameter tube placed in front of the whiskers (10 mm away) was used for stimulation. The air puff (5–10 ms) was controlled by a stimulator (Master-8, AMPI, Israel) and delivered by a Picopump (WPI, Sarasota, FL). Its pressure was adjusted (5–10 psi) such that it would evoke a response of approximately 100 μV in the LFP and 5 mV in the intracellular recordings (Fig. 1C). The stimulus was directed to the whole whisker area, and particularly focused towards one vibrissae. For the stimulus–response curves the applied pressure was 2–38 psi. Time zero for the stimulus was taken as the time of occurrence of the air puff.

2.2. Lidocaine application

Local application of lidocaine was made at the whisker pad of anesthetized rats (Faggin et al., 1997). Several forms of lidocaine application have been tested in order to find the most efficient and reliable way to block the somatosensory input.

2.2.1. Cream

Lidocaine cream (EMLA® cream, 25 mg/g) was applied to the base of the whiskers (1 g). To improve lidocaine skin absorption, a short massage was done with a Q-tip.

2.2.2. Injection

Liquid lidocaine (Xilonibsa®, 10%) was injected at the whisker base (subcutaneous injection, 4–5 injections, 50 μl each).

2.2.3. Aerosol

Liquid lidocaine (Xilonibsa®, 10%) was vaporized on the snout in the whiskers zone (2 sprays, 0.2 ml).

2.2.4. Liquid drop

A drop of liquid lidocaine (Xilonibsa®, 10%) was applied to the base of the whiskers with a pipette (0.4 ml).

3. Results

In order to characterize a reversible and non-invasive method to block sensory transmission from the whiskers, recordings from barrel cortex of 11 rats were included in this study. In 7 of the rats the complete study was performed in both hemispheres. The number of observations that are given refer to responses in one hemisphere following stimulation of the contralateral whiskers.

Intracellular recordings were obtained from a total of 13 neurons (Fig. 1A) recorded in close vicinity to the LFP recording. We obtained sensory-evoked potentials by an air puff to the whisker (10 ms, 5–10 psi; Fig. 1B) (Reig and Sanchez-Vives, 2007). The average amplitude was 6.1 ± 2.6 mV, their average latency being 11.06 ± 2.49 ms (range 8–15.6 ms). Fig. 1C illustrates simultaneously recorded sensory-evoked synaptic potentials in the LFP and in the intracellular recording. In this particular case the evoked synaptic potential was reduced from 6.4 ± 2.0 mV to an average value of 1.2 ± 1.5 mV (a decrease of 81.3% the control amplitude) following lidocaine application. This decrease was also reflected in the LFP evoked synaptic potential from 58.5 ± 31.37 μV to 7.8 ± 21.0 μV (a decrease of 86.5% the control amplitude). Once it had been proven that the LFP accurately reflected the intracellularly recorded synaptic potentials, the following sensory responses were only evaluated

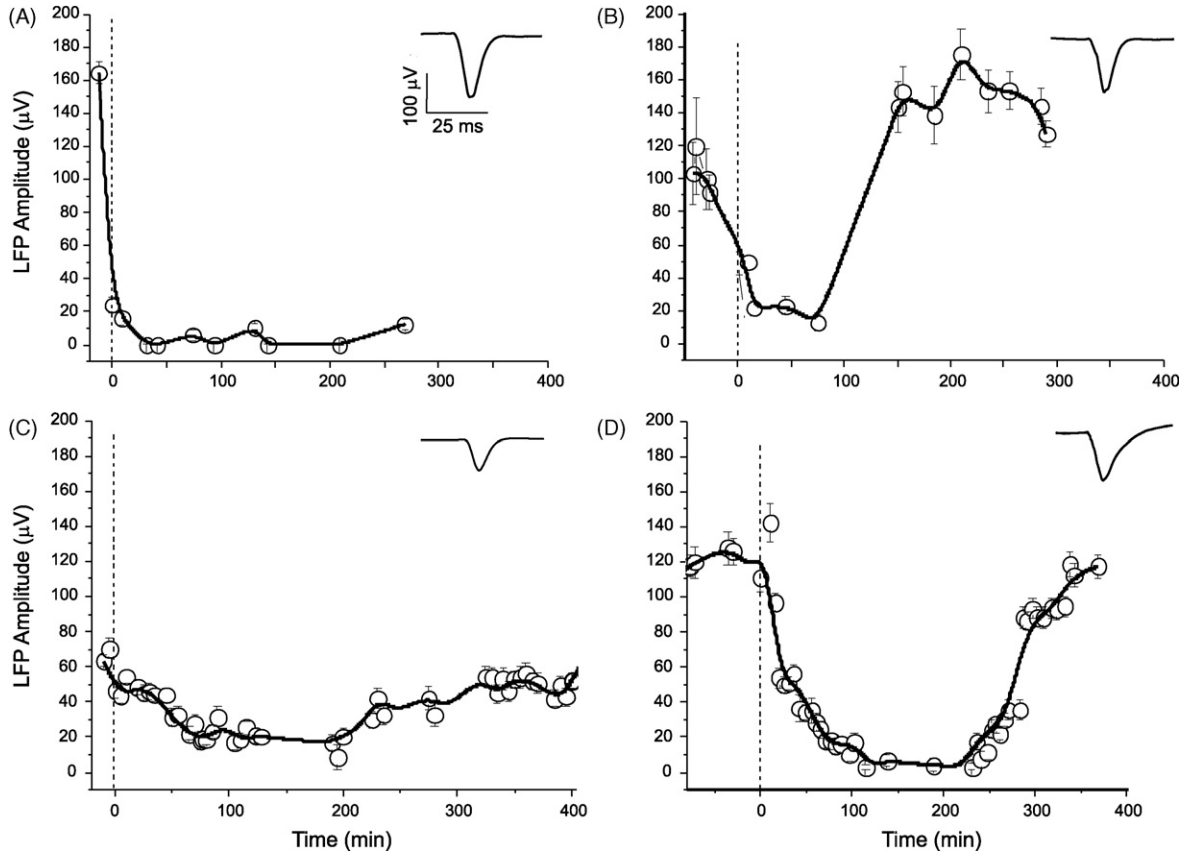

Fig. 2. Time course of action of four different forms of lidocaine application. Zero (dash line) represents the time of lidocaine application. Each circle represents the average amplitude of 60 sensory responses (0.2 Hz) as measured in the LFP in response to the air puff stimuli (5 psi, 5–10 ms duration). (A) Subcutaneous injection of lidocaine (10%, 0.2 ml). (B) Lidocaine in cream, 1 g (25 mg/g). (C) Lidocaine in aerosol application (10%, 2 sprays, 0.2 ml). (D) Liquid drop (10%; 0.4 ml). Insets represent the averaged stimulus-evoked response before lidocaine was applied.

through the LFP. Long (>300 min) and highly stable recordings were needed to monitor the action and recovery of lidocaine application.

Whisker stimulation yielded a sensory-evoked response in the LFP that had an average latency of 13.6 ± 1.07 ms and an average peak amplitude of 77.5 ± 31.2 μV, peak that occurred at a latency of 22.2 ± 1.8 ms ($n = 17$ recordings) in the extracellular recordings. The whisker response evoked in this way was repeated at 0.2 Hz and each of the measured responses were the result of averaging 60 stimuli (300 s). This pattern of stimulation was continued for several hours (3–6 h) in order to monitor the time course of action of lidocaine and its recovery. No significant decays or increases of the sensory-evoked responses were observed over time (Supplementary Material Fig. 1).

Lidocaine was then locally delivered in different forms (local injection, cream, aerosol, drops) and concentrations (2–10%) to the base of the whiskers (Section 2).

Local injection of lidocaine (4–5 injections, 50 μl, 10%) at the base of the whiskers induced the fastest (maximum effect in 30 min) and most complete (97%) blockage ($n = 2$; Fig. 2A). In both cases, 5 h later the response was not yet recovered. This difficult reversibility, together with the fact that it is an invasive technique, suggested that this technique was not ideal for awake behaving animals in spite of inducing an effective blockage. Thus, other forms of application were explored.

In two experiments *in vivo* lidocaine in cream was topically applied to the base of the whiskers (dose 1 g; 25 mg/g) after a sensory-induced response was obtained. We obtained a blockage in one of the two cases 65 min after application, during which it decreased 87% the original amplitude, with a recovery time of 200 min (Fig. 2B). In the second case no effect was observed. Cream application had the advantage of causing less stress than injection to an awake rat, but posed the problem of staying in layers of different thickness and therefore allowing poor the control over the dosage. In an awake animal, the mechanical effect of the cream could also affect the movement of the vibrissae. Overall, cream application did not seem to be a reliable technique to induce temporal blockage.

Aerosol application was carried out in two experiments ($n = 2$). The maximum blockage by aerosol application was of 55% of the control response, with a maximal effect after 105 min and a recovery of the response 320 min after the initial lidocaine application. Even when effective, the aerosol technique presented also a deficit in the control of the applied dose, not so much regarding the amount being sprayed, but rather the distribution of the product over the whisker pad, since small drops remained on the whiskers (Fig. 2C).

A drop of lidocaine (0.4 ml, lidocaine 10%) was found to induce a reliable blockage of the sensory-evoked synaptic potentials (Fig. 2D). Apart from being an easy method to apply to an awake rat (unpublished observations), it seemed to present lesser dosage problems than those reported for the other forms of application. Thus, for a thorough characterization of the lidocaine action, three experiments were performed while doing this form of lidocaine application. In all cases the recordings were carried out in both hemispheres while stimulating whiskers on both sides. The grand average of the effect in 6 cases is represented in Fig. 3B. The average decrease of the response amplitude was of 91.1% the control response in 150 min and with a recovery time of 300 min. Not only

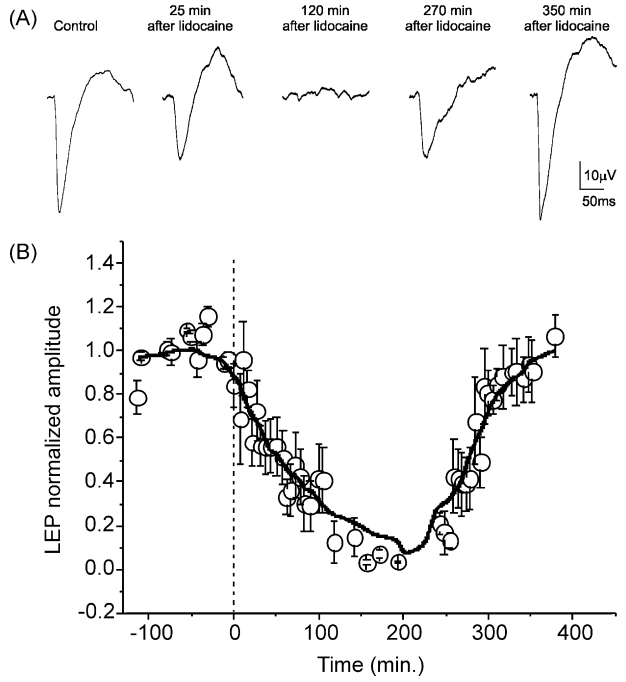

Fig. 3. Effect of topical application of a drop of 10% lidocaine (0.4 ml) to the whisker pad. (A) Waveform average of extracellularly recorded evoked response in control condition, after lidocaine application and during recovery. The stimulus parameters were constant and consisted of 5 ms duration and 5 psi pressure. Each trace represents the average of 60 stimuli occurring at 0.2 Hz. (B) Grand average of the time course of action of topic lidocaine ($n = 6$). Each point represents the average peak amplitude evoked by 60 stimuli per recording, therefore 360 responses in grand average. The sensory-evoked responses were normalized with respect to the average of the control response. The parameters of the air puff stimulation were the same in all cases (5 ms duration, 5 psi). The maximum blockage was achieved in approximately 150 min.

the peak amplitude decreased but also the downward slope of the evoked potential, that was of 2.1 ± 0.8 mV/s in control and slowed down to 0.9 ± 0.6 mV/s in lidocaine, showing afterwards a complete recovery (Fig. 3A).

Intensity of the stimulus *versus* amplitude of the stimulus-evoked response relationship was generated by random modifications of the air puff pressure delivered to the whiskers (5 ms duration; Fig. 4A). Such stimulus–response curve was carried out before and after application of 0.4 ml 10% lidocaine ($n = 6$). The amplitude of the sensory response was represented against the intensity of the stimulation. While the responses were noticeably reduced by this dose of lidocaine, the stimulus–response relationship was maintained in all cases at this dosage. Following the end of lidocaine action (>300 min), the stimulus–response relationship returned to control levels (Fig. 4A).

In order to rule out the possibility that the mechanical effect of a drop of lidocaine on the whisker's base could have an effect decreasing the sensory-evoked response, a drop of the vehicle of lidocaine, 32% ethanol, was applied ($n = 4$). Fig. 4B illustrates the average response ($n = 4$) to different intensities of a 5 ms air puff (0–38 psi). The stimulus–response curve was first obtained in control conditions (black squares), and then obtained again after the topical application of 0.5 ml of 32% ethanol (white circles). No significant difference was found subsequent to the application of a drop without lidocaine, therefore ruling out a mechanical effect.

In order to further test the effect of topical application of liquid lidocaine, we explored the response to one single whisker stimulation (5 ms, 10 psi air puffs) by trimming all the remaining ones ($n = 4$). The rest of the protocol was identical to the one described before. In all cases, 10% lidocaine (0.4 ml) was effective to block the stimulus-induced response. The average response to one whisker stimulation in control condition was 108.5 ± 12.9 μV. Following lidocaine application the response was reduced to an average value of 28.6 ± 4.9 μV, therefore a reduction of 73.68%.

To conclude, topical application of liquid lidocaine applied at the base of the whiskers induces a reversible blockage in barrel cortex sensory responses, and the recovery time can be achieved in approximately 3 h after the maximum effect. This characterization could be useful to induce a reversible blockage of whisker information in both anesthetized and awake preparations in order to explore changes derived from a short term somatosensory deprivation. Longer blockages would require repeated lidocaine applications.

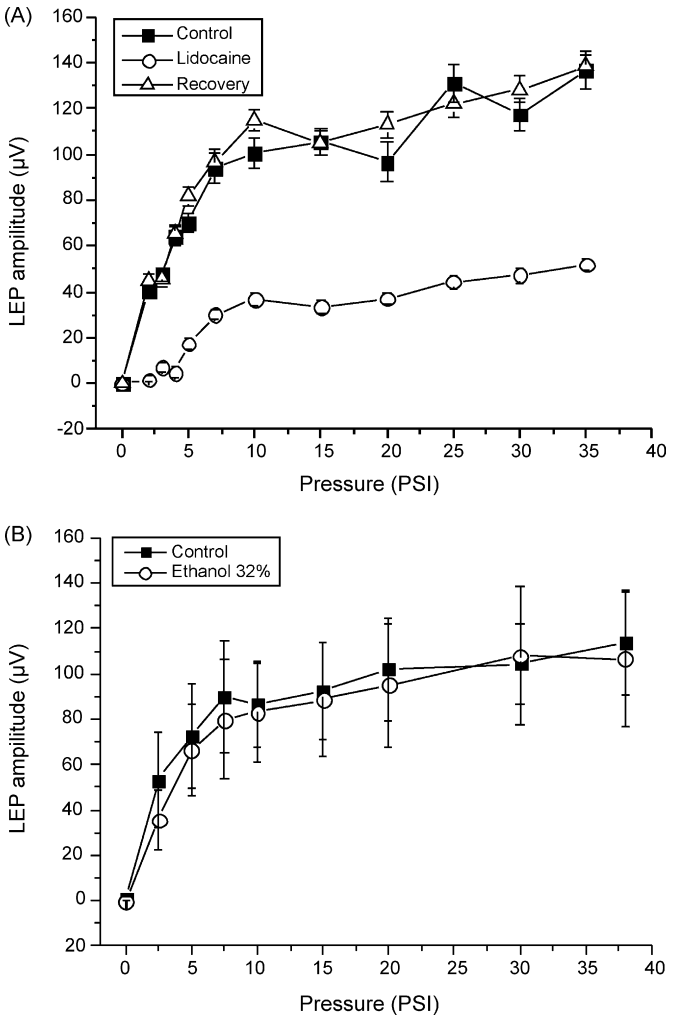

Fig. 4. Stimulus–response relationship in control and lidocaine. (A) Stimulus–response graph to an air puff of 5 ms and randomly varied pressure (0–38 psi). In this case the responses have been studied in control condition, following topical application of 0.4 ml 10% lidocaine on the whisker pad and again after recovery from lidocaine blockade (246 min later). Each point is the average of 60 stimulus that occurred with a frequency of 0.2 Hz. The error bars correspond to S.E.M. Similar results were obtained 6 cases. Notice that lidocaine effect is totally reversible, although it takes 4 h to recover. (B) Stimulus–response relationship in control and lidocaine vehicle (32% ethanol). An air puff of 5 ms duration and randomly varied pressure (0–40 psi) was tested first (control). Next, 32% ethanol was applied in a 0.5 ml drop over the whisker pad, while no significant difference in the response to the same stimulus was observed. This graph is the result of averaging the responses to the same stimulus and conditions in two different rats. Each point is the average of 60 stimulus that occurred with a frequency of 0.2 Hz. The error bars correspond to S.E.M.

4. Discussion

In this study, we explored a new paradigm that would temporarily deprive whisker information and would be appropriate for use in an awake animal. Previous studies in anesthetized animals have used whisker deprivation by subcutaneous injection of lidocaine (Faggin et al., 1997), a method that was found to be efficient. However, this procedure is invasive and probably too stressing to deprive somatosensory information from an awake animal. Apart from this method, the most common form of deprivation in awake animals has been the trimming of whiskers (e.g. Huston et al., 1986; Steiner et al., 1986; Lebedev et al., 2000; Grigoryan et al., 2005). Given that we were interested in a method that allows a short term deprivation (of hours) the vibrissotomia was not ideal, since it requires a certain growth time to recover.

Given these two constraints, we tested different forms of lidocaine application (injection, aerosol, cream, and liquid drop) to the whisker pad. To characterize this new technique, we recorded *in vivo* intracellularly and extracellularly in barrel cortex (S1) the response evoked by an air puff to the whiskers before and after the application of lidocaine. The different forms of application were all effective to temporally deprive somatosensory input, but presented some advantages and disadvantages. The injection technique is an invasive technique which is applicable only in anesthetized animal, but too stressing for the awake. Although the EMLA® cream (Juhlin and Evers, 1990) decreased the response in one out of two cases, the dose was not easy to adjust, plus the remaining dried cream could eventually affect whiskers movement. Aerosol application induced temporal tactile deprivation with the inconvenience of being too imprecise in the application area, given that the aerosol drops often stayed on the whisker's surface.

We found the application of a drop of liquid lidocaine to be the method with more advantages to temporally deprive somatosensory information: easy to apply, not stressing for awake animals, and a course of action that proved to be consistent in 6 cases (Fig. 3B). As we demonstrate in the results, the drop of lidocaine started to reduce the sensory-evoked response in a few minutes, it reached a maximum close to 100% blockage between 100 and 200 min and it was totally reversible after 5–6 h.

We ruled out the possibility that the effect of the lidocaine was due to a mechanical effect of the drop, since a drop of same or even larger volume of the lidocaine vehicle (32% ethanol) did not have an effect on the intensity/response curve. However, in the anesthetized animal we were not able to test what the effect of the different forms of lidocaine may have on the awake animal's whisking movements.

Interestingly, this method can be used in awake animals because it is easy to apply and it is not stressful for the animals (unpublished observations). Repetitive applications over time could provide longer blockages if necessary. By means of this manipulation, the role of whisker information in different cognitive tasks, or the plasticity induced by its short term deprivation, can be explored under experimental conditions involving electrophysiological recordings in awake animals and/or behavioural tasks.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jneumeth.2008.08.012.

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