The Matrix: A new tool for probing the whisker-to-barrel system with natural stimuli

Vincent Jacob a,1,2, Luc Estebané a,c,1, Julie Le Cam a,1, Jean-Yves Tiercelin b, Patrick Parra b, Gérard Parésys c, Daniel E. Shulz a,*

a CNRS, Unité de Neurosciences, Information et Complexité (UNIC), 1 avenue de la Terrasse, 91190 Gif sur Yvette, France
b CNRS, Institut de Neurobiologie Alfred Fessard, 91190 Gif sur Yvette, France
c Laboratoire de Neurobiologie, Ecole Normale Supérieure, 75005 Paris, France

ARTICLE INFO
Article history:
Received 15 January 2010
Received in revised form 10 March 2010
Accepted 16 March 2010

Keywords:
Multi-whisker stimulation
Natural stimuli
Sensory processing
Barrel cortex
Stimulator
Tactile
Somatosensory

ABSTRACT
The whisker to barrel system in rodents has become one of the major models for the study of sensory processing. Several tens of whiskers (or vibrissae) are distributed in a regular manner on both sides of the snout. Many tactile discrimination tasks using this system need multiple contacts with more than one whisker to be solved. With the aim of mimicking those multi-whisker stimuli during electrophysiological recordings, we developed a novel mechanical stimulator composed of 24 independent multi-directional piezoelectric benders adapted to the five rows and the five caudal arcs of the rat whisker pad. The most widely used technology for producing mechanical deflections of the whiskers is based on piezoelectric benders that display a non-linear behavior when driven with high frequency input commands and, if not compensated, show high unwanted ringing at particular resonance frequencies. If not corrected, this non-linear behavior precludes the application of high frequency deflections and the study of cortical responses to behaviorally relevant stimuli. To cope with the ringing problem, a mechanical and a software based solutions have been developed. With these corrections, the upper bound of the linear range of the bender is increased to 1 kHz. This new device allows the controlled delivery of large scale natural patterns of whisker deflections characterized by rapid high frequency vibrations of multiple whiskers.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Since the pioneering work of Woolsey and Van der Loos (Woolsey and Van der Loos, 1970), the whisker to barrel system in rodents has become one of the major models for the study of sensory information processing. Its success is due to an outstandingly high degree of anatomical organization of the neuronal pathway linking the whiskers on the snout of the animal to the postero-medial barrel sub-field (also known as the barrel cortex), the area of the primary somatosensory cortex that receives information from the whiskers. Rodents acquire tactile information on textures and objects through repetitive contacts with multiple whiskers (Carvell and Simons, 1990; Harvey et al., 2001; Sachdev et al., 2001). In certain tactile tasks the performance level was shown to depend on the number of macrovibrissae available on the snout (Krupa et al., 2001; Celikel and Sakmann, 2007). Contact of multiple whiskers with an object as it sweeps across the whisker pad appears in those cases as an important factor for accurate discrimination. Thus, to accurately mimic this observation in the laboratory a whisker stimulator that deflects independently most of the large macrovibrissae is required. Several previous attempts to produce such a device have been successful for small number of whiskers: five whiskers in a row (Brumberg et al., 1996; Rodgers et al., 2006), nine whiskers in a 3 by 3 grid (Drew and Feldman, 2007) and even 16 whiskers in a 4 by 4 array of 16 miniature-solenoid driven actuators (Krupa et al., 2001). Here we present a new device for large scale whisker stimulation that allows isotropic and symmetrical stimulation of 24 whiskers centered on whisker C2.

Although the classical description of a whisk cycle is a protrac-tion on the caudo-rostral plane, followed by a hold period and then a retraction back to the rest position, the contacts of the whiskers on a surface generate unique kinetic signatures of whisker vibrations (Arabzadeh et al., 2005). Moreover, during texture discrimination tasks, particular events occur where the whisker sticks on the surface and then is suddenly released (stick-and-slip events) resulting in a period of high frequency vibrations (Hipp et al., 2006; Ritt et al., 2008; Wolfe et al., 2008). Thus, the trapezoidal or ramp-and-hold stimulation does not cover the full range of rele-
2.1. General overview of the whisker deflection device

The generation of natural high frequency whisker deflections is limited by the mechanical properties of the piezoelectric benders, a technology used in most of the laboratories working on the barrel system. Indeed, when driven with high frequency commands, the benders display a non-linear behavior with a high gain for particular resonance frequencies, usually around 100 Hz. This non-linear behavior precludes the application of high frequency deflections. To overcome these problems, there is a need for a device that can reproducibly and faithfully replicate behaviorally relevant stimuli while recording the system functional responses.

Here we present a new device for large scale whisker stimulation with a linear behavior up to 1 kHz. The stimulation device consists of 24 piezoelectric benders each of them being hold by an independent arm lever and allowing the controlled delivery of spatio-temporal patterns of whisker deflections. We performed a detailed analysis of the mechanical properties of the device and validated it using in vivo electrophysiological recordings. The biological relevance of the stimulations is guaranteed by two characteristics of the device: first, the high degrees of freedom of its mechanical structure makes it possible to align each stimulator at the resting position of the whiskers, and second, the use of a method to cope with the ringing problem increased the upper bound of the linear range of the bender from 100 to 1000 Hz. A first generation stimulator that permitted deflections only in the rostro-caudal axis linearly by rotating and translating the lever arm using the connecting bolt to the rod and using a ball joint on which the bend was glued (Fig. 3).

The lever arms were mounted on a precision translation stage (a one-axis micromanipulator, UL-AC-P, Narishige, Japan) with a 15 mm movement range (Fig. 3). This mechanism allows the movement of the lever arm and of the bendet towards the whisker pad. Whiskers were trimmed to 10 mm length and inserted 3 mm into short polypropylene or metal tubes (see Section 3) of adjusted diameter glued on the tip of the bender. The whiskers were inserted into the tubes by first, adjusting the angle of the lever arm to the resting angle of each whisker and second, by approaching the whisker towards the tubes with the one-axis micromanipulator. In this way, the whiskers were not bended to insert them into the tubes ensuring a stable unconstrained position of the whisker once it was inserted. This was visually verified at the end of the experiment when the Matrix was disconnected from the whiskers.

Whiskers were inserted row after row and we never experienced loss of positioning of the inserted whiskers while manipulating the other whiskers.

Fig. 1. 3D reconstruction of the 24 right macrovibrissae of a 300 g Wistar rat. Only the first 7 mm of each whisker are shown. Whisker positions, vertical and horizontal angles and widths at skin level and at 7 mm from the skin were measured in an anesthetized animal and depicted here (see also Supplementary Table 1). The surface delimited by the whiskers is an open convex polyhedron with the follicles positions as vertex. Whisker rows A, B, C, D and E are figured with colors red, purple, blue, green and yellow, respectively. St. Straddlers. (A) 3D projection. (B) Front view. (C) Top view. (D) Lateral view. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
2.2. Two solutions for holding piezoelectric benders

The whiskers were deflected by attaching them to piezoelectric benders that allow precise and fast displacement of low inertial objects. However, the precision of this displacement is compromised at higher frequencies by the occurrence of dephasing and amplitude variation in the translation of electrical commands into motion. We applied two different strategies to cope with this problem: In a first version of the stimulator (see Jacob et al., 2008), one-directional piezoelectric benders (Polytec-PI, Germany) were used and a certain number of tests, described below, were performed to ensure that indeed the benders were functioning within their linear functioning range at frequencies below the resonance frequency. In a second, more recent version of the stimulator, we applied mechanical and software solutions to multi-directional piezoelectric benders in order to linearize their behavior above the initial resonance frequency. Both solutions are described below.

2.3. First generation stimulator: spatial linearity and normalization of the movement of the bender

In the first version of the stimulator we used long voltage pulses (500 ms) at different amplitudes to study the spatial linear behavior of the benders. The deflections of the benders were recorded with a PSD/laser displacement sensor (LD1607-0.5, Micro-Epsilon, France, Fig. 4A) at a resolution of one micrometer. The movement was measured at the tip of the polypropylene tube where the vibrissa was inserted. We computed a linear regression between the input voltage and the resulting amplitude of the deflection of the bender measured after the ringing period induced by the front edge of the stimulus (Fig. 4B). For all benders the amplitude of the deflection depended linearly ($r^2$ larger than 0.997) on the amplitude of the voltage input (Fig. 4C). Nevertheless different benders showed different amplitudes of deflections in response to the same voltage input. To uniform the amplitude of deflection of the 24 benders, we used the input/output regression function to adjust the voltage input of each bender so as to obtain equal amplitudes of deflection. To control for temporal drifts in the behavior of the benders, the measure of the input/output function was repeated several times at intervals of three to six months. The maximal deviation observed was less than 5% of the initial linear relationship.

Impulse-like stimulations are widely applied to study the linear receptive field of sensory neurons. Square pulses or ramp-and-hold deflections with sufficient initial velocity to optimally drive cortical neurons are usually applied to whiskers. However high-speed deflections of the bender induces unwanted ringing at the resonance frequency (see Fig. 4B). To find the trade-off between speed of deflection and ringing amplitude we studied how different ramp durations from 1 to 50 ms affect the behavior of the bender. The benders were driven with RC-filtered (time constant = 2 ms) voltage inputs that introduced a 1 ms delay that should be subtracted from the response delay. As expected, longer ramp durations resulted in less ringing (Fig. 5A, only ramps of 2, 4, 10 and 30 ms durations are shown). We choose then 10 ms long ramps that in our
Fig. 4. Study of the linear behavior of a piezoelectric bender. (A) Measurement of the displacement of the bender (arrow) with a PSD/laser sensor. (B) Bender displacement to a gradually increasing input voltage of 100 ms duration. (C) Displacement amplitude as a function of the input voltage for rostro-caudal (R-C) and caudo-rostral (C-R) directions. A linear regression line was computed between the input voltage and the resulting amplitude of the deflection measured after the initial ringing period.

Hands induced ringing with amplitude of less than 5% of total deflection amplitude (Fig. 5B) independently of input amplitude (Fig. 5C).

2.4. Second generation stimulator: hardware and software solutions for a linear behavior.

In a more recent development of the stimulator (Fig. 6A), the problem of dephasing and ringing evoked above was corrected using a mechanical and a software approach (Patent application N° FR 09 03752). The transfer function of the bender can be measured over the range of frequencies of interest and the inverse transfer function can be applied to all inputs. However, this correction can only be applied to a piezoelectric bender under two conditions: First, the bender should not display any ringing peak in the frequency range of interest and second, the bender should maintain its transfer function constant during the adjustment of its position within the experimental set-up. Here we describe the solutions we developed to ensure that both aforementioned conditions are verified in the mechanical design of the second generation stimulator. We then present a software solution that builds on top of the mechanical device to provide a fully adjustable 2D whisker displacer with a flat transfer function up to 1 kHz.

The stimulator uses multi-directional piezoelectric benders (CMB-2D, Noliac, Denmark). The displacement achieved by these benders, when they work against no external load is in the range of 250 μm, and show a resonance frequency above 2 kHz. However, the benders are classically fixed rigidly by one of their ends. This causes a strong coupling between the bender and the holding device, hence causing a lowering of the ringing frequency down to near 300 Hz, a frequency lying within the physiological range of the rat whiskers motion. In order to displace the ringing frequency well above that range, we devised a method to uncouple the bender from the structure that holds it (Fig. 6C): We separated the bender from the holder by a layer of elastomer (Sorbothane, 5 mm thick), a material that efficiently transforms high frequency mechanical vibrations into heat. The static stiffness of this material allows the bender to be held in place, and the mechanical energy absorption allows efficient uncoupling of both parts of the device at higher motion frequencies. In addition, an inertia body (a 20 g piece of lead) was also placed between the bender and the elastomer layer in order to ensure rigid holding of the bender at higher frequencies. The uncoupling of the bender from the holder pushed back the ringing frequency on the edge of the kHz range (see Section 3).

The compound bender/elastomer/lead piece was connected to the lever arm through a ball joint and the lever arm was mounted on a one-axis translation stage (Figs. 3 and 6B). These two elements
help respectively to obtain the correct angle of the bender and to approach the bender to the whisker. To apply a correction to the voltage input based on the inverse of the transfer function of the stimulator (see e.g. Maravall et al., 2007; the software is described below) the transfer function of each part should be stable, and this in spite of the adjustments that are necessary to connect the stimulator to each of the 24 whiskers. We designed a particular ball joint and we fine tuned the translation stage so that both display a stable transfer function over their whole range of adjustment. The bead of the ball joint that allows the rotation of the bender is locked by the pressure exerted on it by a spring independently of its angle (Fig. 6B). In addition, changes in the axial position of the one-axis translation stage induced changes in the transfer function of the bender. We corrected this by carefully equalizing the pressure exerted on the stage over the whole translation range. The mechanical structure of the bender we presented so far showed a stable and ringing free transfer function on the kHz range. An additional correction was made though to linearize its behavior by using a software that performs a filtering of the input commands of the bender by the inverse of its transfer function (see Maravall et al., 2007 for an example of this procedure in the range of 0–200Hz) (Fig. 7A). The software was implemented using Elphy (in-house development, Gerard Sadoc) and the Python programming language. The program code and implementation procedure are given as supplementary material (code.py and Supplementary Figure 2). To compute the transfer function of the piezoelectric bender, a pure sinus-based approach was used. A sweep made of a series of sinus of increasing frequency was applied to the piezoelectric bender and the resulting motion was measured using a

Fig. 6. Second generation stimulator. (A) Global lateral view of the device. The tilted rack is an adaptation to fit the stimulator under a two-photon microscope. (B) Schematic drawing of a lever arm. A small steel tube (1) is glued on a 2D piezoelectric bender (2) which is held on a heavy weight (3). A sorbothane layer (4) separates this structure from a ball joint (5). The joint can be unclamped by remotely removing (6) the pressure exerted by a spring on the ball (7). Other mechanical structures are similar to the first generation stimulator.

Fig. 7. Characterization of the second generation stimulator. (A1) Schematic drawing of the two deflection axes of the piezoelectric bender. (A2) Test of independence of the two axes. Input command applied to the horizontal (top) and to the vertical axis (middle). Bottom trace: difference between the motion of the bender in the horizontal plane in response to the vertical axis input command only and to both inputs together. (A3) Scheme of the principle of the software correction performed on the bender. For each axis, the transfer function of the bender is computed and its inverse is applied to the input commands. (B) Gain (left) and dephasing (right) transfer functions for the horizontal axis of the bender under three conditions. (B1) The sorbothane uncoupling is bypassed by a set of metallic bridges that connect together the heavy weight with the ball joint. No software correction is applied. (B2) The sorbothane uncoupling is in place but no software correction is applied. (B3) Both software and hardware corrections are applied.
PSD/laser displacement sensor over the range 0–1000 Hz. The gain and the dephasing between the command and the motion were computed over this range. To obtain the corrected version of a given bender input command from the computed gain and dephasing values, the input command, previously converted into the Fourier domain, was pre-compensated at each frequency by subtracting the computed dephasing from the phase of the input command, and by dividing its amplitude by the computed gain (see Fig. 7A3). This procedure was computed twice, for the x and the y axes of movement of the multi-directional bender. Fig. 7A2 shows that a movement made along one axis does not interfere with the movement on the other axis. Both, the mechanical uncoupling and the software correction were necessary to linearize the behavior of the bender as shown by the following tests. The gain (left) and dephasing (right) transfer functions were calculated when the sorbothane uncoupling was bypassed by a set of metallic bridges that connected together the heavy weight with the ball joint and no software correction was applied (Fig. 7B1), with the sorbothane uncoupling but without software correction (Fig. 7B2), and with both software and hardware corrections applied (Fig. 7B3).

2.5. Driving electronics for the multiple-whisker stimulator

Piezoelectric benders require electrical power/current for dynamic applications. Each bender was driven by a fixed voltage and a variable voltage for each of the two axes. The command signals aimed at each of the 24 × 2 axes were delivered by two 32 channels DAC cards (PD2-AO-32/16, UEI, MA) driven by custom made software (Elphy, G. Sadoc, UNIC-CNRS) running on a PC. A detailed diagram of the electronics can be found in Supplementary Figure 3. An amplification stage was designed to condition properly the voltage commands issued by the DAC. This stage translated the DAC output (±10 V) into a high amperage (maximum 100 mA), ±30 V driving signal that was fed into the piezoelectric bender. We made sure during the design of this amplification stage that it did not significantly low pass filter the command up to 1 kHz. To verify the adequate deflection of the benders during operation we added two signal assessment systems at the amplification stage.

First, each of the 24 amplification line outputs was branched into a secondary amplification stage driving a LED proportionally to the measured voltage. This allowed the detection of hardware failures in the primary amplification stage. It also helped to visually verify the appropriateness between the expected pattern of stimulation and the actual command being fed into the benders. Second, we added to each amplification line a current measurement module, so we could measure the current that was actually flowing through the capacitive benders when applying a voltage command. By this mean, we could ensure that the current flow caused by a voltage command followed closely the “perfect capacitor” electric characteristic expected from well working piezoelectric benders (higher current or no current would mean a broken bender). To implement this current measurement module, we connected serially a resistor downstream the primary amplification stage. We measured the resistor voltage with the ADC circuit of a low cost Arduino board (Arduino Diecimila) after appropriate signal conditioning. By multiplexing the ADC input, we could perform all current flow measurements with a single command fed into a single Arduino board.

2.6. Animal preparation

Male Wistar albino rats were used. Experiments were performed in conformity with French (JO 87-848) and European legislation (86/609/CEE) on animal experimentation. Rats were anesthetized with urethane (1.5 g/kg, i.p.). Atropine methyl nitrate (0.3 mg/kg, i.m.) was injected to reduce respiratory secretions. The heart rate and the electrocorticogram (ECoG) were monitored throughout the experiment. Anesthesia was maintained at stage III-3 through online analysis of the frequency content of the ECoG, of the heart and breathing rates, and the control of reflexive movements (Friedberg et al., 1999). Supplementary doses of urethane (0.15 g/kg, i.p.) were administered when necessary. Body temperature was maintained at 37 °C. The animal was placed in a stereotaxic frame, and the snout was held by a modified head holder (Haidarliu, 1996) allowing free access to the right vibrissae. The whisker stimulator was then connected to the 24 more caudal whiskers. The left postero-medial barrel sub-field (P0-4, L4-8 from Bregma) was exposed. Once the electrode had been positioned on the cortex, the craniotomy was covered with a silicon elastomer (Kwik-Cast, WPI).

2.7. Electrophysiological recordings

Neural activity was recorded extracellularly with tungsten electrodes (FHC, 2–10 MΩ at 1 kHz) vertically lowered in the barrel cortex. Signals were amplified (gain 5000) and filtered (0.3–3 kHz) for spike activity. For each recording site, up to three single units were isolated using a template-matching spike sorter (MSD, Alpha-Omega, Israel). For the control of electrical artifacts that could be elicited by the piezoelectric benders (see Fig. 9), the multi-unit activity was discriminated with a threshold at three standard deviations above noise.

3. Results

3.1. Input/output functions of whisker loaded piezoelectric benders

Trapezoidal deflections of the vibrissae with 10 ms rising and falling ramps were used to quantify the response characteristics of the deflection system once it was connected to the whiskers. The input voltage waveforms and the resulting movement waveform measured with the CDD/laser displacement sensor were compared. The movements of the whisker were measured as close as possible to the plastic tip in which the whisker is inserted. Fig. 8 shows the fidelity with which the voltage command injected into the bender is reproduced by the stimulator and by the connected whisker. We found that a small time lag occurred between the onset of the command and the beginning of the motion of the stimulator. This time lag was 1 ms long for the first generation, and 0.4 ms for the second generation stimulator. We hypothesize that these two different lags are due to the different capacitor characteristics of the two piezoelectric benders used in the two bender generations, combined with the capacitor that was added to the first generation electronic driver.

Since the plastic tubes where the whiskers were inserted are slightly larger than the diameter of the whiskers, one could expect a delay to occur between the motion of the piezoelectric bender and the motion of the whisker because of the separation between the tube and the whisker inside it. However, because of the natural curvature of the whiskers, they were always in contact with the internal wall of the tips and thus they were entrained immediately by the bender. To confirm this, we have recorded (10 kHz sampling rate) the motion of the bender and of the whisker (C2 whisker was used for this measurement) in identical conditions, and we have found that the highest correlation between these two recordings was obtained for a zero delay between the bender and the whisker recordings. If desired, the whisker could have been sealed within the plastic tip with wax. However, following this experiment, we choose not to do so since the input signal and the measured resulting movement presented no visible time delay. In the second, more recent version of the stimulator, the plastic tip was replaced.
by a metallic tube and the same quantitative observations were made.

3.2. Absence of electrical interference with electrophysiological recordings

Single barrel cortex neurons were recorded using extracellular electrodes in urethane-anesthetized adult rats while whiskers were stimulated. For each animal less than 30 min were required to adjust the device to the whisker pad and to connect all 24 whiskers to the piezoelectric benders. Once connected to the whiskers, the 24 benders were relatively close to the site of electrophysiological recordings and their activation could result in an inductive electromagnetic interference. Experiments to control for the absence of electrical artifacts were conducted and the results are presented in Fig. 9. The recording electrodes were not shielded and the stimulator was grounded to the general ground of the preparation. Electrophysiological data were collected from a multi-unit recording in infragranular layers of the barrel cortex while sparse noise stimulation was applied (Fig. 9A). The peri-stimulus time histograms (PSTHs) in Fig. 9 were constructed from events detected by a threshold discriminator at 3 SD above the noise. Identical stimuli were repeated a second time after the stimulator had been removed and the benders, disconnected from the whiskers, were placed in the air just behind them (Fig. 9B). Neuronal responses were recorded exclusively when the stimulator was connected to the whiskers and no induced voltage peaks (i.e. electrical artifacts) occurred during deflections of the benders in the air.

3.3. Wide range of stimulation protocols can be produced by the new stimulator

The purpose of this section is to demonstrate the flexibility and power of the new whisker stimulation device. The new stimulator allows the application of complex spatio-temporal sequences of whisker deflections with great reproducibility and with a very wide range of parameters. In particular, precisely controlled deflections of the whiskers that mimic natural stimuli can be applied.

3.3.1. Linear receptive field obtained with sparse noise stimulation

Benders were driven with voltage pulses of 30 ms duration (with 10 ms plateau) to produce oscillation-free rostro-caudal deflections of 114 μm at 7 mm from the follicle, with an initial velocity of 93°/s. Sparse noise stimulation consisted in stimulating consecutively every whisker in a random order with a 50 ms delay both in the rostro-caudal and caudo-rostral direction. At least 120 random sequences were applied for a total duration of 5–10 min. We built the spatio-temporal receptive fields (STRFs) online using forward correlation methods (Bringuier et al., 1999). The STRF makes a good approximation of the linear receptive field of the neuron. The magnitude of the STRF was calculated by integrating the response to whisker stimulations between 10 and 60 ms after the stimulation onset and plotted it in a color map (Fig. 10).

3.3.2. Reproducing natural time series of whisker deflections

One of the major advantages of the second generation new stimulator is that, in addition to the performances described previously, it allows the faithful reproduction of natural whisker deflections with low variability between trials and with spectral content over a wide range including high frequencies. Fig. 11 shows the reproduction of a natural stimulus having a spectral content that could have not been reproduced by the first version of the stimulator. To record natural time series of whisker deflections, we used a high-speed camera (2500 Hz, Fastcam APX RS, Photron CA, USA) with a spatial resolution of 13 μm per pixel. The camera was placed above the animal's head and a backlight system made of white light LEDs (Phlox, France) illuminated the
whiskers from below. The camera was pointed to whisker C2 of an anaesthetized animal while a motorized rail (Linear motor MLL302, Systro GmbH, Germany) moved a texture (a bar code pattern with a period of 1 mm) across the whisker tip at a constant speed of 60 mm/s and fixed radial distance (90% of total whisker length). We tracked whisker C2 at 2 mm from the follicle by computing the center of mass of squared intensities (pixel value) frame by frame (final spatial resolution of 5 μm). As shown in Fig. 11, under these conditions the whisker at its base showed resonance behavior as well as stick-and-slip events (see e.g. Ritt et al., 2008; Lottem and Azouz, 2009). We then reproduced these movements with the new stimulator by translating them into a voltage waveform input to the bender. When the bender was not corrected, a condition produced by short circuiting the Sorbothane with three metal bridges, the output showed clear periods of resonance vibrations (see arrows in Fig. 11B). We computed the power spectral density (PSD) of the command and of the motion recordings in all three conditions, and we found that the fully corrected stimulator displayed a very similar PSD as the original signal up to 1 kHz, while the uncorrected version departed from the original PSD mainly at the high frequencies. The ‘bridged’ version of the stimulator displayed a major ringing peak starting at 600 Hz. Finally, we observed that the closer the bender to the pad skin, the better the replaying and reproducibility of the movement, probably due to stronger constraint on the whiskers (data not shown).

In conclusion, the combined software and mechanical corrections (see Section 2 for details) provides optimal replaying of natural stimuli, that is waveforms that are particularly demand-
ing for the stimulator. With these corrections a wide avenue of empirical possibilities is opened up by the Matrix.

4. Discussion

The whisker to barrel cortex system became in the last decades an important model for the study of sensory processing. However, one of the inherent difficulties in stimulating a tactile system is that contrary to audition or vision, the sensory organ has to be physically contacted by the stimulating device. Several methods have been used to deflect whiskers. Manual deflections are easy to produce even on awake animals, but are not subject to any control and then cannot be used in a reproducible manner (Brecht and Sakmann, 2002). Using a computer-controlled device allows to stimulate the whiskers with a time-accurate onset. Individual whiskers have been traditionally stimulated using a galvanometer (Stephen, 1969; Nicolelis and Fansenlow, 2002) and eventually moving magnet motors (Rajan et al., 2006), although with those methods whiskers are stimulated once at a time. More recent systems have been developed and applied to awake animals. Air puffs have been used for stimulating whiskers in head-restrained preparations (Hutson and Masterton, 1986; Nunez et al., 1994) but they unavoidably deflect several whiskers at a time. More recently a method for whisker deflection on freely moving rats was developed by attaching a small iron particle to one whisker and by delivering a brief magnetic pulse (1–5 ms) through an electromagnetic coil (Melzer et al., 1985; Ferezou et al., 2006). In both cases however the trajectory of whisker deflections is not controlled and not precisely known. In addition, the magnetic field affects simultaneously all the whiskers having a metallic attachment and thus is not suitable for studies where spatio-temporal multi-whisker stimulations are needed.

It is generally accepted that the cortical responses are strongly modulated when two or more whiskers are deflected simultaneously or slightly out of phase (Brumberg et al., 1996; Carvell and Simons, 1988; Drew and Feldman, 2007; Ego-Stengel et al., 2005; Erchova et al., 2003; Ghazanfar and Nicolelis, 1997; Ghazanfar et al., 2000; Higley and Contreras, 2003; Kleinfeld and Delaney, 1996; Mirabella et al., 2001; Shimegi et al., 1999; Simons, 1985). Similarly, many vibrisseas are used for solving certain tactile tasks (Carvell and Simons, 1990; Sachdev et al., 2001; Krupa et al., 2001). In the past, a 16 whisker stimulator based on miniature solenoids has been used by Krupa et al. (2001). This system has the advantage that the spatial dimension of each solenoid is small and the whiskers can be held at their resting position. Although the kinetic properties of the movement are reasonably good, complex high frequency kinetics like natural whisker movements have not been tested yet and since only one single direction of stimulation can be applied, it is unlikely that this device can be upgraded to a version providing deflections with multiple directions.

One of the major advantages of our device is that it allows the deflection of 24 whiskers in any spatio-temporal configuration within a large parametric space. Piezoelectric benders have been used in number of studies. Their main advantage is that the amplitude and direction of the movement can continuously vary in a controlled manner. A few other laboratories used them for stimulating up to nine whiskers although the resting position of the whiskers was not maintained (Drew and Feldman, 2007). We showed here that the spatial dimensions of piezoelectric bimorphs can be compatible with the stimulation of a large number of whiskers while holding their natural position at rest. We also present a solution to cope with the ringing problem which was until now precluding the application of the high temporal frequencies (200–1000 Hz) encountered in natural scenes (but see Andermann and Moore, 2008 for modified piezoelectric benders able to apply small movements up to 800 Hz). We applied complex patterns of stimulation defined both at single and at multiple whisker levels. The stimulation varied spatially by changing the whisker identity and/or the amplitude and the direction of whisker deflection. It varied also temporally by controlling the interval of time between the whisker stimulation and/or by defining the kinetics of the deflection of the stimulated whiskers.

It is commonly accepted that the temporal profile of the angular velocity of deflection constitutes a relevant property of the stimulation to elicit cortical responses (Arabzadeh et al., 2005). Protraction velocities during free whisking are around 500–900 °/s whereas retraction velocities reach 1500 °/s (Gao et al., 2001; Grant et al., 2009). During texture discrimination, whisker deflection can reach a few thousands of degrees per second (Carvell and Simons, 1990; Ritt et al., 2008). While non-compensated piezoelectric benders show a limitation to reach those high velocities because of the ringing behavior, our corrected device can be used to deflect whiskers at those velocities relevant for natural tactile exploration by rats. For example, when the stimulator is used at 5 mm from the follicle it can produce a rapid deflection of 180 μm at 800 Hz corresponding to a velocity of 1650 °/s.

4.1. Limitation of the stimulation device in studying responses in the awake animal

As we have shown here, the new stimulation device can be used in an anesthetized preparation. The connection of all 24 whiskers to the benders takes less than half an hour which is a reasonable period of time for an acute electrophysiological experiment. It is conceivable to use this stimulator in head-posted awake animals. However this probably would need to condition the animal to stay calm and not to whisk. One disadvantage of the system described here is that it is out of its possibilities to be used in head-posted animals solving a tactile task that needs whisking. Although this must be recognized as a limitation in the utility of this system, there are a number of observations that show that passive and active whisker deflections induce similar responses in the barrel cortex, making it pertinent to study the response of the system to passive whisker deflections. First, the exploratory strategies used by rats to discriminate objects are still not fully known. While texture discrimination tasks depend on whisking (see e.g. Guic-Robles et al., 1989; Carvell and Simons, 1990) some tactile tasks do not seem to depend on active whisking to be solved. For example, aperture or distance discrimination can be performed at very high levels of success with whisker contacts induced by head movements without whisking (Krupa et al., 2001). Hence, cortical processing of sensory information can be studied in conditions in which the whiskers are deflected passively like when using whisker stimulators in anesthetized animals. Second, voltage-sensitive dyes imaging of active sensory processing in awake, freely moving animals show that sensory responses to active touch are similar to those evoked by a passive stimulus in anesthetized and in quiescent awake animals (Ferezou et al., 2006). Finally, recent experimental data (Lottem and Azouz, 2009) showed that whisker contacts produced by a passive exposure to a texture or by active whisking produce similar responses in the trigeminal ganglia.

In conclusion, we have presented a novel stimulator for deflecting macrovibrissae individually or conjointly, in a controlled manner and with high fidelity. With this device we have shown that it is possible to probe the whisker somatosensory system with a range of spatio-temporal patterns of deflections having temporal frequencies encompassing those produced naturally by the exploring animal. Our stimulation device can be used to evaluate in a rapid manner the properties of spatio-temporal receptive fields of somatosensory neurons using sparse noise stimulation. It can also consistently reproduce high dimension tactile scenes inspired
from natural multi-whisker stimulations and therefore enlarge the possibilities for studying the complex interactions acting between sensory neurons receiving inputs from distinct whiskers.

Acknowledgements

We thank Yves Boubenc for providing the high-speed camera data. This work was supported by ANR (07-NEURO-025-01, NAT-ACS) and FACETS (EC FP6-015879).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jneuneth.2010.03.020.

References


