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Lateralization of responses to vibrissal stimulation: Connectivity and information integration in the rat sensory-motor cortex assessed with fMRI

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ABSTRACT

Rats move their whiskers or vibrissae to gain sensory information about the world surrounding them. A single whisker can work as an independent detector but normal whisking involves the use of several vibrissae in a bilateral fashion. Here we used blood oxygen level dependent (BOLD) contrast to acquire functional magnetic resonance images (fMRI) of the rat brain activity during uni- and bilateral whisker stimulations with different timing schemes under Isoflurane anesthesia. Experiments were performed to assess the integration of bilateral information produced by normal whisking behavior. First, we showed that it was possible to obtain BOLD whisker activations using Isoflurane harmless for the animals and thus allowing for future repetitive/longitudinal studies. Second, we obtained different BOLD activation patterns depending on the number of stimulated whiskers and timing of the stimulation scheme. Third, we found lateralization of BOLD activations in the somatosensory-motor cortex. It manifested itself in considerably larger activations in the right hemisphere during equal bilateral whisker stimulation. Fourth, we found Granger Causality Analysis (GCA) to be a useful tool in information integration analysis, as it reproduced the stimulus specific Cross-correlation Analysis results. Both analyses showed that the amount of whiskers stimulated and the timing of stimulation lead to specific dynamic connectivity patterns. Finally, by adding directionality information GCA revealed meaningful lateralization of information processing in the rat whisker system consistent with the observed BOLD activation patterns.

Introduction

"Barrels" in the cortex were first described by Woolsey and Van der Loos in 1969. There is a well-established one-to-one relationship between every whisker on a rodent's snout and each one of the barrels in the contralateral primary and secondary somatosensory cortex (Brumberg et al., 1996). Each of these barrels can be considered to work as an independent detecting unit, making this an ideal model to study sensory signal integration when one or more whiskers are stimulated.

Rodents, i.e. rats, regularly use all whiskers to explore their environment. They move them in a bilateral, symmetric, back-and-forth fashion with frequencies ranging between 1 and 12 Hz (Carvell and Simons, 1990; Fee et al., 1997; Gao et al., 2001; Welker et al., 1964). Shuler and colleagues (Shuler et al., 2002) showed that some discrimination tasks could not be performed with a set of whiskers only on a single side. Even if they move their whiskers on the two pads synchronously, there is a degree of asymmetric motion to accommodate for rotational head velocity (Towal and Hartmann, 2006). Asymmetric whisking has also been reported to occur during object contact (Sachdev et al., 2003). The neurobiological mechanisms used to process information from a single whisker might differ from those during stimulation of several whiskers. E.g. Mirabella and colleagues (Mirabella et al., 2001) showed that the total cortical response of several whiskers is less than the summation of the signals from individual whisker stimulations. Consequently, in order to fully describe the processing of information integration in the rodent whisking model, one should not limit studies to a single whisker (SW) moving on one side of the snout. Instead, multi-whisker (MW) stimulations in a bi- and unilateral as well as asynchronous fashion appear to be prerequisite. Bi- and unilateral whisking of rats could be considered analogous to bi- and unimanual activities in humans. Human experiments have shown that handedness can be a source of anatomical (Bergvall et al., 1986; Kertesz et al., 1986) and functional (Jancke et al., 1998; Kim et al., 1993) differences and consequently of lateralization of information processing in the brain. Rats are right handed animals in

Abbreviations: Iso, Isoflurane; CCA, Cross-correlation analysis; GCA, Granger causality analysis; MWt, Mann–Whitney tests; KW, Kruskal–Wallis tests by ranks; Pcc, Pearson cross-correlation tests.

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73% of cases (Guven et al., 2003). Accordingly, in this study we investigated if lateralization of vibrissal information processing exists in the rat brain.

Historically, electrophysiology has been the golden standard used to study processing of vibrissal information: responses to changes in stimulation frequency, amplitude, acceleration, etc. (Gibson, 1987; Simons, 1978). Other techniques have also been implicated in describing this sensory system, e.g. intrinsic signal imaging, (Devor et al., 2005) or voltage sensitive dye-based imaging (Petersen et al., 2003). However, they all present fundamental limitations due to the fact that they can just cover a specific area of the brain/cortex and/or are invasive. If there is an interest in studying the integration of information from whisker signals we need images that cover the whole of the brain, moreover both hemispheres. fMRI together with its BOLD contrast (Ogawa et al., 1990), offers an approach for depth independent imaging of the whisker system covering the whole brain in a short period of time and in a non-invasive and recoverable way. fMRI was already used by Yang et al. to image individual whiskers' responses (Yang et al., 1996) and was already combined with optical imaging (Horikawa et al., 2001) establishing the validity of this model. Since then a large body of studies has appeared using fMRI and this model (Alonso Bde et al., 2008; Hewson-Stoate et al., 2005; Lu et al., 2003; Sachdev et al., 2003; Sheth et al., 2004).

Understanding the integration of brain activity necessitates knowing how separate activated structures specifically interact for a given task. Such an interaction can be assessed by functional connectivity analysis (Friston et al., 1993; McIntosh et al., 1994) investigating the correlation between fMRI activity in different brain regions during task performance. A well-established, basic technique is the cross-correlation analysis (CCA). This technique addresses the interaction in terms of correlation between the temporal evolution of the signals in (two) different regions. This correlation approach has been used in the past to study fMRI activations (Cauda et al., 2009; Honey et al., 2007; Hui et al., 2009) and is the basis of "functional correlation" as defined by Friston et al. (1993). Nevertheless care has to be taken in drawing conclusions from correlation analyses alone as the direction of the information flow, i.e. causal relations, cannot be obtained. In contrast to CCA, Granger causality analysis (GCA), well established for electrophysiological research (Bernasconi and Konig, 1999), provides information of causality i.e. including the direction of connectivity. It can provide directionality of the interaction working without any apriori assumption of the underlying Granger model (Granger, 1969). It is based on the idea that if the evolution of a time series can be predicted based on the previous time series of a different region, then the second region "granger causes" the first. This analysis has been successfully used in the past to assess connectivity between fMRI data (Abler et al., 2006; Deshpande et al., 2008, 2009; Roebroeck et al., 2005; Stilla et al., 2007).

In this study we used BOLD fMRI to image the activation produced by different bi- and unilateral MW and SW stimulations in the brain as a whole. We then used CCA and GCA analysis to discuss how the obtained activations were integrated in somatosensory and motor cortices. To our knowledge such a study of lateralization and information integration in the bilateral whisking model has never been performed with fMRI before.

Methods

Animal preparation

All experiments were approved by a local ethics committee. A total of 10 mature rats (Sprague–Dawley, 350 to 450 g), were used for experiments. Anesthesia was induced with 5% Isoflurane (Iso). Immediately after, rat whiskers were trimmed on both sides of the snout. (For details see the section: Trimming and Stimulation Protocols). Rats were then mounted on a Plexiglas cradle in a way which allowed the whiskers to move freely (Fig. 1A) and placed inside the scanner. Iso anesthesia and monitoring of physiological functions as previously described (Hess et al., 2007; Knabl et al., 2008) continued throughout the whole experiment.

During the experiments Iso was kept at values between 1.1% and 1.3%. This resulted in a stable average breathing rate of 65 ± 5 respirations per minute (rpm) on all animals as recorded by a pressure sensor (Smiths Medical PM, Inc. USA). Respiration rates of 65 rpm led to a constant blood pCO_2 level of 38 mmHg $\pm$ 10% over a period of 5-6 h as shown with a complete physiological monitoring setup in previous *in-vivo* tests in our group (Hess et al., 2007, compare to Ramos-Cabrer et al. (2005)). As experiments were intended to be as physiological and non-invasive as possible, no cannulations or intubations were performed.

No fatalities or unusual behavior were observed in any of the animals on the following days.

Trimming and stimulation protocols

We performed two fMRI experiments on each animal with two different trimming protocols on different days. For trimming protocol 1 we cut all the whiskers of the A row together with outliers and short whiskers of all rows (A to E). This trimming left 16 whiskers on each side of the snout for the following Multi-whisker stimulation (MW) (Fig. 1C). Protocol 2 cut all the whiskers except for the C1 on both sides of snout for the Single whisker stimulation (SW) (Fig. 1D). Protocol 1 always preceded protocol 2 and 10 days were left between the experiments.

Whiskers were stimulated with a pneumatically-driven device integrated into the holding cradle (Fig. 1A). Two inverted combs situated 2 cm from both sides of the snout allowed for uni- and/or bilateral stimulations at frequencies ranging between 0 and 10 Hz. The

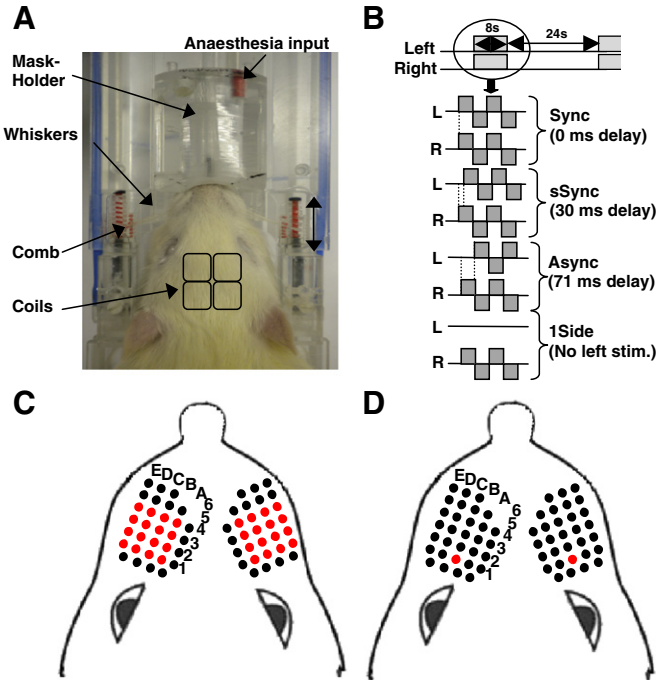

Fig. 1. Experimental setup and whisker trimming protocols. Fig. 1A shows an image of the experimental setup for bilateral whisker stimulation. Fig. 1B shows a scheme of the event-related blocks used to stimulate rats in our experiments. A schematic of each of the four stimuli (Sync, Async, sSync and 1Side) is presented. Fig. 1C shows trimming protocol 1 (MW). Cut whiskers are represented with black circles while those untouched are highlighted as red circles. Fig. 1D shows in equal manner trimming protocol 2 (SW).