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

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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 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.

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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 bilateral 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 opposite way.
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% Isofluorane (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 pCO2 level of 38 mmHg ± 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 the 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
stimulation was performed in the rostro-caudal direction along the side of the rat's head. Amplitude of the comb motion was 10 mm with a moving frequency of 7 Hz. The space between comb teeth was large enough to leave some flexibility for the whiskers to move but not to get free, avoiding painful pulling stimuli. Combs were driven from an external console triggered by the scanner and running a custom-programmed user interface developed in LabView (LabView, National Instruments, Austin, USA).

During fMRI experiments (54 min) 100 stimulation periods were carried out each consisting of 8 s stimulation followed by 24 s rest. During the experiment four different stimuli were enacted. A schematic of the stimulation paradigms can be seen in Fig. 1B: A synchronous stimulation (Sync), in which both sides were stimulated at the same time, an asynchronous stimulation (Async) where the left side was stimulated in counter-phase with right side (inter stimuli interval (ISI) of 71 ms), a single side stimulation (1Side) where just the right side was stimulated, and finally a slightly symmetric stimulation (±Sync) in which the left side was stimulated using an ISI of 30 ms. For both Async and ±Sync the delays were implemented on the left-hand comb.

MRI acquisition and data analysis

fMRI experiments were performed on a 4.7 T BRUKER Biospec scanner, horizontal magnet (200mT/m, 40 cm free bore) equipped with an actively RF-decoupled coil system. A whole-body birdcage resonator enabled homogenous excitation and a 4-channel rat brain surface array coil (Bruker Biospin, Germany) was used to receive the signal.

All experiments followed the same order of events: standard scanner setup, a motion control scan as previously described (Hess et al., 2007; Knabl et al., 2008) followed by the fMRI experiment and finally anatomical image acquisition. fMRI data was acquired with a Gradient-Echo Echo Planar imaging sequence (GE-EPI): 2 excitations, TEeff=24.4 ms, TR=4 s, in-plane resolution 390×390 μm, 1 mm slice thickness, matrix 64×64, FOV of 25×25 mm. A total of 22 axial slices were obtained covering the brain from Bregma 6.12 to −15.72 mm, and slice 14 was positioned at Bregma −3.48 mm according to Paxinos Atlas (Paxinos, 2007) using the smallest anterior-posterior distance between the posterior tip of the corpus striatum and the anterior tip of the hippocampus on the horizontal anatomical reference image as reference. Eight baseline volumes (32 s) were acquired previous to the stimulation in order to saturate the MRI signal and avoid effects of sudden noise reactions on the animal as the scanner started. These volumes were then discarded from analysis.

Anatomical data was obtained on the same 22 slices with a RARE sequence: TE = 51 ms, TR = 3000 ms, in-plane resolution 97×97 μm, 0.5 mm thickness, matrix 256×256, FOV of 25×25 mm.

Functional data analysis was performed using BrainVoyager QX V1.10 (Brain Innovation B.V. Netherlands). Motion correction was performed by the trilinear interpolation algorithm. Slice time correction was performed with a Cubic Spline. A 2D Gaussian smoothing of the data (2 pixel kernel FWHM) was performed in the in-plane direction. In this way data fulfilled the statistical General Linear Model (GLM) conditions where data approximates a random Gaussian field (Worsley and Friston, 1995). Finally, high pass temporal filtering (9 cycles) of the data was performed to remove slow drifts in the MR signal due to physiological or scanner noise.

Single subject GLM analysis was performed using BrainVoyager. Stimulus specific contrasts were calculated convolving the model with a two gamma variate hemodynamic response function. BrainVoyager FDR corrected maps (Z-score maps, one per contrast) were thresholded at p > 0.05 which is at Z > 1.96, and a minimum of four activated voxels together was considered as a positive finding. The individual contrast specific Z-score maps were registered using a 9 degree affine transformation driven by the individually-segmented brain masks. For quantitative single case analysis, groups of the significantly-activated voxels were identified as belonging to certain brain structures by overlaying a digital atlas derived from the Paxinos rat brain atlas (Paxinos, 2007) which contained, among others, the most essential structures for the whisker processing sensory-motor system. Finally in MagnAn, the size of the activated volumes and the BOLD signal amplitude for all stimuli were calculated for each brain structure.

Cross-correlation analysis (CCA) was performed on the time profiles of the significantly activated voxels of the brain structures of the somatosensory and motor system as defined by our GLM analysis (see Fig. 2B). Pearson cross-correlation (Pcc) coefficients were calculated between all pairs of regions and thresholded at p < 0.05. Granger Causality Analysis (GCA) was performed on an IDL software package developed in our group which was validated against Seth toolbox (Seth, 2010). A multivariate vector autoregressive model was used to detect directional influences between the different brain structures (Kus et al., 2004). Connections and their strengths were derived from the direct Directed Transfer Function (dDTF) as introduced by Deshpande et al. (2009). This matrix includes partial coherence calculations that eliminate mediated influences. All the calculations used a lag time of 1 volume (4 s) and all resulting values were thresholded to p < 0.001.

Finally, group average activation patterns of the registered Z-score maps were visualized on the background of the anatomical images.

Statistics

Group maps were only used for display purposes while all the statistical values were obtained from single animal data. The data was non-parametric. Therefore, statistical tests comparing two populations were done with Mann–Whitney tests (MWt), and comparisons between multiple populations were done with Kruskal–Wallis (KW). Bonferroni tests were applied to our results if multiple comparisons were performed. All error bars in graphics represent square error of the mean (SEM). All statistical analyses were performed with Sigma Plot software (Systat Software Inc. USA).

Lateralization

In order to assess differences in hemispherical information processing we used a lateralization index (Li) calculated per single subject and region according to:

\[
\text{Lateralization Index} = \frac{\text{Left Activation} - \text{Right Activation}}{\text{Left Activation} + \text{Right Activation}} \times 100
\]

Results

Different activation patterns depending on the type of stimulation

Fig. 2 presents group Z-score maps for different MW and SW stimulations.

For MW-Sync stimulations, activation was found −2.5 mm from Bregma bilaterally in primary (SI) and secondary (SII) somatosensory cortex (Fig. 2A). These activations were of similar size in both hemispheres. This aggregation contained some dorsal sections of motor cortex and the retrosplenial cortex. The axial slice through motor cortex (2.5 mm frontal to Bregma) showed bilateral activations of motor areas together with a strong activation in the cingulate and prelimbic cortex. Smaller bilateral activation was also found in somatosensory jaw areas (SII), upper lip perception (SIULP) and in the insula. Bilateral activations in sub-cortical structures like the caudate and putamen were also significant (cf. Fig. 2B). Async and ±Sync for MW stimulation produced similar activation patterns compared to the Sync case. As can be visually appreciated (and will be quantified later,
Fig. 3C–E) there was a trend for larger volumes in right hemisphere somatosensory structures compared to their left counterparts. Finally, the 1Side stimulation (right) produced activations in the left SI and SII, contralateral to the stimulation. No major activations were found in the right somatosensory cortex, ipsilateral to stimulation. The motor cortex slice showed activations also mainly in the left, contralateral cingulate, primary (MI) and secondary (MII) motor cortex and SIj for this stimulation. A small bilateral activation in the caudate putamen was found, being on the right side smaller than on the left.

SW activations showed similar trends to MW stimulations with respect to the activated areas (Fig. 2A) with a trend toward smaller extension (discussed and quantified in Fig. 3C and F).

Quantification of Sync, Async, sSync and 1Side

Z-score maps demonstrated that different stimuli produced different patterns of fMRI activation. To precisely quantify these differences and due to the high response variability between animals, single animals had to be analyzed separately.

Intensity values for BOLD activations in different brain regions are presented as supplementary material (Supplementary 1). These overall small values together with their large SEMs made it impossible to derive conclusive statistical analysis from this data. We therefore decided to focus on the significantly activated volumes.

The total number of voxels activated over the whole brain as a response to a given stimulus is considered first (Fig. 3A).

Even if no significant differences were found (p > 0.7, KW), the data showed a tendency of decreasing overall activation volume from Sync, followed by sSync and Async to the 1Side stimulation. This decrease was best described linearly (R² = 0.956). Table 1 presents numbers of activated voxels in the regions related to whisker activity.

These areas were: SI, SII, MI, MII, cingulate (Cg), putamen (Put); insula (Ins) and somatosensory jaw area (SIj).

The lateralization of BOLD responses

Group activation maps showed a tendency for the right hemisphere to show larger activations compared to left (cf. sSync and Async in Fig. 2A). We studied these effects in more detail using the lateralization index (see Methods and Fig. 3C–E). For MW stimulations right hemisphere
structures showed significantly larger responses (−7.3% and −9.4% Li.) then the left hemisphere when performing sSync and Async stimulations (p < 0.032, p < 0.002, MWt). In contrast no differences were found for the Sync stimulations (p > 0.342, MWt, −3.29% Li.). Results were independent of the number of whiskers stimulated, as the same relation was found for SW stimulation (statistical data not shown). 1Side experiments, when the whiskers of the right side of the snout were stimulated, produced a Li. favorable to left structures in the MW and SW experiments as expected (p < 0.003% p < 0.015, MWt, 24% and 12% Li).

Fig. 3D depicts lateralization effects in SI and SII and Fig. 3E in MI and MII. The Sync stimulation showed no differences for any of the four regions (p > 0.4, MWt in all cases). In contrast, sSync and Async stimulations had lateralization effects (right side BOLD activations being significantly larger) in SI, SII and MII structures (p < 0.05 MWt in all cases). This trend was observed also in MI, although it did not achieve statistical significance (p = 0.3 MWt).

In all the experiments presented in this study, the right side of the snout was stimulated constantly at 7 Hz while the left side was subjected to the delays during the sSync and Async stimulations (see Fig. 1B for stimulation protocol details). A control study was performed on four animals to check if the lateralization effects, i.e. right side dominance, were still present when the stimulation of the right side of the snout was delayed (Fig. 4).

In this case, right side structures still presented larger activated volumes for SI (81±8 vs. 62±7, Li: −13%) and MII (90±11 vs. 72±10, Li: −11%) with statistically significant differences (p < 0.015 & p < 0.021, MWt).

**Dependence of activation on number of whiskers stimulated**

As can be inferred from Fig. 2, MW stimulations produced larger volumes of activation than SW stimulation. Fig. 3F shows the difference in average number of voxels for the whole brain depending on the number of whiskers stimulated. Total numbers of voxels activated by our four stimulations in the MW stimuli paradigm were on average 33% larger than their counterparts in SW stimulations (p < 0.001, MWt).
deviation for four different stimuli (Sync, sSync, Async and 1Side) and for left and right hemispheres.

In particular, activations in SI (Fig. 3G), MI, and MII (Fig. 3H) were larger for MW than for SW stimulation (p < 0.045, p < 0.021 and p < 0.017, MWt, respectively). A non-significant relationship was found for SI (p = 0.978, MWt, Fig. 3G). For other brain structures like cerebellum, cingulum, hypothalamus and VPM significant differences were only found for cerebellum (p < 0.007, MWt). All other structures showed no differences (p > 0.1 MWt).

CCA analysis

A total number of 32 significant correlations between somatosensory and motor structures were found for the four stimuli. As can be seen in Fig. 5A all stimuli yielded significant SI-SII correlations in the left hemisphere while just Async and Sync did this in the right hemisphere.

Asyn stimulion produced the only inter-hemispheric correlation in the somatosensory cortex, where left SII was correlated with the right SI (p < 0.0001, Pcc). In motor cortex (Fig. 5B) the intra-hemispheric correlations between MI and MII were present for all stimuli in the left hemisphere, 1Side correlation is missing on the right hemisphere. On top of this, for the Sync stimuli we also observed inter-hemispheric correlations between all motor areas. Finally, large amounts of correlations between SII and motor cortex were found for the Sync (4) and Async (3) stimuli (cf. Fig. 5C), while none was found for the sSync. The 1Side stimulation presented two correlations between left and right hemispheres. The number of correlations between the right somatosensory cortex with any of the motor cortex structures was larger than that of the left somatosensory cortex for bilateral stimuli (10 vs. 3 respectively).

GCA analysis

A total number of 105 significant causal connections between somatosensory and motor structures were found for the four stimuli (connectivity increased with respect to CCA as now the majority of interactions have two directions and consequently count double). The GCA analysis was able to reproduce 24 of the 32 correlations found in the CCA i.e. 75%. As can be seen in Fig. 5D, except for the Async all other stimuli presented SI-SII significant connectivity intra-hemispherically. Compared to Fig. 5A a larger number of inter-hemispheric interactions for the stimulations were found (9 from GCA vs. 1 from CCA). For motor cortices (Fig. 5E) we found 12 connections for the Sync stimuli, 9 for the Async, 8 for the 1Side, and 4 for the sSync. Regarding the somatosensory-motor interaction (Fig. 5F) large amounts of connections were found from the somatosensory cortex (SI and SII on both hemispheres) to the motor cortex for the Sync and Async stimulation (8 connections for each, 3 of 1Side and sSync). For Sync and Async stimuli we found strong feedback from MI and MII back into SI, but no input back to SII. The sSync and 1Side stimuli showed no connections leaving SII.

**Discussion**

**Methods appraisal**

Our experiments needed a large number of repetitions and long scanning times to obtain BOLD whisker activations. This was probably
Cross-correlation analysis (CCA)

Granger Causality analysis (GCA)

Fig. 5. CCA and GCA analysis between BOLD signals in somatosensory and motor cortex. Figs. 5A–C show the results of the CCA analysis. Results are plotted on two Paxinos atlas slices, left slice: barrel somatosensory field (-2.5 mm from Bregma) and right slice: motor field (2.5 mm from Bregma). Overlaid are the significant correlations following a color coding scheme (Red = Sync, Orange = sSync, Blue = Async and Black = 1Side). The L and R indicate the left and right hemisphere. Fig. 5A shows a schematic with significant correlations in the somatosensory cortex. Fig. 5B shows a schematic of correlations in the motor cortex and Fig. 5C shows a schematic with significant correlations between somatosensory and motor cortex. Figs. 5D–F show the results from the GCA in the same manner as the CCA analysis. In Fig. 5F points indicate a connection in all possible directions.

necessary because of the following: whisker stimulation is a weak physiological stimulation that produces in the best case 0.3% BOLD signals at 4.7 T in our setup, and this is small compared to other stimulation paradigms. Nevertheless, Iso provided a baseline value (Maekawa et al., 1986). Nevertheless, Iso provided a better analysis tool as it provided meaningful directionality information that the CCA could not give. Generally, the application of these two techniques to fMRI data are restricted by the limited temporal resolution of fMRI, which is in the order of seconds, and the variability of the hemodynamic response across different brain structures (see e.g. Aguirre et al., 1998). Detailed discussion (see e.g. Deshpande et al., 2010) and corrections for these effects were not in the scope of the current study.

Laterality

Laterality of functions in the brain and handedness have been found to exist not only in humans, but also in animals (cats, dogs, toads, birds etc.). It appears to be biologically reasonable to specialize one of the hemispheres in performance of a particular task, although the flow of information between hemispheres has been shown to be crucial in the performance of bilateral tasks (Geffen et al., 1994; Kennerley et al., 2002).

Human studies (Jancke et al., 1998; Kim et al., 1993) have shown differences between left and right hemisphere activations in right-handed persons. Right hemisphere activations were larger when a uni-manual activation of the left hand was performed. In contrast, equal right hand manipulations produced in the contralateral (left) brain less activation. The authors suggest that these differences were due to the fact that the right hemisphere is less trained and therefore requires a larger amount of neuronal substrate implicated in performing the task, whereas the left hemisphere, as a trained system, needed fewer resources to perform it (entrainment or sharpening of the response). Although this opinion is not unequivocal (see Karni et al., 1995), we argue that the observations in our experiments point to similar conclusions. During bilateral sSync and Async stimulations - physiologically relevant for the animal for object discrimination - the right sensory-motor structures showed considerably larger activations than their left counterparts (Figs. 2 and 4) independent of whether the delays were implemented on the right or on the left stimulating comb. We believe that this finding indicates natural functional asymmetry of the rats’ whisker system, with the right whisker pad (but the left brain hemisphere) being better tuned for discrimination – it shows, so to speak, the “right-whiskeredness” of the rats.

Correlation and Causality

fMRI combined with MEG studies in humans has suggested that information processed in the somatosensory cortex starts with detection
in the SI contralateral to the stimulated side. Next, there is a propagation to SI of the same hemisphere and finally connections are established to the other hemisphere (Disbrow et al., 2001). If we look at Fig. 5A and D, we can see that the majority of the experimental stimuli showed SI-SI connections on the left and right hemispheres. These relations were stable over at least 4 s indicating a prolonged interaction between these areas. Inter-hemispheric connections were sparser but were also found for the four stimuli corroborating previous findings. MI, a higher order motor region in rats (comparable to SMA in humans) showed large amounts of connectivity between motor structures (Fig. 5B and E) and between somatosensory and motor cortex (Fig. 5C and F). These large amounts of interactions indicate that MI has a pivotal role in motor coordination.

Apart from this, GCA revealed remarkable asymmetry in the directionality of the inter-hemispheric connections of the somatosensory cortices (cf. Fig. 5D): the majority of them were directed from right to left. Considering functional asymmetry between the two hemispheres demonstrated by BOLD effect – a lesser activation of the left hemisphere in bilateral sSync and Async paradigms – we did not find this surprising. Several authors show that the net effect of ipsilateral stimulations on the following contralaterally induced SI activations and vice versa is inhibitory (Manns et al., 2004; Shuler et al., 2001). We assume that GCA elucidated both excitatory (intra-hemispheric) and these inhibitory inter-hemispheric relations. Thus, the prevalence of right-to-left directed inhibitory inter-hemispheric connections corroborates our finding of functional asymmetry in the rat brain reflecting lateralization of vibrissal responses. It is plausible that these directed inhibitory influences contribute to a sharpening of the response in the left, more important for the task hemisphere.

In this study we have shown that it is possible to obtain BOLD-fMRI activations in the rat brain during whisker stimulation under mild isoflurane anesthesia. This will allow for future repetitive and longitudinal studies on this useful model of somatosensory and motor cortex (Fig. 5C and F). These large amounts of activations indicate that MI has a pivotal role in motor coordination.

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2012.05.045.

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