Published in final edited form as:

NMR Biomed. 2008 May ; 21(4): 410–416. doi:10.1002/nbm.1259.

Frequency-dependent tactile responses in rat brain by fMRI

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Abstract

We measured frequency-dependent fMRI activations (at 11.7T) in the somatosensory cortex with whisker and forepaw stimuli in the same α -chloralose anesthetized rats. Whisker and forepaw stimuli were attained by computer-controlled pulses of air puffs and electrical currents, respectively. Air puffs deflected (± 2 mm) the chosen whisker(s) in the right snout in the rostral and caudal direction, whereas electrical currents (2 mA amplitude, 0.3 ms duration) stimulated the left forepaw with subcutaneous copper electrodes placed between the second and fourth digits. In the same subject, unimodal stimulation of whisker and forepaw gave rise to significant BOLD signal increases in corresponding contralateral somatosensory areas of whisker barrel field ($S1_{BF}$) and forelimb ($S1_{FL}$), respectively, with no significant spatial overlap between these regions. The BOLD responses in $S1_{BF}$ and $S1_{FL}$ regions were found to be differentially variable with frequency of each stimulus type. In the $S1_{BF}$, a linear increase in the BOLD response was observed with whisker stimulation frequency of up to ~ 12 Hz, beyond which the response seemed to saturate (and/or slightly attenuate) up to the maximum frequency studied (i.e., 30 Hz). In the $S1_{FL}$, the magnitude of the BOLD response was largest at forepaw stimulation frequency between 1.5 and 3 Hz, beyond which the response diminished with little or no activity at frequencies higher than 20 Hz. The volume of tissue activated by each stimulus type followed a similar pattern as the stimulation frequency dependence. These results of bimodal whisker and forepaw stimuli in the same subject may provide a framework to study interactions of different tactile modules, both with fMRI and neurophysiology (i.e., inside and outside the magnet).

Keywords

activation; deactivation; hyperemia; integration; multi-sensory

INTRODUCTION

The somatosensory cortex integrates inputs from different parts of the body into somatotopic maps. Thus reliable models of precisely controlled sensory stimuli provide excellent tools for studies in experimental neuroscience. In recent years, there have been widespread applications

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of rodent fMRI in the neurosciences (1,2). Development of tactile (e.g., forepaw (3,4), whisker (5,6)) and non-tactile (e.g., visual (5,7), olfactory (8,9)) models in rodent fMRI could play a major role in establishing the physiological basis of BOLD contrast (10,11) because these sensory models in physiologically controlled anesthetized subjects provide reproducible functional responses (12,13). Given the recent trend in electrophysiology (14) and optical imaging (15) applications with NMR methods – either simultaneously or concurrently – it is important to provide identical sensory stimuli to rodents, both inside and outside the magnet.

Forepaw stimulation in the rat is a very popular model in many fMRI laboratories (16,17) and can be easily applied identically inside and outside the magnet (18,19). A current impulse is delivered to the forepaw via small subcutaneous electrodes, the pulse amplitude being typically in the range of a few mA and the duration a fraction of a ms. Whisker stimulation is a more difficult model to apply reproducibly inside the magnet primarily because of space constraints. Unfortunately our prior approach for whisker stimulation inside the magnet (20) (i.e., using Lorentz force (21) to move a small conducting copper wire attached to the whiskers) is not easily reproduced outside the magnet. A good alternative is mechanical movement of whiskers (22), but this works poorly inside a magnet because the non-magnetic manipulator must span the entire length of the bore making dexterity quite limited at the site of whisker stimulation.

Here we describe the use of a non-magnetic whisker stimulation device that can be easily used – either simultaneously or concurrently – with almost any other sensory stimuli (e.g., forepaw, olfactory, visual), both inside and outside the magnet. A home-built whisker stimulation device using air puffs was applied in conjunction with electrical forepaw stimulation to measure the tactile responses from the corresponding somatosensory areas of whisker barrel field ($S1_{BF}$) and forelimb ($S1_{FL}$), respectively. The air puff approach (23) can provide deflections with a wide range of amplitudes (e.g., ± 2 mm) and various directions (e.g., rostral-caudal), and in addition this method may be more easy to apply identically both inside and outside the magnet. We describe the frequency-dependent behavior of the BOLD response in $S1_{BF}$ and $S1_{FL}$ regions.

METHODS

Animal preparation

All experiments were conducted on adult male rats ($n = 16$; Sprague-Dawley; 200–300 g; Charles River, Wilmington, MA) which were tracheotomized and artificially ventilated (1–2% halothane during surgery + 70% N_2O /30% O_2). Intraperitoneal line administered α -chloralose (~40 mg/kg/hour; i.p.) and *D*-tubocurarine chloride (~0.3 mg/kg/hour; i.p.). A cannulated femoral artery was used for monitoring systemic parameters (pCO_2 , pO_2 , pH). The abdomen was covered with a heated pad to maintain core temperature at 37 °C.

Sensory stimuli

The design of the naturalistic whisker stimulator is shown in Fig.1A. In most cases ($n = 12$), only the middle three rows of whiskers on the right snout were spared and all other whiskers, including the ones on the left snout, were shaved. The middle three rows were chosen for consistency in thickness and length of the whiskers across subjects and therefore their activation generally is believed to result in comparable columns in $S1_{BF}$ (24–26). In a few cases, ($n = 4$) only the C2 or D2 whisker (on the right snout) was spared and all other whiskers shaved near the level of facial fur. The spared whiskers were trimmed to equal length (~20 mm) and then taped together with a 20×6 mm piece of lightweight masking tape to ensure that the whiskers moved in unison.

Air puffs were used to stimulate the whiskers via the air-induced flutter of the tape. Pulses of compressed air (up to 41.3 kPa or 6 PSI) were delivered in a computer-controlled way by a home-built system using solenoid units (Cole-Parmer Instrument, Vernon Hills, IL). The trigger signals for the solenoid were recorded as the time stamps for stimulus onset. A pair of teflon tubes (2 mm diameter) positioned rostral and caudal (± 2 cm) from the taped whiskers delivered the air puffs. While air puff from one tubing seems sufficient for low frequency stimuli, the repositioning of the whisker with the additional air puff (in the opposite direction) provides for higher frequencies.

The direction of air flow was from rostral to caudal along the whisker row. Under these conditions the air puff stimuli deflected all the taped whiskers by 2 mm (rostral-caudal). After ensuring that the taped whiskers were properly deflected by the air puffs, the rats were moved into the center of the bore of the magnet in the fMRI experiments. Whisker stimuli were tested with varying the frequency (up to 30 Hz) for duration of 30 s and were interleaved with an interstimulus interval (ISI) of 300s (time from end of one stimulus to the start of next). This very long ISI ensured that there were no interaction effects between consecutive stimuli. The duration of the air puff was dependent on the frequency (ν) based on the relationship $\nu = 1/(2t)$, where t (period for movement from rostral to caudal position) varied from 125 ms to 16.7 ms, giving ν of 4 Hz to 30 Hz respectively.

In the same subjects ($n = 16$), forepaw stimulation was achieved by a pair of thin needle copper electrodes inserted under the skin of the left forepaw, between the second and fourth digits as shown in Fig. 1B. The electrical pulse duration was 0.3 ms with amplitude of 2 mA achieved by an isolation unit (WPI, Sarasota, FL).

All stimulus presentation was controlled by a μ 1401 analog-to-digital converter unit (CED, Cambridge, UK) running custom-written script for providing block design (off-on-off) electrical stimuli of 30 s in duration with at least 300 s in between consecutive stimuli, irrespective of modality. The maximum stimulation frequency tested was 30 Hz. The different stimuli were given in a random order to avoid habituation effects.

fMRI studies

All fMRI data were obtained on a modified 11.7T Bruker horizontal-bore spectrometer (Bruker AVANCE, Billerica, MA) using a ^1H surface coil (1.4 cm). All fMRI data were collected with sequential sampling echo planar imaging sequence (27): field of view of $2.56 \times 2.56 \text{ cm}^2$; image matrix of 64×64 ; slice thickness of 2 mm; repetition delay of 1000 ms, and echo time of 13 ms; and voxel size of $400 \times 400 \times 2000 \mu\text{m}^3$. Sixteen dummy scans were carried out before fMRI data acquisition. Neuroanatomy was imaged with either RARE (28) or FLASH (29) contrast.

All fMRI data were subjected to a translational movement criterion using a center-of-mass analysis (30). After masking of non-brain tissues by thresholding each image within a series, the masked raw images were converted into binary maps (i.e., brain vs. background). Removal of image intensity information (i.e., binary maps) assured that the analysis was not biased by stimulation-induced effects. For each binary map in the series two center-of-mass values were calculated, one for each in-plane direction. If either center-of-mass value in a series deviated by more than $1/4$ of a pixel, the entire dataset was discarded from further analysis. Data which did not pass the movement analysis test were not analyzed further. Single run data were used to create activation maps and time courses. Activation foci for $\Delta S/S$ maps were obtained by applying Student's t test comparison of resting and stimulated data. All activation maps were overlaid on the corresponding anatomical images.

RESULTS

Localized BOLD activations in S1_{BF} and S1_{FL}

The maximum BOLD activations in the contralateral cortex during forepaw and whisker stimuli were detected in coronal slices located at ~1 mm anterior and ~2.5 mm posterior, respectively, to bregma. With either stimulation type, there were no stimulus induced variations in systemic physiology and no significant BOLD signal changes were observed in the ipsilateral side during stimulation.

Rostro-caudal whisker movement (8 Hz), caused by air puffs, reproducibly activated the contralateral S1_{BF} (Fig. 1C). Repeated trials in the same subject (Fig. 1C; top row) and across subjects (Fig. 1C; middle row), where the middle three rows of whiskers were stimulated, produced BOLD activations in approximately the same large areas of the contralateral S1_{BF}. BOLD reproducibility was observed even for single whisker (C2) stimulation in the same subject (Fig. 1C; bottom row), however the activated area was much smaller than when many whiskers were stimulated together. Electrical stimulation of the forepaw (3 Hz) induced activity patterns in contralateral S1_{FL} (Fig. 1D). There were no significant differences in the localization of the BOLD response in the contralateral S1_{FL} region with repeated trials in the same subject (Fig. 1D; top row) and across subjects (Fig. 1D; bottom row).

Frequency dependence of BOLD responses

In the same subject, independent stimulation of three rows of whiskers on the right snout (Fig. 2A) and the left forepaw (Fig. 2B) activated contralateral somatosensory areas corresponding to S1_{BF} and S1_{FL}, respectively. For whisker and forepaw stimuli the responses peaked – both in terms of magnitude and area of activation – at 12 Hz (Fig. 2C) and 1.5 Hz (Fig. 2D) respectively where the maxima of the averaged BOLD magnitudes were ~4% and ~12%.

BOLD responses to whisker movement at all the tested frequencies showed a characteristic time course (Fig. 2C) in which the signal increased rapidly after stimulus onset, reached a peak level within 6 s, then decreased slightly to a lower sustained plateau level, and then returned to the baseline after stimulus offset. With forepaw stimulation the BOLD response was sustained for the duration of the stimulation (Fig. 2D) even when the duration was lengthened to several minutes (data not shown). This is most likely a reflection of the nature of forepaw stimuli in which strong and highly synchronous barrages of identical efferent inputs invade the contralateral S1_{FL}, causing consistent activity of cortical neurons. It is unlikely that the forepaw stimulus is out of physiological range, because the systemic physiology (data not shown) was unaffected by the stimulus as observed similarly with whisker stimuli.

To further differentiate between the generally stable BOLD response during forepaw stimulation (Fig. 2D) and declining response during whisker stimulation (Fig. 2C), we compared *t*-maps for the first and last 10 s of stimulation of each type. Furthermore we compared these periods of stimulation with the pre-stimulus and post-stimulus periods to depict differences between these two stimuli, as shown in Fig. 3. While slight activation area variations were observed in the early vs. latter activation phases for each type of stimulation, the activated areas with forepaw stimulation were generally more consistent over time. With forepaw stimulation, the effect of using either the pre-stimulus or post-stimulus periods had little effect on the size of the activated areas. This suggests that although the post-stimulus signal was significant (Fig. 2D), in comparison with the signal during stimulation, the statistical significance was still very high. However with whisker stimulation, the size of the activated area was reduced when the post-stimulus period was used, which suggests that the weaker BOLD response during whisker stimulation (Fig. 2C) is only marginally greater than the magnitude of the signal fluctuations during the post-stimulus period. These activation

differences, both in time and space, between the two stimuli suggest further multi-modal (e.g., blood flow, neuronal activity) studies to be conducted.

During either tactile stimulus, the responses peaked both in terms of magnitude (Figs. 4A,B) and area of activation (Figs. 4C,D) at designated stimulation frequencies. For whisker stimulation, the coronal slice at ~2.5 mm posterior to bregma showed a peak BOLD activation in magnitude (Fig. 4A) and area (Fig. 4C) at 12 Hz stimulation frequency. The $S1_{BF}$ responses were only marginally different at higher stimulation frequencies, but significantly attenuated at frequencies lower than 12 Hz. For forepaw stimulation, the coronal slice at ~1 mm anterior to bregma showed maximal BOLD activation in magnitude (Fig. 4A) and area (Fig. 4C) at 1.5 Hz and slightly lower at 3 Hz stimulation frequencies, whereas the responses were significantly lower at other frequencies. While the magnitude of the maximal $S1_{BF}$ response was approximately $\frac{1}{3}$ that of the maximal $S1_{FL}$ response (i.e., ~4% vs. ~12 %), the peak area of activation at these locations were quite comparable (i.e., 107 ± 12 vs. 107 ± 14 voxels). The current results can be compared with prior fMRI results. The averaged BOLD response with 12 Hz whisker stimulation (Fig. 4A) is in good agreement with prior findings under similar experimental conditions (20,22). Similarly the averaged BOLD response with 3 Hz forepaw stimulation (Fig. 4B) is in good agreement with prior results (18,19).

DISCUSSION

The frequency dependencies of whisker and forepaw stimuli have not been demonstrated previously in the same animal by fMRI, perhaps due to technical challenges in the experimental setup. This study demonstrates BOLD activations in distinct contralateral somatosensory areas during independent whisker and forepaw stimuli at varying frequencies. In the same subject, stimulation of whisker and forepaw gave rise to significant BOLD signal increases in corresponding $S1_{BF}$ and $S1_{FL}$ areas, respectively, with no significant spatial overlap between these regions (Figs. 1C,D). The success of being able to apply both stimuli to the same subject relied on a home-built non-magnetic, air puff stimulator for moving the whiskers. Air puffs allowed precise control of stimulus onset, frequency, and duration thereby facilitating reproducible and consistent stimulation to the same subject in conjunction with forepaw stimulation (Figs. 2A,B). We used this method to stimulate even a single whisker reproducibly in the same subject (Fig. 1C, bottom row). It should be noted, however, that prior studies of $S1_{BF}$ (31–33) have shown that the cortical responses (e.g., blood flow or neuronal activity) to stimulation of an individual whisker vs. several rows of whiskers are non-linear, both in terms of magnitude and area of activation.

The BOLD responses in $S1_{BF}$ and $S1_{FL}$ were found to be variable with stimulus frequency of each type (Fig. 4). The peak magnitude of the BOLD response was larger with forepaw stimulation (Figs. 4A,B). However the peak area of activation for the two stimuli were quite comparable (Figs. 4C,D) which partially agrees with previous assignments by Welker (34) who showed that 16–20% of the somatosensory cortex is designated each for the limbs and whiskers.

In the $S1_{BF}$, the BOLD response magnitude increased linearly with whisker stimulation frequency up to 12 Hz and plateaued above 12 Hz (12 – 30 Hz) (Fig. 4A). The area of activated pixels in the $S1_{BF}$ at the different stimulation frequencies followed a similar pattern (Fig. 4C). In agreement with these observations, previous studies have reported that blood flow (35) increases linearly in the rat somatosensory cortex with whisker movement frequency of 1.5 to 10.5 Hz, whereas the total spike rate (36) increases linearly with whisker movement frequency of 12 Hz and beyond which the electrical activity is slightly decreased. Since whisker stimulation frequency range of 4–12 Hz correspond to active whisking frequencies (36), it is expected that maximal responses to whisker movement will be within this range as has been

observed by a variety of methods (37,38). However it should be categorized that fMRI responses to electrical stimulation of the whisker pad directly may differ from physical movement of whiskers (39).

In the $S1_{FL}$, the magnitude of the BOLD response was largest at forepaw stimulation frequency of 1.5 – 3.0 Hz, beyond which the response diminished with little or no activity at frequencies higher than 20 Hz (Fig. 4B). The volume of tissue activated followed a similar pattern as the magnitude trend (Fig. 4D). The impact of the forepaw stimulation frequency on BOLD or related hemodynamic responses has been extensively investigated (3,4,40–44). Our current results are consistent with these results where the maximal response is detected with stimulation frequencies of 1–3 Hz.

Cortical perception of the physical world relies on the formation of multi-dimensional representation of stimuli impinging on the different sensory systems. Activation studies in experimental neuroscience assume that a sensory stimulus may have very different neurophysiologic outcome(s) when paired with a near simultaneous event in another modality (45,46). Before approaching this level of complexity in future fMRI studies, reliable measures must be obtained of the relatively small changes in the BOLD signal and other neurophysiologic markers (electrical, blood flow) induced by different peripheral stimuli. The demonstration of both whisker and forepaw stimuli given to the same subject, which can be applied identically both inside and outside the magnet, may be used in studies of multi-sensory interactions in anesthetized rats, *en route* to a rudimentary understanding of the functioning brain where various sensory cues presumably interrelate. This model can also be used for mapping of adjacent somatosensory representation by fMRI and to study cortical reorganization or plasticity (47).

Acknowledgments

The authors thank scientists and engineers at MRRC (mrrc.yale.edu), and QNMR (qnmr.yale.edu). FH is grateful to Drs. Alan P. Koretsky and Afonso C. Silva for access to their 11.7T scanner (at NINDS, NIH) to obtain preliminary data which was subsequently used in an NCR grant application (S10 RR-016761) for acquisition of a similar scanner at Yale. This work was supported by grants from National Institutes of Health (R01 MH-067528, R01 DC-003710, P30 NS-52519 to FH), and the Hungarian Research Foundation (OTKA-T34122 to PH).

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Fig. 1. Schematics of (A) whisker stimulation device and (B) location of forepaw stimulation electrodes. (C) BOLD responses to right whisker stimulation (8 Hz) in slices positioned ~2.5 mm posterior to bregma. Reproducibility in the same subject for stimulation of three rows of whiskers (top row), among different subjects for stimulation of three rows of whiskers (middle row), and in the same subject for stimulation of the C2 whisker (bottom row). (D) BOLD responses to left forepaw stimulation (3 Hz) in slices positioned ~1 mm anterior to bregma. Reproducibility in the same subject (top row) and across subjects (middle row) during forepaw stimulation. The lowest and highest thresholds were $p < 0.001$ and 0.0001 , respectively. All data from single trial runs where the stimulation period was 30 s in duration.

Fig. 2.

BOLD data from the same subject where (A) three rows of whiskers on the right snout and (B) the left forepaw were independently stimulated at different frequencies. Lowest and highest thresholds were $p < 0.001$ and 0.0001 , respectively. All data from single trial runs where the stimulation period was 30 s in duration. For whisker stimulation the response peaked at 12 Hz, whereas for forepaw stimulation the maximum response was observed 1.5 Hz. Averaged BOLD time courses for (C) 12 Hz of whisker stimulation (across subjects; $n = 6$) and (D) 1.5 Hz of forepaw stimulation (across subjects; $n = 8$). The horizontal bar represents the stimulation period and the statistical significances of the stimulated signals compared to the pre-stimulus periods are shown.

Fig. 3.

BOLD data from the same subject where (A–D) three rows of whisker on the right snout and (E–H) the left forepaw were independently stimulated. All data from single trial runs where the stimulation period was 30 s in duration. In $S1_{BF}t$ -maps were generated by comparison of the first and last 10 s of whisker stimulation (12 Hz) with pre-stimulus (A and B, respectively) and post-stimulus (C and D, respectively) periods. Likewise, in $S1_{FL}t$ -maps were generated by comparison of the first and last 10 s of forepaw stimulation (1.5 Hz) with pre-stimulus (E and F, respectively) and post-stimulus (G and H, respectively) periods. In each case, the portions of the data used to create the respective t-maps are indicated below. Lowest and highest thresholds were $p < 0.001$ and 0.0001 , respectively. With either stimulus type, slight activation area variations were observed in the early vs. latter phases of the stimulation. However the activated areas with forepaw stimulation were generally more consistent over time.

Fig. 4.

Frequency-dependent averaged BOLD magnitude (A,B) and area of activation (C,D) for whisker and forepaw stimuli, respectively. The BOLD response for whisker (A,C) and forepaw (B,D) stimuli peaked at 12 and 1.5 Hz, respectively, both in terms of BOLD magnitude and area of activation. fMRI data from coronal slices located ~1 mm anterior and ~2.5 mm posterior to bregma were analyzed to assess the BOLD responses for forepaw and whisker stimuli, respectively. All statistical comparisons are with 12 Hz data for whisker stimulation and 1.5 Hz data for forepaw stimulation.