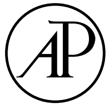

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Experimental model for functional magnetic resonance imaging of somatic sensory cortex in the unanesthetized rat

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Abstract

Functional magnetic resonance imaging (fMRI) has evolved into a method widely used to map neural activation in the human brain. fMRI is a method for recording blood oxygen level-dependent (BOLD) signals. These signals change with local cerebral blood flow coupled to neural activity. However, the relationship between BOLD signals and neural function is poorly understood and requires the development of animal models. Here we use an unanesthetized rat preparation to study BOLD responses to whisker stimulation in somatic sensory barrel cortex. Five rats were trained to tolerate restraint in a holder and fMRI noise with positive reinforcement. For maximal immobilization, the head was fastened to the holder with nuts screwed on threaded bolts attached to the head. On scanning day, residual stress was alleviated with injections of diazepam, and the rats were restrained in the holder and transferred into the scanner. After >75 min to allow the tranquilization to abate, structural images were acquired from three coronal brain slices. Subsequently, functional images were taken utilizing 4-min epochs without stimulation alternated with equivalent epochs during which the right caudal whiskers were stimulated with three air puffs/s. After 4 weeks, fMRI could be repeated in four rats. In seven of the nine functional runs, head motion was minimal and whisker stimulation resulted in a statistically significant ($P \leq 0.05$) increase in BOLD signal in barrel cortex predominantly on the contralateral side. The results provide encouragement that long-term fMRI studies on cerebral function in unanesthetized rats may be feasible with our procedure.
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Keywords: Whisker; Barrel; Rat; Awake; fMRI; BOLD

Introduction

A number of protocols have recently been developed for functional magnetic resonance imaging (fMRI) in animals to investigate cerebral activation under physiological and pathophysiological conditions. Particularly, the relationship between neural activity, cerebral blood flow (Gotoh et al., 2001), and blood oxygen level-dependent (BOLD) signals (Kwong et al., 1992; Ogawa et al., 1992) needs further examination (Arthurs and Boniface, 2002). In the rat, the study of Yang et al. (1996) demonstrated the activation of barrels in the somatic sensory barrel cortex with a physiological stimulus, i.e., whisker stimulation. In that study the animals were anesthetized and ventilated. Anesthesia and ventilation, however, introduce several problems. Anesthesia suppresses neural activity and behavioral responses and, thus, profoundly diminishes local cerebral energy metabolism (Ueki et al., 1992) and blood flow (Nakao et al., 2001). Ventilation requires endotracheal intubation of the animal. Temporary intubation via the larynx with removable catheters is wrought with complications in small animals and

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tracheotomy excludes longitudinal studies. Therefore, protocols have been developed for use with awake primates (Logothetis et al., 1999; Stefanacci et al., 1998), rats (Lahti et al., 1998), and rabbits (Wyrwicz et al., 2000). However, in these studies head motion during imaging was not monitored, and the protocol of Lahti et al. (1998) requires the insertion of ear bars under anesthesia to immobilize the heads. In the present study, we introduce a protocol with which whisker-related cerebral activation can be examined in behaviorally conditioned, restrained rats without anesthesia. Sedation with diazepam was required to minimize stress on the animals while they were restrained in the bore of the magnet and barred from access to their accustomed rewards. Post hoc tracking of head motion showed that in the majority of functional runs excursions of the head lay within acceptable limits. Unilateral whisker stimulation resulted in statistically significant increases in BOLD signal in somatic sensory cortex on both sides; the change in BOLD signal was greatest on the side contralateral to stimulation. Scanning could be repeated after 1 month in the majority of animals, thus demonstrating the potential of our preparation to monitor changes in neural function in the same animals over an extended period of time.

Materials and methods

All methods were approved by the Vanderbilt University Animal Care Committee and were in accordance with NIH-approved procedures. Three male and two female adult Long Evans rats (350–550 g) were obtained from an in-house breeding colony where they were raised in standard cages in a 12:12 light/dark cycle with access to food and water ad libitum.

Preexperimental training

The rats were prepared for fMRI in a two-step procedure. In the first step, the rats were habituated to body restraint and fMRI noise. In the second step, they were familiarized with immobilization of their heads with head posts. In both steps, toleration of restraint was positively reinforced with treats. The training protocol was adapted from that developed in this laboratory for single- and multiunit recordings in awake rats (Sachdev et al., 2000). It is described in detail below.

Step 1. Initially, the rats were merely handled by their trainer every day for a week. Then, their chow diet was reduced and they were gradually accustomed to restraint through daily practice and chocolate milk rewards. In the first practice sessions, they were slid into a loosely fitting felt sleeve, wrapped in a cotton cloth, inserted to the neck into a Plexiglas tube, and liberally fed chocolate milk. When they were at ease with the immobilization, the animals were exposed to tape recordings of the sound bursts generated by gradient switching in the magnet during fMRI. Once fully accustomed to restraint and noise, the rats in the sleeve were placed into a casting molded in silicon resin to contour the ventral half of their body. They were held in place with Velcro straps while chocolate milk was dispensed into the rostral tip of the mold to entice the rats to push their noses deep into it, thus familiarizing them with sitting in this position. The animals typically showed no signs of distress, e.g., extraneous movements, squealing, sneezing, nose bleed, or respiratory arrest, when transferred from immobilization in the tube to restraint in the casting. One day of training in the casting was sufficient to ensure that the rats were comfortable in the new situation and daily practice was continued for a week prior to head post implantation.

Step 2. To accomplish a maximum of immobilization during imaging, three thread-bearing nylon posts were attached to the skull. The rats were anesthetized with intramuscular injections of ketamine (90 mg/kg b.w. Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (10 mg/kg b.w. Xylazine; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO). The scalp was incised longitudinally and the muscles were retracted from the cranium. Three 1.5 mm × 3.0 mm craniotomies were drilled with a burr; two were located posterior of sutura lambdoidea, one over each cerebellar hemisphere, and the third was located immediately anterior of bregma. The posts were fashioned from 26 mm-long # 10–32 nylon machine screws (Small Parts, Inc., Miami Lakes, FL). The heads of the screws were thinned and shaped to 1 mm × 2 mm rectangles so that the screws could be inserted into the craniotomies and gently rotated until they locked under the bone. Finally, they were cemented into place with orthodontic resin (Dentsply International Inc., Milford, DE). The preparation is shown in Fig 1A. To facilitate scouting for barrel cortex, markers made of plastic tubing (3 mm long and 1 mm ∅) filled with vitamin E in mineral oil were cemented on the cranium 2 mm posterior of bregma.

The rats were re-acclimated to body restraint in the sleeve and casting 1 week after implantation (Fig. 1B). The radio frequency surface coil consisting of one loop of insulated, tin-coated copper wire (1.4 mm thick; ~25 mm ∅) was mounted on the head round the posts (Fig. 1C). A Velcro strap was wrapped over the head around the shell. The strap contained slits for the posts and the contact ends of the surface coil. Then, the animals were exposed to scanner noise while being fed chocolate milk.

Full immobilization of the head was practiced 2 days prior to imaging. Each rat was wrapped into the sleeve and placed into the casting. The surface coil was placed on the head, the animal was strapped on the holder with Velcro straps, and the head was covered with a 5 mm-thick contoured True-Shape Plastic™ (Tru-Scan Inc., Annapolis, MD) top (Fig. 1D). Slits provided passage for the contact ends of the surface coil and the head posts. The top was strapped onto the casting with two Velcro straps; the threaded head posts were fastened against the True-Shape Plastic™ top with hexagonal #10–32 nylon nuts (Small

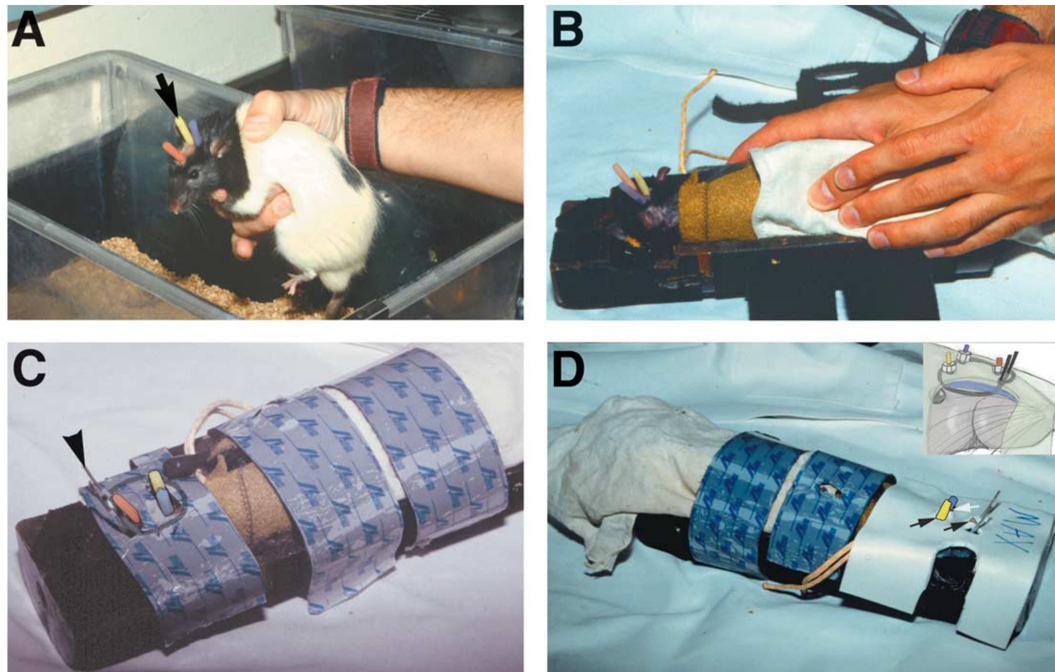


Fig. 1. Preparation of the rat for fMRI. (A) Threaded Nylon head posts (red, yellow, and blue; arrow) were implanted into the cranium in prior surgery. (B) The rat is taken from the cage and placed into a felt sleeve. The sleeve is tightened at the neck with a string and the rat in the sleeve is wrapped in cloth and placed in the bottom casting of the holder. (C) The surface coil is placed over the rat's head and Velcro straps are wrapped around rat and casting. The black loop on the strap over the rat's head indicates the position of the surface coil and its contact ends can be seen protruding through slits in the strap (arrowhead). (D) Head and thorax are covered with the contoured plastic top of the holder; openings on the top and at the side provide for head posts, coil ends, and whiskers. The head posts have been pushed through the top ready to be fastened with nylon nuts (not shown to provide an unobstructed view of the openings). Gently tightening the nuts on the post threads down to the points indicated by the arrows raises the head posts until the head is seated flush against the underside of the top wedging Velcro and surface coil firmly between the head and the top (insert). Finally, the top is fastened to the casting with Velcro straps wrapped around at both ends.

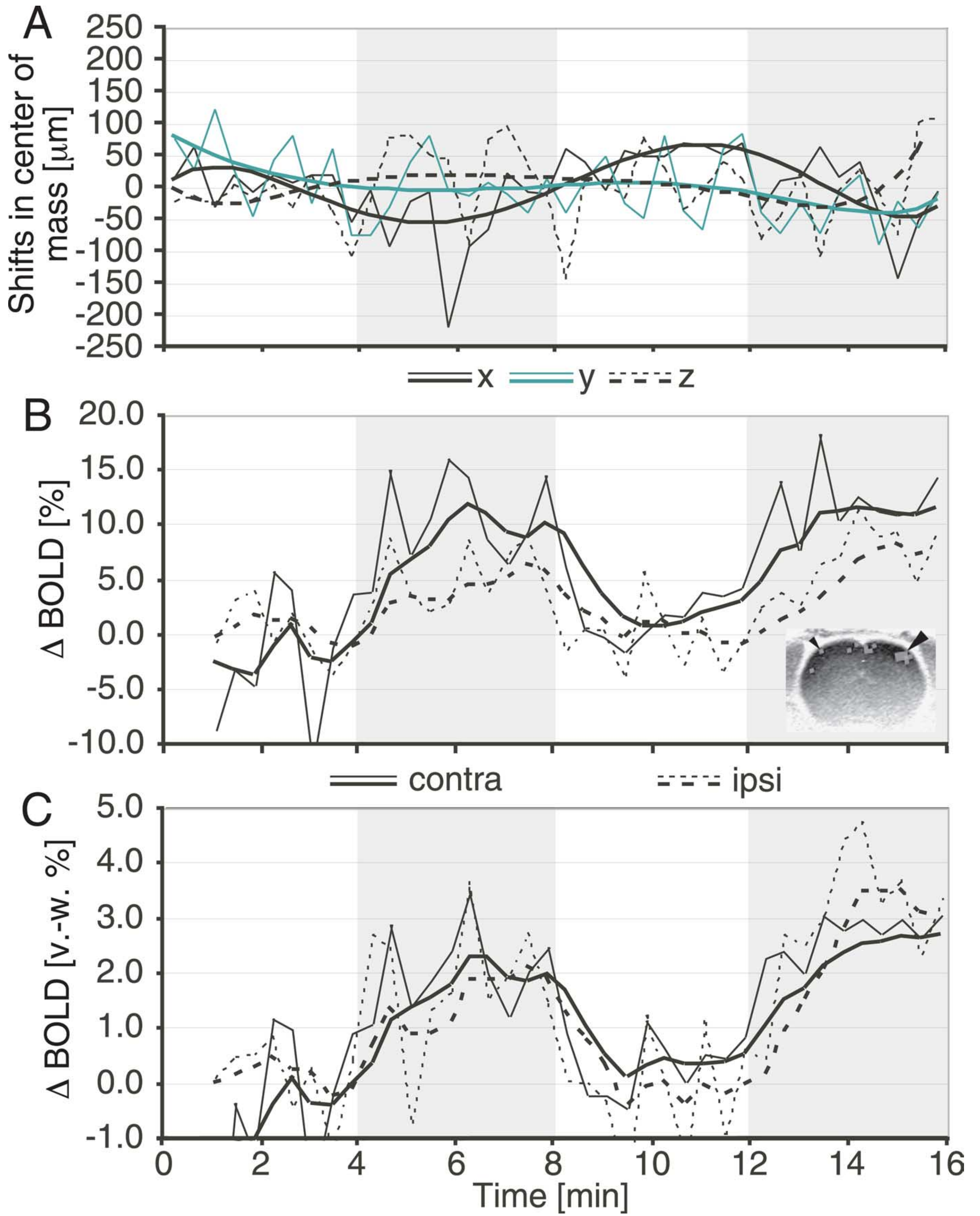
Parts Inc., Miami Lakes, FL), and the rats were exposed to scanner noise under these conditions. After 20 min, the animals were released from restraint, fed chocolate milk, and returned to their cages.

Magnetic resonance imaging

Preparation of the rats

To minimize stress and further facilitate the rats' adjustment to restraint and the scanning environment, the rats were sedated with intraperitoneal injections of diazepam (12 mg/kg b.w. Valium; Elkins-Sinn, Cherry Hill, NJ) more than 1 h before functional imaging commenced. The initial injection constituted approximately half of the total dose. The other half was spread out over two to five injections depending on body weight. The supplements were administered in 5 to 10-min intervals until the animals appeared calm and their movements were smooth. Then, they were restrained in the casting and the surface coil was mounted on their heads as during practice. The whiskers in the three posterior arcs[1] of rows C, D, and E on the right side were taped together with a 20 mm × 20 mm piece of lightweight masking tape (#512; Minnesota Mining and Manufacturing CO., MN) to ensure that they were stimulated in unison. The tape protruded ~30 mm from the holder. Air puffs (duration: 30 ms; pressure: 50 psi; frequency: three puffs/s) were directed onto the tape through a jet (2 mm inner ∅ at the tip) mounted ~30 mm above it. A polyethylene hose (4.3 mm inner ∅; 6.4 mm outer ∅) connected the jet with a compressed air tank in the control room. The puffs were generated with a control unit that triggered a solenoid valve (James Long Co., Caroga Lake, NY). The rats in the holder were taped onto a plastic pipe (7 m long; 10 cm ∅) cut open at midlength and the surface coil was connected to the tuning rod inside the pipe. After it was ensured that the taped whiskers were properly deflected by the air puffs, the rats were moved into the center of the bore of the magnet and scouting for scanning planes commenced. Functional imaging began >75 min after the last diazepam injection.

[1] The large caudal whiskers on the snout are arranged in five rows that Woolsey and Van der Loos (1970) designated A (dorsal) to E (ventral). In each row, the whiskers are numbered in rising order beginning with 1 at the caudal end. The whiskers with the same number form an arc. Four separate whiskers, named α to γ, straddle the rows posteriorly.


A
Shifts in center of mass [μm]
250
200
150
100
50
0
-50
-100
-150
-200
-250
x
y
z
B
Δ BOLD [%]
20.0
15.0
10.0
5.0
0.0
-5.0
-10.0
contra
ipsi
C
Δ BOLD [v.-w. %]
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
0
2
4
6
8
10
12
14
16
Time [min]

MR data acquisition

Magnetic resonance images were acquired with a 4.7T/ 440 Spectroscopy Imaging Systems Corporation imaging spectrometer equipped with actively shielded gradient coils (Varian, Palo Alto, CA). The surface coil on the head was used as transmitter and receiver. After global shimming with final line width of less than 100 Hz, transverse and sagittal T_1-weighted proton images [repetition time (TR)/ echo time (TE) = 400/16 ms] were acquired to determine slices of interest. Based on the atlas of Paxinos and Watson (1986), coronal slices were selected at bregma +1.0 mm, bregma −2.0, i.e., the slice in which the barrel representing whisker C3 is situated, and bregma −3.5 mm, i.e., the slice in which the barrel representing whisker C1 is situated (Chapin and Lin, 1984). Structural images were recorded at a slice thickness of 1.2 mm in a field of view of 30 mm × 30 mm and at a data matrix size of 128 pixels × 128 pixels. T_2^*-weighted BOLD images were acquired from these slices in subsequent fashion with a FLASH sequence (TR/TE = 100/18 ms, RF flip angle = 40°). Using these settings, the data matrix size was set at 64 pixels × 64 pixels. Each functional run consisted of four 4-min epochs during which 10 images were captured per slice. Epochs without whisker stimulation were alternated with epochs with whisker stimulation. Hence, the BOLD signal in each voxel was recorded 20 times with and 20 times without stimulation. Each image took 6.4 s to acquire and the whole functional run lasted ~16 min. After completion, the rats were removed from the scanner, the masking tape was detached from the whiskers, and the rats were released from restraint into their cages. They were housed singly in the animal care facility until fMRI was repeated 4 weeks later. However, in one rat the head posts separated from the skull before it was scheduled for the second fMRI and the animal had to be euthanized. The other four rats were rescanned.

Data analysis

The fMRI data were analyzed with STIMULATE 5.0 (Strupp, 1996). Functional scans were examined at a raster matrix size of 64 pixels × 64 pixels, limiting the dimensions of the smallest rendered tissue volume to 470 μm × 470 μm × 1200 μm. Head motion was assessed by reviewing time lapse movies made from the image volumes recorded for each slice. Images with visible contamination of head motion, i.e., image blurring, were deleted. Not more than one image per epoch had to be eliminated. Then, the image volumes from the three slices were combined and shifts in center of mass were calculated (Pratt, 1991). In two of the nine functional runs, these shifts were so great that three standard deviations of the motion exceeded 0.5 pixels or 235 μm in any one direction and the results from these runs were discarded. The BOLD signal recorded during the epochs of stimulation was compared with that recorded during the epochs without stimulation voxel by voxel using Student's *t* tests. The first volume of images was excluded from the tests to avoid T_1-weighted effects. Voxels in which the signal strength increased statistically significantly at $P \leq 0.05$ were considered activated, provided that the significance did not result from monotonic shifts in baseline, and these voxels were overlaid on the corresponding anatomical images. Activated voxels in barrel cortex were outlined on each hemisphere separately, and the mean BOLD signal was determined for each time point. The averages were transformed into percent differences from baseline, i.e., the signal strength averaged over the epochs without stimulation. For each side, the mean percent differences of the epochs with whisker stimulation were averaged across the seven functional runs with acceptable head motion to represent activation of barrel cortex. Side-to-side percent differences were calculated for each functional run by subtracting the percent BOLD signal change in barrel cortex contralateral to stimulation from that on the ipsilateral side and expressing the difference in percent of the latter. The median of the percentages from the seven acceptable functional runs was taken to represent cortical activation related to ascending somatic sensory inputs. Furthermore, the mean BOLD signal change on each side and of each functional run was multiplied by the number of corresponding activated voxels as fraction of the total number of activated voxels of the seven acceptable functional runs, and the resulting products were used to compute the means for each side. These means, thus, take into consideration differences in the size of the activated tissue among runs. Each time course of percent BOLD change was smoothed by convolution with a shift-invariant linear temporal filter, the temporal impulse response was modeled with a gamma function, and the resulting hemodynamic response curves were used to com-

Fig. 2. Functional activation. (A) Typical motion in the mediolateral (*x,* solid black lines), dorsoventral (*y,* solid gray lines), and rostrocaudal direction (*z,* dashed black lines) during an acceptable functional run. (B) Time courses of the mean percent changes in statistically significant ($P \leq 0.05$) BOLD signal in barrel cortex contra- and ipsilateral to stimulation from the seven functional runs with acceptable head motion. The insert shows typical activation at bregma −2.0. Areas of activation are superimposed on the coronal anatomical slice (large arrowhead, contralateral; small arrowhead, ipsilateral). (C) Time courses of volume-weighted mean percent BOLD signal changes ($N = 7$). Epochs of whisker stimulation are shaded. Thin lines represent measurements and thick lines represent either polynomial fits (A) or hemodynamic response curves (B and C). The response curves model changes in blood flow associated with whisker stimulation. Although in the example in A the head appeared to move mediolaterally concomitant with stimulation, the motion remained smaller than the smallest functional unit, i.e., one barrel, and Spearman's test did not indicate a statistically significant correlation between head motion in any direction and the BOLD signal change measured in this animal. The BOLD signal distinctly increased concomitant with whisker stimulation in barrel cortex in both hemispheres (B). The response appeared consistent regardless of the size of the activation, because weighting the responses for the volume of activated tissue did not degrade the average response (C).

pare the magnitude of changes in blood flow (Boynton et al., 1996).

Results

At a range of ±235 μm in all directions, the center of mass of the slices was permitted to shift only within 0.10 mm^3, i.e., approximately the average volume of a barrel representing the large caudal whiskers. On occasion, head motion appeared associated with the epochs of whisker stimulation (Fig. 2A). However, the tolerated movements were small compared with the volume of the cortical tissue activated by the nine stimulated whiskers. In seven of the nine functional runs, head motion remained within a barrel and was not statistically significantly correlated with the changes in BOLD signal (Spearman's test, $P \geq 0.05$).

Whisker stimulation increased the BOLD signal in barrel cortex on both sides. The volume of the activated tissue contralateral to stimulation was on average twice as large (mean = 0.56 mm^3; SD = 0.40 mm^3) as that ipsilateral to stimulation (mean = 0.22 mm^3; SD = 0.31 mm^3). In the coronal slice shown in the insert of Fig. 2B, the medial-to-lateral extent of the contralateral activation spans ~2000 μm, i.e., all five rows of barrels were covered. However, on average, statistically significant activation spanned only three barrels consistent with the three rows of whiskers stimulated. In contrast, on the ipsilateral side, statistically significant BOLD signal increases spanned only one barrel.

Fig. 2B shows the average time courses of statistically significant percent BOLD signal changes and the average hemodynamic response curves from the seven functional runs with acceptable head motion. The BOLD signal strength in barrel cortex increased during each epoch of whisker stimulation. Typically, the increase appeared greater during the second than during the first epoch, particularly on the contralateral side. However, the hemodynamic response function in Fig. 2B suggests that the second peak appeared greater because the baseline had increased during the preceding epoch. Indeed, the difference in BOLD signal change between the first pair of epochs was greater (10%) than the difference between the second pair (7%).

Averaging the change in BOLD signal in barrel cortex during both epochs of whisker stimulation resulted in a mean signal increase of 11.3 ± 4.6 (SEM) percent contralateral to whisker stimulation and 3.6 ± 1.4% on the ipsilateral side; the median side-to-side percent difference was 60%. Data from single activated voxels were included in these averages, although the BOLD signal in single voxels may change more spuriously than that in multiple, contiguous voxels. The contribution of spurious signal changes must have been negligible, however, because volume weighting did not degrade the measured response (Fig. 2C). In contrast, the side-to-side percent difference in activation increased from 60% to 100% when weighted for the volume of activated tissue.

Four rats were imaged twice. In two rats, one functional run was contaminated with head motion above threshold. In the remaining two rats, BOLD responses from the first and second imaging session could be compared (Fig. 3). In Rat 1, the magnitude of the BOLD response in barrel cortex was about equal on both sides in both sessions. It averaged 9% in the first imaging session and 4.5% in the second imaging session. Regardless of the twofold variation in magnitude, the change in BOLD signal observed in both sessions cross-correlated statistically significantly on the contralateral side (Spearman's test; $r = 0.65$; $P = 0.0001$) and approached significance on the ipsilateral side ($r = 0.30$; $P = 0.07$).

In Rat 2, in contrast, the BOLD response to whisker stimulation was about equal during both sessions, but the magnitude of the response was distinctly higher on the contralateral side (9%) compared with the ipsilateral side (6%). The side-to-side percent difference increased from 40% in the first session to 60% in the second session. In spite of the greater consistency in magnitude of BOLD signal change compared with Rat 1, the time course data of the first and the second session remained slightly less cross-correlated contra- ($r = 0.57$; $P = 0.0002$) and ipsilateral to stimulation ($r = 0.27$; $P = 0.10$).

In summary, unilateral whisker stimulation resulted in statistically significant increases in BOLD signal in barrel cortex that were on average larger in extent and greater in magnitude contralateral than ipsilateral to stimulation. Even though the degree of activation may have varied greatly between imaging sessions, the stimulus-related changes in BOLD signal were tightly cross-correlated and the side-to-side differences in activation were consistent.

Discussion

In the present study we demonstrate that the experimental protocol developed in this laboratory can be successfully used to noninvasively image local cerebral activation in response to whisker stimulation in the unanesthetized rat. fMRI could be carried out repeatedly over an extended period of time. Positive reinforcement and habituation conditioned the rats to tolerate restraint and scanner noise. Sedation was considered necessary to reduce stress on the animals at a time when positive reinforcement was not available. Conditioning combined with sedation resulted in head motion within the limits of one barrel in somatic sensory cortex in the majority of functional runs. The cortical response to whisker stimulation observed in the present study and factors that may have played a crucial role in the BOLD signal changes are discussed in detail below.

Cortical activation

We anticipated a high variability in cortical activation, because the efficacy of the stimulus and the animals' attentiveness and/or sedative state may vary considerably be-

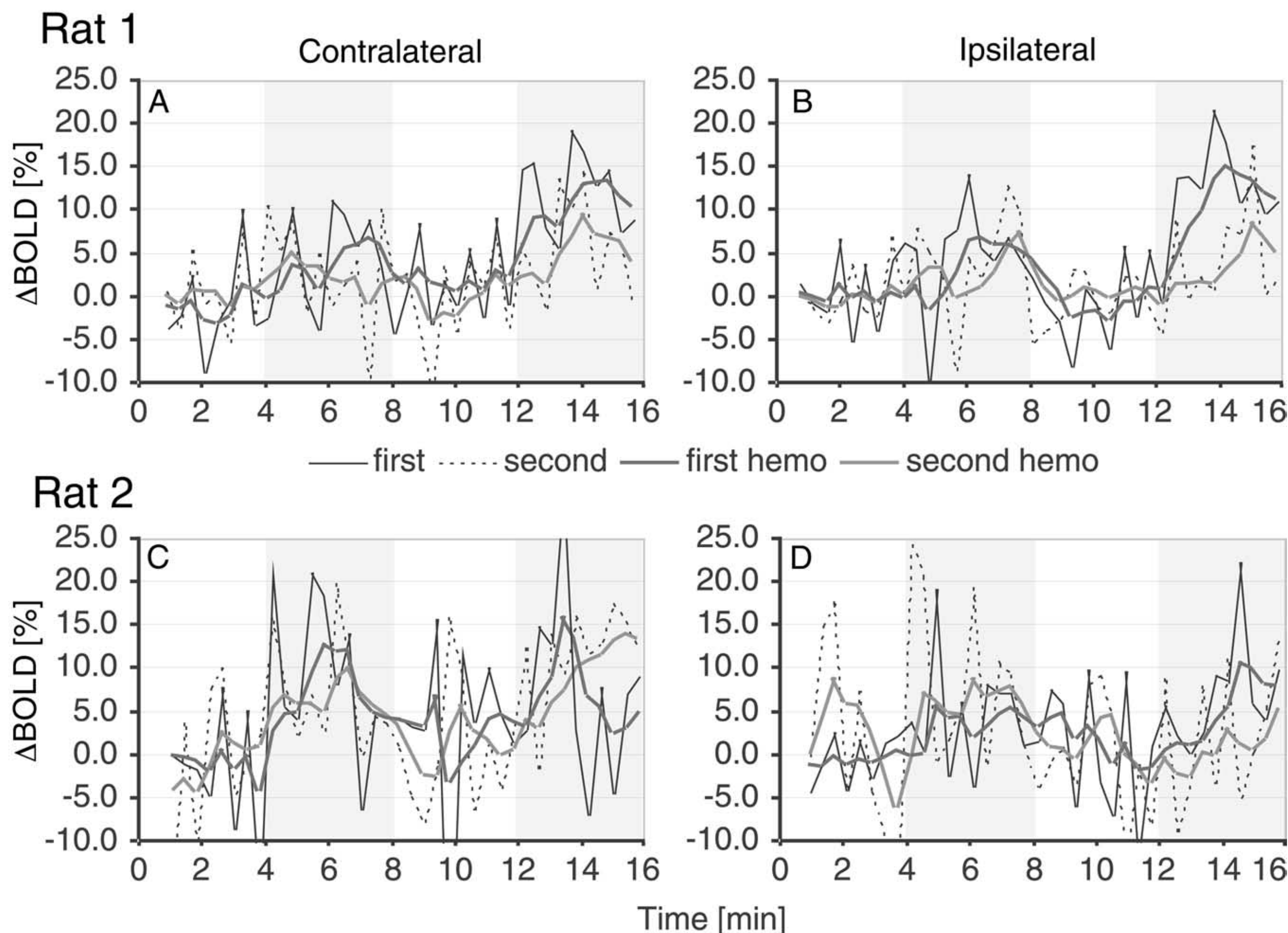


Fig. 3. Repeated measures recorded in two rats [Rat 1 (A and B) and Rat 2 (C and D)]. The plots show the time courses of mean percent change in BOLD signal in barrel cortex contra-(A and C) and ipsilateral (B and D) to whisker stimulation during the first (thin solid lines) and the second (thin dashed line) imaging session. The thick lines represent the hemodynamic response curves derived from the measurements (red, first session; blue, second session). Epochs of whisker stimulation are shaded. In spite of the varying efficacy of stimulation and the potentially wide range of the arousal of the animals, whisker stimulation resulted in distinct activation in barrel cortex during both sessions. In both functional runs, changes in BOLD signal correlated statistically significantly on the contralateral side and approached significance on the ipsilateral side.

tween imaging sessions. In spite of these sources of variation, we observed robust stimulus-related BOLD signal changes. The stimulation of caudal whiskers on the right side of the snout resulted in statistically significant increases in BOLD signal in barrel cortex contra- and ipsilateral to stimulation. On average, activation was stronger on the contralateral side.

The contralateral activation covered roughly the number of barrels corresponding to the number of whiskers stimulated. On average, its BOLD signal increased 11%. This increase is slightly higher than that found by Gyngell et al. (1996) in the α-chloralose anesthetized rat after electrical stimulation of the forepaw at the same frequency of stimulation and with the same magnetic field strength. The authors used approximately half the TE, i.e., 60 ms, a fourfold TR, i.e., 70 ms, and half the flip angle, i.e., 22.5°, as employed in the present study. These settings may have yielded greater sensitivity for changes in BOLD signal at the risk of greater susceptibility related losses in signal strength (Elster, 1994). More conspicuously, a physiological stimulus, i.e., whisker stimulation, was applied in the present study and the neural responses, therefore, would be expected to reflect more physiological levels of the size of cortical representation.

Small, but consistent activation was found in barrel cortex ipsilateral to stimulation. Because the whiskers on the nonstimulated side were not clipped and may have touched the holder, this BOLD response may have been the result of whisking concomitant with stimulation. However, compared to the contralateral side, the activation was on average less than half in magnitude and only one-third in size. Touching the holder with all whiskers would have led to a greater BOLD signal in a larger area. Because the ascending whisker-to-barrel pathway is completely crossed, the observed ipsilateral increase in BOLD signal, therefore, is more likely the result of activation by callosal input from the opposite hemisphere (Champney et al., 2001; Shuler et al., 2001; Pidoux and Verlay, 1979). Interestingly, this activation appears to depend on intact whisker input. Clipping

all whiskers on the side contralateral to stimulation abolishes the ipsilateral response (Gotoh et al., 2001), as does lidocaine anesthesia of the contralateral cortex (Shuler et al., 2001).

Methodological considerations

Sedation

The rats were fed chocolate milk during training but could not receive any treats in the scanner and therefore needed to be tranquilized to alleviate stress. Tranquilization was furthermore employed to stabilize heart rate and respiratory rate prone to influence the BOLD response (Mitra and Pesaran, 1999) and minimize the risk of acidosis and hyperventilation. Acidosis and hyperventilation may increase or decrease local cerebral blood flow by roughly 50%, respectively (Table 28.3 in Sokoloff, 1996), compromising the BOLD signal. At 12 mg/kg i.p, the dose of diazepam was in the sedative range, i.e., > 6 mg/kg (Söderpalm et al., 1989). We were not able to monitor heart rate, respiratory rate, arterial blood pressure, arterial blood gases, or pH in the present study. Though functional imaging commenced more than 1 h after the injection of the tranquilizer, sedation may still have affected the physiological state of the animals, thus contributing to the variability of the results. On the other hand, sedation allowed us to scan the animals unprovoked and calm. Sedation may become obsolete once the animals are provided with access to the food reward that they are accustomed to. Rewards during imaging would result in head motion owing to periodic licking and chewing. However, the rats could be trained to sit quietly during functional imaging and expect rewards only between runs. Furthermore, monitoring the physiological state of the animals will enable us in the future to select results from more comparable runs and thus reduce the variability of the BOLD response.

Spatial resolution

The ability to map cortical activation in small brains with fMRI is governed mainly by finding a workable compromise between the smallest volume of tissue that provides a recordable BOLD signal and the size of the functional cortical units that need to be imaged. In the present study, a pixel size of 470 μm $\times$ 470 μm fulfilled these requirements at 4.7 T. This size is slightly larger than the average barrel representing caudal whiskers in layer IV of rat barrel cortex in a coronal section. Yet, it is small compared with the width of three barrels that were anticipated to be most activated by the stimulation of whiskers in three rows (Melzer et al., 1985). A slice thickness of 1200 μm appears large compared to the size of one barrel. It was set to cover three barrels, thus, enabling us to capture the maximal BOLD signal strength when three whiskers are stimulated in a row. The chosen voxel size eventually increased the probability that the observed changes in BOLD signal were related to whisker stimulation even in single voxels. One consequence of this choice was that volume weighting would not be expected to degrade the BOLD response.

Temporal resolution

Recording robust BOLD signal changes related to local blood flow with the equipment used in the present study required acquisition times that were adequate to cover the adjustments of blood oxygenation after stimulus onset. Deoxygenated and oxygenated hemoglobin concentrations may reach their plateaus within 2.5 and 4.5 s, respectively (Shtoyerman et al., 2000). The image acquisition time was chosen to be sufficiently long to conservatively bracket this time interval. Shorter image acquisition times diminished the strength of the BOLD signal and increased its variability and more images would have had to be recorded to measure a statistically significant change. Prolonging the duration of the functional runs, however, increases the risk of head motion and, thus, the probability of recording spurious cerebral activation.

Acknowledgments

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