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Mapping Rabbit Whisker Barrels Using Discriminant Analysis of High Field fMRI Data

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Abstract

High field (>4T) functional magnetic resonance imaging (fMRI) techniques provide increased spatial resolution that enables the noninvasive, repeatable study of the sensory cortices at the level of their basic functional units. The examination of these units is important for studies of sensory information processing, learning- or experience-related brain plasticity, or the fundamental relationship between hemodynamic and neuronal activity. However such units cannot always be distinguished from their surrounding areas by conventional activation mapping methods such as correlation or hypothesis tests, which only consider temporal variation within each individual voxel. We report a novel method to detect individual functional units based upon the joint characterization of high order dependency among multiple voxels using discriminant analysis. Our results in the whisker barrel cortex of the awake rabbit indicate that the proposed method can differentiate reliably small clusters of activated voxels corresponding to individual whisker barrels within larger areas of functional activation, even in the case of adjacent whiskers in unanesthetized subjects. This method is computationally efficient, requires no specific experimental design for fMRI acquisition, and should be applicable to studies of other sensory systems.

Introduction

Advances in high-field functional magnetic resonance imaging (fMRI) techniques have facilitated more detailed functional analyses of brain structures at the level of individual functional units. The higher signal to noise ratio (SNR) provided by increased magnetic field strength has allowed fMRI, through blood oxygenation level dependent (BOLD) contrast and other techniques, to achieve the spatial resolution necessary for noninvasive, repeatable functional mapping of small brain anatomic structures, such as the cortical barrel fields or ocular dominance columns. The ability to image such functional units is desirable, as they provide a highly localized substrate for studies of information processing and sensory- or learning-induced neuronal plasticity, as well as the basic properties of the BOLD signal, at the level of the fundamental columnar structure of the cortex. However, achieving sufficient spatial specificity to resolve these structures in echo planar imaging (EPI) data remains a significant challenge. EPI images typically have low spatial resolution, and the analysis of functional time series is often complicated by subject movement and physiological noise. Furthermore, the widespread increase in blood flow and potential spreading of neuronal

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activity that accompanies the BOLD response in regions such as the whisker barrel cortex makes the distinction of individual functional units from neighboring voxels quite difficult by methods such as correlation analysis or hypothesis tests that consider only independently the temporal variation of each voxel.

A number of previous studies have attempted to map functional units including rat whisker barrels (Yang et al., 1996), rat olfactory glomerulae (Kida et al., 2001, 2002), iso-orientation columns in cat visual cortex (Duong et al., 2000, Menon et al., 1999), and human ocular dominance columns (ODC) (Menon et al., 1999, Cheng et al., 2001, Goodyear et al., 2001). A high-field study of rat whisker barrel cortex using multi-slice gradient-recalled echo (GRE) imaging has used Student's paired t-test to locate barrels (Yang et al., 1996). However, it is unclear if this method can identify such small structures with sufficient spatial specificity in low resolution EPI data, particularly without the use of anesthesia, which can attenuate the spatial extent of the BOLD response (Armstrong-James et al., 1988). In other studies, particular features of the BOLD signal that are dependent on a high temporal sampling rate, such as the reported early negative BOLD response (i.e., "initial dip") (Duong et al., 2000, Kim et al., 2000) or specific experimental designs such as differential imaging (Menon et al., 1999, Cheng et al., 2001, Goodyear et al., 2001) were used to distinguish the basic functional units in fMRI data. A more versatile approach to resolving individual functional units that is applicable to a wide variety of fMRI experiments without imposing limitations on the experimental design or temporal sampling rate and sufficiently robust for application to lower-resolution EPI data and unanesthetized subjects would therefore be highly desirable.

In this study, we propose an approach to identify individual cortical whisker barrels. Voxels representing stimulated whisker barrels and their surrounding regions can be differentiated if their BOLD responses can be jointly characterized using discriminant analysis. This joint characterization of the multiple voxels involves high order statistics that can potentially provide a more complete representation of the data structure, compared to methods that only consider the second order statistics (Friston et al., 1999, 2000). Our proposed analysis method consists of a two-step process, where the first step identifies activated and non-activated voxels by correlation analysis, and the second step groups the activated voxels into sub-regions. The grouping is implemented by correlating the activated voxels with temporal components obtained from a discriminant analysis method that can characterize high order dependency among multiple voxels, such as kernel principal discriminant analysis (KPCA) (Schölkopf et al., 1998) or independent component analysis (ICA) (McKeown et al., 1998). Our results demonstrate that this approach can resolve tightly localized clusters of activity in the barrel cortex during the stimulation of single and multiple adjacent and non-adjacent whiskers. The effectiveness of ICA and KPCA for the differentiation of whisker barrels was also compared. The whisker barrel cortex is well-suited for this analysis due to the clearly-defined topographical correspondence between individual whiskers and their functional representation in the cortex. However, as our proposed analysis method does not rely upon a particular experimental design for the fMRI data acquisition, it should be flexibly applicable to functional studies in other sensory cortices.

Methods

fMRI Data Acquisition

Two Dutch-belted female rabbits (2-3 kg) were used in this study. The rabbits were surgically prepared for the experiments as described previously (Wyrwicz et al., 2000), which included securing four nylon restraining bolts to the skull with nylon skull screws and dental cement. The headbolt was implanted stereotactically with lambda 1.5 mm below bregma, and was used to secure the radiofrequency (RF) coil and the rabbit's head in the

same position to obtain a constant imaging angle and slice positioning among subjects. Animals were restrained by means of a cloth bag and secured in an acrylic imaging cradle during each experiment. Each subject was habituated to the imaging environment prior to the experiments. fMRI data were acquired from both subjects in order to verify the reproducibility of the results across animals, but the results presented are from a single subject in order to eliminate potential inter-subject variability due to individual anatomy or differences in slice orientation resulting from the headbolt implantation. All procedures were carried out under NIH guidelines, NorthShore University HealthSystem Research Institute IACUC approved protocols.

fMRI experiments were performed on awake rabbits using a Bruker (Billerica, MA) Biospec 9.4T imaging spectrometer operating at a proton frequency of 400 MHz. A single-turn, 40mm-diameter, circular RF surface coil was used for both transmission and reception. A multi-slice, single-shot gradient-echo EPI pulse sequence, with a repetition time (TR) of 2 s and an echo time (TE) of 13 ms, was used to map brain activation. Coronal images in a plane perpendicular to the surface coil were collected from four consecutive slices (1.0 mm thickness) using a 80×80 matrix size, which was zero-filled to 128×128 , and a 30×30 mm² field of view (FOV), corresponding to an in-plane resolution of 234×234 μ m². The angle of the headbolt implantation with respect to the surface of the cortex allowed us to image all three barrels of whisker row A within a single 1 mm thick coronal slice.

The whisker stimulation was induced using a 15mm-diameter, six-turn, circular coil that was placed in the magnet bore and was driven by an alternating current. Either one (A3) or two whiskers (A1 and A3, A2 and A3) on the left side of rabbit face were selected for stimulation in each experiment. The vibration amplitude was ± 750 μ m; the vibration frequency was 75Hz. The stimulus paradigm for each trial consisted of a baseline period (25 images), followed by a stimulation period (20 images) and a rest period (20 images). The first five images were removed from each trial to ensure stable image intensities. Ten trials were collected in each experimental session.

Data Analysis

All fMRI data were registered using a 2-D affine transform-based method to remove small head motion artifacts. The proposed two-step analysis approach was then used to detect activated voxels in the somatosensory cortex and identify activated sub-regions corresponding to whisker barrels.

In the first step of fMRI data analysis with the proposed method, each voxel's time course was correlated with a boxcar function corresponding to the experimental paradigm with a significance level of 0.05. In the second step, the activated voxels in the somatosensory cortex were further analyzed by either temporal KPCA or ICA. KPCA is a nonlinear extension of PCA. It incorporates nonlinearity into analysis via a kernel function that implicitly projects the input fMRI data into a high dimensional feature space where a linear PCA is performed that is equivalent to a nonlinear PCA in the input space. Given a set of d -dimensional data $\mathbf{x}_i \in \mathbb{R}^d$, $i = 1, 2, \dots, n$, the principal components (PC) are obtained by computing eigenvalues $\lambda > 0$ and eigenvectors $\mathbf{V}$ satisfying:

$$\lambda \mathbf{V} = \frac{1}{n} \sum_{i=1}^n (\Phi(\mathbf{x}_i) \cdot \mathbf{V}) \Phi(\mathbf{x}_i), \quad (1)$$

where Φ is a nonlinear mapping from $\mathbb{R}^d$ to a higher dimensional feature space $\mathcal{F}$ and $(\cdot)$ is the inner product operator. Since $\mathbf{V}$ lies in the space spanned by $\Phi(\mathbf{x}_1), \dots, \Phi(\mathbf{x}_n)$, we have

$\mathbf{V} = \sum_{i=1}^n \alpha_i \Phi(\mathbf{x}_i)$. By defining a $n \times n$ matrix $\mathbf{K}$ with entries $K_{ij} = (\Phi(\mathbf{x}_i) \cdot \Phi(\mathbf{x}_j))$ and replacing the inner product with a kernel function $k(\mathbf{x}_i, \mathbf{x}_j)$, the eigenvalue problem becomes $\lambda \mathbf{a} = \mathbf{K} \mathbf{a}$, where $\mathbf{a} = (\alpha_1, \alpha_2, \dots, \alpha_n)^T$. The feature projection on the k^{th} PC in F can be obtained by:

$$(\mathbf{V}^k \cdot \Phi(\mathbf{x})) = \sum_{i=1}^n \alpha_i^k (\Phi(\mathbf{x}_i) \cdot \Phi(\mathbf{x})), \quad (2)$$

where $\mathbf{V}^k$ is the k^{th} eigenvector in F . In our study, the following polynomial kernel function was used for KPCA: $k(\mathbf{x} \cdot \mathbf{y}) = [(\mathbf{x} \cdot \mathbf{y}) + 1]^m$, where $(\mathbf{x} \cdot \mathbf{y})$ is the inner product of feature vectors $\mathbf{x}$ and $\mathbf{y}$ and the kernel order m was experimentally determined to be 11. For the implementation of KPCA, the kernel matrix $\mathbf{K}$ is first computed based on the kernel function to represent feature variation in the projected feature space, and then single value decomposition (SVD) is performed on the matrix. The ten most significant principal components (PC) were examined. In general, the 1st most significant PC characterizes the average BOLD signal of the activated voxels, and other PCs show temporal patterns that can be used to differentiate sub-regions in the somatosensory cortex by correlation analysis.

ICA is a linear transform that is able to characterize high order dependencies among data samples. Given a set of zero-mean d -dimensional data $\mathbf{x}_i \in R^d$, $i = 1, 2, \dots, n$, ICA aims to estimate an un-mixing matrix $\mathbf{W}$ so that $\mathbf{x}$ is transformed into m components $\mathbf{s}_m$, $m \leq d$, which have minimum dependencies to each other: $\mathbf{s} = \mathbf{W}\mathbf{x}$. ICA incorporates the nonlinearity into decomposition by minimizing an objective function that approximates the mutual information between different components $\mathbf{s}_m$. Various objective functions have been proposed to approximate the mutual information and lead to different solutions (Hyvärinen, 1999a). For ICA implementation, FastICA was used with the nonlinear contrast function *Tanh* (Hyvärinen, 1999b). The data were pre-whitened prior to ICA analysis, reduced in dimension using PCA, and then decomposed into 15 independent components (ICs). Since an appropriate initialization of the un-mixing matrix $\mathbf{W}$ for optimal decomposition is hard to obtain, typically a random initialization is used that can produce suboptimal results.

Components obtained from temporal KPCA or ICA in the second step were correlated with all voxels in the somatosensory cortex where each component is expected to characterize one or several activated sub-regions. Finally, the activated sub-regions corresponding to barrels were identified by examining their spatial location in the correlation maps. The average temporal profiles of those identified barrels were calculated. For comparison, Student's *t*-statistics maps were also calculated in the study.

Results

The rabbits used in this study adapted well to the restraint and habituation, and displayed no obvious signs of movement during the experiments. As expected, both subjects showed stimulus-related BOLD activation in the somatosensory cortex contralateral to the stimulus presentation; the results compared in this section were from the same subject, in order to eliminate differences due to inter-subject variation in physiology or slice orientation. No activation was observed on the ipsilateral side.

Fig. 1 illustrates the distribution of layers within the somatosensory cortex overlaid on a gradient echo image obtained at the same spatial location as the EPI images analyzed in this study. The relative proportions of the cortical layers are based on histological studies of the rabbit brain (Gould et al., 1986, McMullen et al., 1994). The approximate thickness of the

layers I-III is 0.66mm, the thickness of layer IV is 0.4mm, and the thickness of layers V and VI is 1.44mm. Thus, the approximate thickness of the entire cortex is 2.5mm.

Fig. 2 shows fMRI activation maps in the somatosensory cortex of a representative subject during the stimulation of a single whisker (A3). For comparison, results are shown for correlation analysis alone (i.e., the first step of our proposed two-step method) and for the additional steps employing KPCA and ICA decomposition, as well as for Student's t-test. Fig. 2a shows the results from correlation of the fMRI data to a boxcar function corresponding to the experimental paradigm, with a significance threshold of $p < 0.05$ and the Bonferroni correction. A large cluster of activated voxels can be seen in the somatosensory cortex extending approximately through the first four cortical layers. The t-statistics map obtained at a significance level of $p < 0.05$ with the Bonferroni correction is also shown for this region (Fig 2b). The activation detected by the t-test method appears in the same region as that in Fig 2a, but is separated into two distinct clusters. The activation maps determined by correlating the fMRI data with components obtained by KPCA and ICA decompositions, with a significance threshold of $p < 0.05$ and cc values above 0.35, are shown in Figs 2c and 2d, respectively. The correlation with components obtained from both KPCA and ICA detected regions of activation which were similar in area to one another and visibly smaller than those detected by either correlation alone or the t-test. Note that the most highly correlated BOLD response in both cases appears primarily in layer IV.

Fig. 3 shows the averaged time courses, normalized to the baseline average, for the activated regions from the single whisker experiment detected using correlation analysis (Fig. 3a), Student's t-test (Fig. 3b), and the proposed two-step method using both KPCA and ICA (Figs. 3c, d). Little variation in the magnitude or shape of the temporal profiles is present, although the noise level is higher for the smaller activated regions due to the lower number of averaged voxels.

Fig. 4 shows fMRI activation maps in the somatosensory cortex of a representative subject during the stimulation of two adjacent whiskers (A2 and A3). Fig. 4a shows the results from correlation of the fMRI data to a boxcar function corresponding to the experimental paradigm, with a significance threshold of $p < 0.05$ and the Bonferroni correction. A single, large cluster of activation can be seen in the somatosensory cortex, extending approximately through the first four layers of the cortex, with no separation evident between the two barrels. Fig. 4b shows the t-statistics map of the somatosensory cortex, with a significance threshold of $p < 0.05$ and the Bonferroni correction. As in Fig 4a, a large region of activation is revealed, with no definition of the individual whisker barrels. The activation maps determined by correlating the fMRI data with components obtained by KPCA and ICA decompositions, with a significance threshold of $p < 0.05$ and cc values above 0.35, are shown in Figs. 4c and 4d, respectively. For both KPCA and ICA, the regions of activation detected by the analysis are visibly smaller than those obtained by correlation or t-test alone. However, the correlation with the component from ICA cannot differentiate the two adjacent barrels at the current threshold level. The activation detected by KPCA appears more spatially focused than that detected by ICA, and is distinctly separated into two clusters at this significance threshold. Note that the most highly correlated BOLD response in both cases appears primarily in layer IV. As expected, one of the activated clusters detected by KPCA appears in the same position as the cluster corresponding to the single whisker (A3) stimulation, as shown in Fig. 2. As in the case of the single whisker experiment (Fig. 3), the averaged temporal profiles (not shown) for each of these detected regions showed little variation in the shape or magnitude of the BOLD signal.

The activated regions detected by the proposed two-step approach using KPCA during the stimulation of whiskers A2 and A3 were quantified for multiple experiments ($n = 9$) within a

single subject in terms of several parameters, including their depth within the cortex, distance from the midline, width, and separation, for comparison with known physiological properties of the whisker barrels. These results are summarized in Table 1. The average depth of cluster corresponding to A2 was 1.14 ± 0.06 mm, and that of the cluster corresponding to A3 was 1.12 ± 0.04 mm, placing both clusters within the depth range of cortical layer IV. The average distance between the center of the cluster corresponding to A2 and the brain midline was 7.43 ± 0.30 mm; for the cluster corresponding to A3, this distance was 8.32 ± 0.35 mm. The average separation between the center of A2 and A3 is 0.87 ± 0.15 mm. At the selected threshold level of $p < 0.05$ and cc values above 0.35, the average widths of the clusters corresponding to A2 and A3 in the coronal section are 0.72 ± 0.18 mm and 0.67 ± 0.14 mm, respectively, and their standard deviations are less than the size of one voxel.

To further examine the performance of the proposed analysis method, another experiment was performed in which the non-adjacent whiskers A1 and A3 were stimulated, as shown in Fig. 5. Fig. 5a shows the results from correlation of the fMRI data to a boxcar function corresponding to the experimental paradigm, with a significance threshold of $p < 0.05$ and the Bonferroni correction. As in the case of the adjacent whiskers (Fig 4a), a large cluster of activated voxels is present in the somatosensory cortex extending approximately through the first four cortical layers, with no evident separation between the individual whisker barrels. Fig. 5b shows the t-statistics map of the somatosensory cortex, with a significance threshold of $p < 0.05$ and the Bonferroni correction. Although two focal areas of high t-values can be seen, the region could not be separated into distinct clusters at this significance level. The activation maps determined by correlating the fMRI data with components obtained by KPCA and ICA decompositions, with a significance threshold of $p < 0.05$ and cc values above 0.35, are shown in Figs. 5c and 5d, respectively. In this case, with a greater distance between the whisker barrels compared to the previous experiment involving A2 and A3, both KPCA and ICA were able to separate the activated region shown in Fig. 5a into two distinct clusters. The position of the lower cluster, presumably corresponding to A3, remains consistent with the results observed in the previous experiments shown in Figs. 2 and 4, and once again the most highly correlated BOLD response in both cases appears primarily in layer IV. However, although both methods could distinguish two separate clusters in this region, correlation to the component obtained from KPCA was able to achieve visibly greater spatial specificity, as compared to ICA. In all cases, the temporal profiles (not shown) from these activated regions showed little variation in the shape or magnitude of the BOLD signal.

Discussion

Our results demonstrate that the proposed two-step analysis method, which incorporates nonlinearity into the discriminant analysis to jointly characterize multiple voxels, provides a superior ability to resolve individual whisker barrels in awake animals as compared to either correlation to the experimental paradigm or Student's t-test alone. In the single whisker stimulation experiment (Fig. 2), the proposed approach, using both KPCA and ICA, detected activated regions that were more spatially specific and accurate than those detected by correlation or t-test alone. The adjacent and non-adjacent double whisker stimulation experiments (Figs. 4 and 5, respectively) demonstrated the ability of the two-step approach to distinguish clusters corresponding to individual whisker barrels where correlation or t-test alone could not do so at this significance level.

The relative position of the clusters corresponding to barrels A1-A3, as illustrated in Figs. 2, 4, and 5, corresponds well to the orientation of the row A whiskers within the barrel cortex. Differences in separation between the adjacent barrels are due primarily to the curvature of the cortical surface in three dimensions as captured by our slice and represented in a 2D

image. As shown in Table 1 for the barrels corresponding to whiskers A2 and A3, the proposed method implemented using KPCA produced highly consistent results across multiple experiments. The standard deviations of the depth and width of barrels A2 and A3, and the standard deviation of the distance between the two barrels were smaller than the size of a single voxel, while the standard deviation of the distance of barrels A2 and A3 to the brain midline was only slightly greater than the size of a single voxel. Factors such as small differences in imaging artifacts, positioning, and noise conditions in fMRI data acquired at different times could account for these variations. The depths, widths and separation of the A2 and A3 barrels measured by the proposed method correspond well to the values reported from histological studies of the rabbit whisker barrel cortex (Gould et al., 1986, McMullen et al., 1994).

After the second step of analysis with the proposed method, particularly in the case of KPCA, the clusters of activation which initially spanned a large area beyond the extent of the stimulated whisker barrels were divided into smaller foci of activation. The large clusters detected after the initial correlation analysis likely reflect the spreading of neuronal response within the somatosensory cortex from the initial site of activation. Previous studies have used voltage sensitive dyes (Laaris et al., 2002, Petersen et al., 2003) and electrophysiology (Armstrong-James et al., 1992) to map neuronal excitation in the whisker barrel cortex. Sensory input from the thalamus is initially received in layer IV of the stimulated barrel. From layer IV, this information projects first vertically within each barrel unit, and then to neighboring barrels over approximately 20 ms. Thus, at the time resolution of fMRI, which in our study was 2 s, it is not surprising that the BOLD activation appears to spread over an area much larger than the stimulated barrels themselves. However, it is notable that the spread of BOLD activation in our experiments, as expected, was always in the medial direction with respect to A3, and did not extend laterally beyond the barrel cortex.

As the components obtained from KPCA and ICA decomposition in the second step of analysis do not necessarily have a clearly-defined physiological relationship to the BOLD response, it is difficult to relate them to a specific structural characteristic or signal feature to explain their ability to subdivide these larger activated regions into more focal clusters. A direct comparison of *in vivo* MR results to histology is problematic, due to the shrinkage and distortion that occur during histological slicing and the difficulty of obtaining slices that reflect the precise orientation of the images. However, previous histological studies can provide important information such as the relative proportions of the cortical layers and the distribution and orientation of whisker barrels within each slice. In particular, the consistent depth of these clusters, which placed them primarily within layer IV of the cortex, was notable in our results. As described previously, layer IV represents the primary location of thalamic input to the whisker barrel cortex, and the initial site of activation within each barrel from which neuronal activity spreads to the other layers. Such laminar specificity has also been observed in the visual cortex by Harel et al. (2006), who found that the BOLD signal exhibited a peak in layer IV during stimulation. Silva et al. (2002) demonstrated that the onset of the BOLD response in layer IV occurs consistently earlier, compared to other layers in the rat somatosensory cortex. Our results suggest that discriminant analysis of voxels in the temporal domain by introducing nonlinearity into the decomposition is able to resolve subtle features of the BOLD response that are more specifically localized to the stimulated barrels as opposed to the surrounding septal neurons and the barrels to which the activation subsequently spreads. The excitation of cortical neurons in layer IV requires synchronous input from the projecting cells of the thalamus, and the greatest level of synchrony in these thalamic cells is produced by stimulation of the principal whisker of their receptive field (Bruno et al., 2006). The BOLD response of neurons in layer IV located in the barrels corresponding to the stimulated whiskers could display unique temporal characteristics that distinguish these voxels from their surroundings. Notably, we observed

that for each stimulation paradigm only one component derived from the discriminant analysis produced results that were both consistent in their position and in agreement with the anatomy of the barrel cortex, as shown in Table 1. Thus, evaluation and interpretation of the results from the discriminant analysis must take into account the known properties of the region under investigation.

Our results agree well with a study by Yang et al. (1996), who observed BOLD activity primarily in layer IV of the posteromedial barrel subfield during whisker stimulation in the rat. Although the authors used t-statistics maps to localize the whisker barrels, there are some key differences between those experiments and our study. The whiskers (D1 and D4) stimulated by Yang et al. were relatively far apart and not present within the same coronal slice. Furthermore, the rats used in this study were anesthetized. Other studies (Austin et al., 2005; Peeters et al., 2001) have examined the effects of anesthesia on the BOLD response in rats, and observed that anesthetized subjects exhibited a decrease in the spatial extent of BOLD activation. This effect would significantly decrease the horizontal spreading of activation from the site of the principal whisker barrel in anesthetized subjects (Armstrong-James et al., 1988), facilitating the resolution of individual whisker barrels. In our experiments, we were unable to resolve individual barrels for adjacent whiskers in awake animals using t-statistics regardless of the threshold level. In the correlation analysis between PCs/ICs and voxels in the somatosensory cortex, the width of the clusters corresponding to the stimulated whisker barrels was found to vary depending upon the threshold used. However, the threshold level shown in the results using the proposed method consistently produced barrel widths comparable to those measured in histological studies of the somatosensory cortex (Gould et al., 1986; McMullen et al., 1994).

The implementation of KPCA or ICA in the second step of analysis involved a number of considerations related to the experimental design. Temporal as opposed to spatial analysis was used for both KPCA and ICA because it can directly provide temporal profiles to identify subtle differences between whisker barrels and their surrounding regions. The choice of a kernel or objective function is a consideration for implementing KPCA or ICA, respectively. However, while the polynomial kernel function used in KPCA was heuristically selected and shown to be effective in our experimental studies, we found that other commonly-used kernel functions, such as the radial basis function (RBF) kernel, can also be used to obtain comparable results. The objective function *Tanh* in FastICA was chosen because it is suitable for general purpose use (Hyvärinen, 1999b), and can provide consistent results for fMRI study in a computationally efficient way to approximate the mutual information (Correa et al., 2007).

Additional factors that influenced the performance of the proposed method were the decomposition criteria of KPCA vs. ICA in the second step of the analysis. Temporal KPCA attempts to separate different sources of signal and noise in terms of temporal co-variation of brain voxels in the feature space projected by a kernel function, whereas temporal ICA, in contrast, attempts to obtain components with a minimum mutual dependency in the temporal domain. In terms of the decomposition criteria, therefore, KPCA may be more appropriate than ICA for the task of resolving individual functional units because voxels corresponding to whisker barrels are highly correlated to one another but differ in subtle ways from the surrounding brain regions. In KPCA the separation rule for the components is based upon the identification of differences in co-variation that can capture the subtle differences between barrels and neighboring voxels. For ICA, in contrast, the separation of components is based upon an assumption of complete independence between the temporal variations of barrels and their neighboring regions which apparently does not hold. This difference likely accounts for the superior spatial specificity achieved by KPCA-based analysis in our study, as compared to ICA. Nevertheless, we did observe that ICA was able to separate clusters of

activated voxels associated with whisker barrels in cases where correlation or t-test alone were unable to do so at an equivalent significance level. There are two possible reasons. One is that a sub-optimal ICA decomposition could generate components that retain dependency with one another, which could help to differentiate barrels from their surround regions. The other is that these sub-regions may contain subtle variations that are independent to each other, and ICA could potentially identify sub regions based upon such independence.

Although KPCA or ICA could be applied directly to the entire image to identify whisker barrels, this process would require the generation and examination of an impractically large number of components. Thus, the rationale for the two-level analysis approach is to localize the somatosensory cortex region in the first level of analysis and subsequently identify the sub-regions corresponding to the whisker barrels by examining a smaller number of components in the second level of analysis. One advantage of this approach, therefore, is that it considerably decreases the computational load by reducing the number of components required for the analysis. In addition, analysis of the entire brain slice would include sources of both signal and noise that are not directly related to the mapping of whisker barrels, which could complicate and degrade the performance of the discrimination process.

In summary, our results have shown that the proposed two-step analysis method can reliably resolve BOLD activation in the somatosensory cortex corresponding to individual whisker barrels, even in the case of adjacent whiskers. The improvement provided by this method lies in the ability of KPCA or ICA to jointly characterize the interactions of multiple voxels and detect subtle temporal differences that could reflect distinctive activity of layer IV cortical neurons. Future studies at higher temporal sampling rates could provide more detailed information about the nature of the changes distinguished by the decomposition step. However, in general this method is robust with respect to the choice of kernel and decomposition parameters, particularly in the case of KPCA which does not rely upon the assumption of independence to achieve its optimization. The major advantages of the proposed approach are that it can provide reliable detection of whisker barrels from relatively low SNR EPI data without the need for particular fMRI experimental designs, such as short TR or anesthesia of animals, and that it is computationally efficient. The proposed method should also be applicable to the analysis of cortical functional units in other sensory systems, such as the olfactory glomerulae and ocular dominance columns, which exhibit distinctive spatiotemporal properties in their BOLD response.

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References

- Austin VC, Blamire AM, Allers KA, Sharp T, Styles P, Matthews PM, Sibson NR. Confounding effects of anesthesia on functional activation in rodent brain: a study of halothane and α -chloralose anesthesia. *NeuroImage* 2005;24:92–100. [PubMed: 15588600]
- Armstrong-James M, George MJ. Influence of anesthesia on spontaneous activity and receptive field size of single units in rat Sm 1 neocortex. *Exp Neurol* 1988;99:369–387. [PubMed: 3338529]
- Armstrong-James M, Fox K, Das-Gupta A. Flow of excitation within rat barrel cortex on striking a single vibrissa. *J Neurophysiol* 1992;68:1345–1358. [PubMed: 1432088]
- Bruno RM, Sakmann B. Cortex is driven by weak by synchronously active thalamocortical synapses. *Science* 2006;312:1622–1627. [PubMed: 16778049]
- Cheng K, Waggoner RA, Tanaka K. Human ocular dominance columns as revealed by high-field functional magnetic resonance imaging. *Neuron* 2001;32:359–374. [PubMed: 11684004]

- Correa, Nicolle; Adali, Tülay; Calhoun, Vince D. Performance of blind source separation algorithms for fMRI analysis using a group ICA method. *Magn Reson Imaging* 2007;25:684–694. [PubMed: 17540281]
- Duong TQ, Kim DS, Uğurbil K, Kim SG. Spatiotemporal dynamics of the BOLD fMRI signals: toward mapping submillimeter cortical columns using the early negative response. *Magn Reson Med* 2000;44:231–242. [PubMed: 10918322]
- Friston K, Phillips J, Chawla D, Büchel C. Revealing interactions among brain systems with nonlinear PCA. *Hum Brain Mapp* 1999;8:92–97. [PubMed: 10524598]
- Friston K, Phillips J, Chawla D, Büchel C. Nonlinear PCA: characterizing interactions between modes of brain activity. *Philos Trans R Soc Lond B Biol Sci* 2000;355(1393):135–146. [PubMed: 10703049]
- Goodyear BG, Menon RS. Brief visual stimulation allows mapping of ocular dominance in visual cortex using fMRI. *Hum Brain Mapp* 2001;14:210–217. [PubMed: 11668652]
- Gould HJ III. Body surface maps in the somatosensory cortex of rabbit. *The Journal of Comparative Neurology* 1986;243:207–233. [PubMed: 3944277]
- Harel N, Lin J, Moeller S, Ugurbil K, Yacoub E. Combined imaging-histological study of cortical laminar specificity of fMRI signals. *Neuroimage* 2006;29:879–887. [PubMed: 16194614]
- Hyvärinen A. Survey on independent component analysis. *Neural Computing Surveys* 1999a;2:94–128.
- Hyvärinen A. Fast and robust fixed-point algorithms for independent component analysis. *IEEE Trans on Neural Networks* 1999b;10(3):626–634.
- Kida I, Hyder F, Xu F, Shulman R. Functional MRI of rat olfactory glomerulae: Mapping a single neuronal unit. *Proc Intl Soc Magn Reson Med* 2001;9:1492.
- Kida I, Xu F, Shulman R, Hyder F. Mapping at glomerular resolution: fMRI of rat olfactory bulb. *Magn Reson Med* 2002;48:570–576. [PubMed: 12210928]
- Kim DS, Duong TQ, Kim SG. High-resolution mapping of iso-orientation columns by fMRI. *Nat Neurosci* 2000;3:164–169. [PubMed: 10649572]
- Laaris N, Keller A. Functional independences of layer IV barrels. *J Neurophysiol* 2002;87:1028–1034. [PubMed: 11826066]
- McKeown MJ, Makeig S, Brown GG, Jung TP, Kindermann SS, Bell AJ, Spjnowski TJ. Analysis of fMRI data by blind separation into independent spatial components. *Hum Brain Mapp* 1998;6:160–188. [PubMed: 9673671]
- McMullen NT, Smelser CB, Rice FL. Parvalbumin expression reveals a vibrissa-related pattern in rabbit SI cortex. *Brain Research* 1994;660:225–231. [PubMed: 7820691]
- Menon RS, Goodyear BG. Submillimeter functional localization in human striate cortex using BOLD contrast at 4 Tesla: implications for the vascular point-spread function. *Magn Reson Med* 1999;41:230–235. [PubMed: 10080267]
- Peeters RR, Tindemans I, De Schutter E, Van der Linden A. Comparing BOLD fMRI signal changes in the awake and anesthetized rat during electrical forepaw stimulation. *Magnetic Resonance Imaging* 2001;19:821–826. [PubMed: 11551722]
- Petersen CC, Grinvald A, Sakmann B. Spatiotemporal dynamics of sensory responses in layer 2/3 of rat barrel cortex measured in vivo by voltage sensitive dye imaging combined with whole-cell voltage recordings and neuron reconstructions. *J Neurosci* 2003;23:1298–1309. [PubMed: 12598618]
- Schölkopf B, Smola A, Müller K. Nonlinear component analysis as a kernel eigenvalue problem. *Neural Computation* 1998;10:1299–1319.
- Silva AC, Koretsky AP. Laminar specificity of functional MRI onset times during somatosensory stimulation in rat. *Proc Natl Acad Sci* 2002;99:15182–15187. [PubMed: 12407177]
- Wyrwicz AM, Chen NK, Li L, Weiss C, Disterhoft JF. fMRI of visual system activation in the conscious rabbit. *Magn Res Med* 2000;44:474–478.
- Yang X, Hyder F, Shulman R. Activation of single whisker barrel in rat brain localized by functional magnetic resonance imaging. *Proc Natl Acad Sci* 1996;93:475–478. [PubMed: 8552664]

Figure 1.

Distribution of cortical layers in the primary somatosensory cortex. This figure illustrates the relative proportions of the cortical layers in the whisker barrel cortex overlaid on a gradient echo image obtained at the same location as the EPI data analyzed in this study. The approximate thickness of the layers is 0.66mm for layers I–III; 0.4mm for layer IV, and 1.44mm for layers V–VI. The full thickness of the cortex is therefore approximately 2.5mm. These relative proportions are based upon previous histological studies of the rabbit brain (Gould et al., 1986, McMullen et al., 1994).

Figure 2.

Analysis results for single-whisker (A3) stimulation. The correlation map (a) with the experimental paradigm and the t-statistics map (b), obtained at a significance level of 0.05 with the Bonferroni correction, show large clusters of activated voxels extending through layers I-IV of the contralateral somatosensory cortex. In the case of the t-statistics map, two distinct clusters are detected. Applying a second step of analysis which correlates voxels in the somatosensory cortex to components obtained from KPCA (c) and ICA (d) at a significance level of 0.05 and a cc value threshold of 0.35 produced visibly smaller clusters of activation, similar in area and location to each other, with the most highly correlated activity present at the level of cortical layer IV. The color bar in (a) is also applicable to (c) and (d).

Figure 3.

The average time course of the activated voxels in the somatosensory cortex as detected by (a) correlation analysis, (b) Student's t-test, and the proposed method using KPCA (c) or ICA (d) the in the second level of analysis. Note the similarity of all the profiles in terms of the amplitude, duration, and shape of the BOLD response. The higher noise level in (c) and (d) reflects the smaller number of voxels averaged within the activated regions.

Figure 4.

Analysis results for adjacent double-whisker (A2 & A3) stimulation. The correlation map (a) with the experimental paradigm and the t-statistics map (b), obtained at a significance level of 0.05 with the Bonferroni correction, show large clusters of activated voxels extending through layers I-IV of the contralateral somatosensory cortex, with no separation evident between the individual whisker barrels. Applying a second step of analysis which correlates voxels in the somatosensory cortex to components obtained from KPCA (c) and ICA (d) at a significance level of 0.05 and a cc value threshold of 0.35 produced visibly smaller clusters of activation with the most highly correlated activity present at the level of cortical layer IV. The component obtained from KPCA produced activated clusters of higher spatial specificity, as compared to ICA, and was able to separate fully the two adjacent barrels. The color bar in (a) is also applicable to (c) and (d).

Figure 5.

Analysis results for non-adjacent double-whisker (A1 & A3) stimulation. The correlation map (a) with the experimental paradigm and the t-statistics map (b), obtained at a significance level of 0.05 with the Bonferroni correction, show large clusters of activated voxels extending through layers I-IV of the contralateral somatosensory cortex. No separation was achieved between the individual whisker barrels at this significance level, although two foci of activation can be seen in the t-statistics map. Applying a second step of analysis which correlates voxels in the somatosensory cortex to components obtained from KPCA (c) and ICA (d) at a significance level of 0.05 and a cc value threshold of 0.35 produced visibly smaller clusters of activation with the most highly correlated activity present at the level of cortical layer IV. The component obtained from KPCA produced activated clusters of higher spatial specificity, although both discriminant analysis methods were able to separate the clusters corresponding to the non-adjacent barrels at this significance level. The color bar in (a) is also applicable to (c) and (d).

Table 1

Location measurements (average $\pm$ standard deviation) of whisker barrels A2 and A3^a identified by the proposed method using the KPCA decomposition

Whisker barrel	Depth ^b (mm)	Distance to midline ^b (mm)	Width of barrel in coronal section (mm ²)	Distance between barrels ^b (mm)
A2	1.14 $\pm$ 0.06	7.43 $\pm$ 0.3	0.72 $\pm$ 0.18	0.87 $\pm$ 0.15
A3	1.12 $\pm$ 0.04	8.32 $\pm$ 0.35	0.67 $\pm$ 0.14	

^aData for calculation were acquired from the same rabbit in nine experiments on three different days.

^bDepth, distance to midline, and distance between barrels were measured from the center of barrel.