Full length article

Model-free fMRI group analysis using FENICA

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Abstract

Exploratory analysis of functional MRI data allows activation to be detected even if the time course differs from that which is expected. Independent Component Analysis (ICA) has emerged as a powerful approach, but current extensions to the analysis of group studies suffer from a number of drawbacks: they can be computationally demanding, results are dominated by technical and motion artefacts, and some methods require that time courses be the same for all subjects or that templates be defined to identify common components.

We have developed a group ICA (gICA) method which is based on single-subject ICA decompositions and the assumption that the spatial distribution of signal changes in components which reflect activation is similar between subjects. This approach, which we have called Fully Exploratory Network Independent Component Analysis (FENICA), identifies group activation in two stages. ICA is performed on the single-subject level, then consistent components are identified via spatial correlation. Group activation maps are generated in a second-level GLM analysis.

FENICA is applied to data from three studies employing a wide range of stimulus and presentation designs. These are an event-related motor task, a block-design cognition task and an event-related chemosensory experiment. In all cases, the group maps identified by FENICA as being the most consistent over subjects correspond to task activation. There is good agreement between FENICA results and regions identified in prior GLM-based studies. In the chemosensory task, additional regions are identified by FENICA and temporal concatenation ICA that we show is related to the stimulus, but exhibit a delayed response. FENICA is a fully exploratory method that allows activation to be identified without assumptions about temporal evolution, and isolates activation from other sources of signal fluctuation in fMRI. It has the advantage over other gICA methods that it is computationally undemanding, spotlights components relating to activation rather than artefacts, allows the use of familiar statistical thresholding through deployment of a higher level GLM analysis and can be applied to studies where the paradigm is different for all subjects.

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Introduction

The most common approach to the analysis of fMRI data is to use knowledge of the timing of the presentation of stimuli and typical responses to formulate and evaluate a General Linear Model (GLM; Friston et al., 1995). The conformity of voxel time courses to known periods of task processing, modulated by a standard hemodynamic response, is evaluated to infer activation related to the task. While powerful and simple, the sensitivity of this approach is compromised if signal fluctuations do not show the expected time course. This may occur if activation arises only during a part of task execution, if the activated region exhibits non-standard hemodynamic response or if activation is temporarily unrelated to task processing but nonetheless impacts on neuronal response to the task (Fox et al., 2007).

There are a number of scenarios in which the timing of activation is either difficult to predict or does not conform to model expectations (Cunnington et al., 2003). Complex neuropsychological task designs such as emotion induction often cannot be split into well-defined phases, and task onsets and durations may be difficult to identify in self-paced designs. Exact specification of stimulus timing can also be challenging with patients who may be not able to execute a task promptly and reliably. Sensitivity is also reduced, and distinct

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responses become merged, when a task engages distinct networks in different processing stages (Fangmeier et al., 2006). Response magnitude may also deviate from basic model predictions in that it may not be constant with repeated trial presentations; stimulus repetition can lead to habituation (Breiter et al., 1996; Poellinger et al., 2001), or conversely, repetition enhancement (Henson, 2003; Grill-Spector et al., 2006).

Model-conform responses are dependent on model-conform hemodynamic coupling. Differences in the hemodynamic response function (HRF) across subjects, brain regions and sessions have been reported (Aguirre et al., 1998; Menz et al., 2006; Cunnington et al., 2003; Neumann et al., 2003; Handwerker et al., 2004). As an extreme example, it has been shown that activation in the hippocampus can lead to negative BOLD signals, most likely as a result of a small local perfusion response to elevated oxygen consumption (Schrödel et al., 2008). Moreover, specifying a valid HRF for a group becomes problematic in the presence of pathology or medication.

Fully exploratory analysis methods such as Independent Component Analysis (ICA), introduced by McKeown et al. (1998), do not require the specification of a model or a HRF. They have been shown to offer a powerful approach to the identification of activation in response to tasks and in the study of resting-state networks, for which no a priori information about temporal evolution is available (Beckmann et al., 2005). A number of group ICA (gICA) approaches have been developed to extend ICA from the analysis of a single data set to the analysis of data from a group of subjects (Calhoun et al., 2009). The most widely adopted method is to concatenate single-subject data in time prior to decomposition (Calhoun et al., 2001; Beckmann et al., 2005) (for a comparison of toolboxes using temporal concatenation ICA see Schöpf et al. (2010b)). The computer memory requirements associated with processing all data sets simultaneously generally necessitate data be downsampled. This may be undesirable when high resolution features are of interest — for instance, in presurgical fMRI of a single subject with a large number of runs (Shimony et al., 2009) or where small structures are important for interpretation (e.g. the substantia nigra in a basal ganglia resting-state network (Robinson et al., 2009); partial voluming introduced by downsampling can destroy such fine features. In addition, temporal concatenation ICA (concatICA) yields a large number of components (in the approximate range 30–300, depending on the amount of data and the criteria for selecting the optimum number of components). Independent sources relate not only to activation but also physiological and technical noise, and have to be inspected and subjectively identified by an experienced investigator. Another approach to gICA, Tensorial ICA (Beckmann and Smith, 2005) requires that the time course be the same for all subjects, precluding its application to most complex fMRI paradigms and resting-state studies. An early approach to gICA was to perform single-subject ICA decompositions and attempt to identify related components by matching these to one or more templates, which needed to be defined (Deluca et al., 2006). This is time-consuming, introduces a priori information, and is prone to omission and bias.

In addition to isolating common components amongst subjects, gICA methods face the challenge of attributing statistical significance to group components. For spatial concatenation gICA this has been achieved via a mixture modeling approach (Beckmann et al., 2006) or by back-projecting group components to elicit single-subject responses which are entered into a second-level GLM (Calhoun et al., 2001). Without calling the validity of these methods into question, both are sufficiently unfamiliar to the fMRI community large to lead to a lack of confidence in gICA thresholds, and the results of inter-group gICA comparisons (e.g. between patients and controls) in particular.

Promising clustering and hierarchical-modeling approaches, also based on single-subject ICA have recently been introduced by Esposito et al. (2005) and Varoquaux et al. (2010). Esposito's algorithm introduces a complex similarity measure by taking into account spatial and temporal characteristics for clustering. As temporal resting-state network (RSN) patterns do not imply very diverse temporal characteristics this leads to unpredictable outcomes if clustering parameters are not adjusted properly. Furthermore, rank ordering of the extracted components is biased by visual selection of component maps. Varoquaux's algorithm introduces a hierarchical model pattern selection using non-parametric noise description for model-order selection on a multi-subject level, which has been tested on resting-state data. Though the method is fully automated, and yields activity maps and meaningful and reproducible features, the algorithm tends to split components of common origin, and produce undefined components as well as activity-related maps and resting-state networks.

In order to overcome these limitations, we have developed a novel approach which we call Fully Exploratory Network Independent Component Analysis (FENICA). FENICA was introduced in the context of group ICA methods for identifying resting-state fMRI data, and is predicated on the spatial consistency of network activity across subjects (Schöpf et al., 2010a). The method is based on single-subject ICAs, followed by grouping and second-level analysis of spatially consistent components across subjects. As well as being computationally much less demanding than temporal concatenation group ICA, FENICA yields activation maps without the need for visual inspection, and operates in a statistical framework similar to that used in conventional GLMs. We assess the performance of this method in the study of task-related activation by applying it to data from three experiments covering a wide range of experiment designs and stimuli, and compare it to common group approaches such as concatenation ICA (concatICA) and GLM.

Material and methods

Studies

Study I: motor experiment

Twenty-eight healthy subjects (mean age 27.3 ± 7.1 years, 16 females) participated with written informed consent in the study, which was approved by the Ethics Committee of the Medical University of Vienna, Austria.

Subjects were asked to perform a go/no-go motor task. Interstimulus-intervals (ISI) were 15 s, comprising a white crosshair for 10 s followed by a yellow crosshair for 5 s. The yellow crosshair indicated that a trial was imminent. A green crosshair, presented for 1 s, signalled a “go” trial, for which subjects were asked to perform the button press sequence (right→left→right button) using the index and middle fingers of the right hand. “No go” trials were signalled by a red crosshair, presented for 1 s. Each run consisted of 14 “go” trials and 5 “no go” trials.

Measurements were performed with a 3 Tesla Medspec 5300 system (Bruker Biospin, Ettlingen, Germany) using single-shot gradient-recalled EPI (TE = 40 ms, TR = 1000 ms, FOV = 230 × 190 mm, flip angle = Ernst angle, 14 slices of 5 mm thickness (1 mm gap), matrix size 64 × 96). Slices were aligned to the line connecting the anterior and posterior commissures. During the experiment 330 image volumes were acquired.

Study II: cognitive experiment

The 28 subjects who participated in Study I also took part in this experiment.

The Tower of London (TOL) paradigm was presented. Subjects were asked to determine the minimum number of moves required to achieve a target configuration of balls on pegs from a given starting configuration, moving only one ball at a time. The balls were of three different colors and were arranged on three pegs of different lengths. There were three balls on the left peg (of length 3 units), two balls on
the middle peg (of length 2 units) and one ball on the right peg (of length 1 unit). The starting and target configurations were presented simultaneously, along with two response options (the correct minimum number of moves and another plausible, but false, answer). The task was presented until subjects indicated their response by button press. The interstimulus interval was 12 s, during which a crosshair was displayed.

The same measurement parameters were used for the acquisition of functional images as in Study I. For each subject 475 image volumes were acquired.

Study III: chemosensory experiment

Twenty-two healthy subjects (mean age 29.0 ± 5.6 years, 13 females) were included in the experiment. Subjects were not taking any medication known to interfere with chemosensory perception. All subjects were informed about the aim of the study and gave their written informed consent prior to inclusion in the study, which was approved by the Medical Ethics Committee of the Ludwig Maximilians-University of Munich, Germany.

A computer-controlled air-dilution olfactometer (OM6b, Burghart Instruments, Wedel, Germany) was used for repeated chemosensory stimulation without any simultaneous stimulation of mechanoreceptors in the nose (Kobal, 1985; Kobal and Hummel, 1988). Monorhinal pulses of CO₂ (50–60%) were randomly applied to the left and right nostrils in 500 ms bursts, engendering a painful stinging sensation. To avoid respiratory air flow in the nose, the subjects were trained to breath using velopharyngeal closure (Kobal, 1985). Stimuli were embedded in a constant-temperature of 36.5 °C and relative humidity of 80% to prevent mechanical or thermal alterations of the stimulated nasal mucosa. Stimuli were presented in a sparse event-related design, with 40 stimuli and an interstimulus interval of 20 s (±4 s), with identical timing for all subjects.

Functional images were acquired with a 3 T MRI scanner (Signa HDx, GE Healthcare, Milwaukee, WI, USA) using single-shot gradient-recalled EPI (TE = 35 ms, TR = 2100 ms, FOV = 240×240 mm, flip angle = 90°, 37 slices of 4 mm thickness and a matrix size of 64×64). Slices were acquired parallel to the line connecting anterior and posterior commissures. For each subject 475 image volumes were acquired.

Preprocessing

The same measurement parameters were used for the acquisition of functional images as in Study I. The starting and target configurations were presented simultaneously, along with two response options (the correct minimum number of moves and another plausible, but false, answer). The task was presented until subjects indicated their response by button press. The interstimulus interval was 12 s, during which a crosshair was displayed.

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Data analysis

Preprocessing

Image preprocessing for all three studies was performed with SPM5 (http://www.fil.ion.ucl.ac.uk/spm/) including slice-timing and motion correction, normalization to an MNI template and spatial smoothing using a Gaussian kernel (FWHM = 9 mm). To correct for artefacts related to drift, breathing, motion and heartbeat, ROI time courses were extracted, for each subject, in white matter and the ventricles (in the cornu occipitale). These were entered into a first-level SPM design matrix as nuisance regressors. Beta estimates for the two regressors were multiplied by the respective ROI time courses and subtracted from the data (for full details see Weissenbacher et al. [2009]).

FENICA

Analysis with FENICA consisted of two stages (see Fig. 1): single-subject ICA (Stage 1) and second-level GLM analysis of components matched through cross-correlation analysis (Stage 2).

Stage 1: Single-subject ICA was performed using probabilistic ICA (Beckmann and Smith, 2004) as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) version 3.05, a part of FSL (FMIRI’s Software Library, www.fmrib.ox.ac.uk/fsl). The optimum number of components to be estimated for each subject was determined using the Minimum Description Length criterion (MDL; Rissanen, 1978).

Stage 2: For all N subjects ICA returned N_{TOTAL} components, whereby c_{k,m} were denoted as the spatial map of component m of subject k. The number of components for subject k was referred to as N_k. Correlation coefficients of component m_1 of subject k_1 and component m_2 of subject k_2 were given by equation 1 in Fig. 1 forming a correlation matrix CC of size N_{TOTAL} × N_{TOTAL} for all N subjects.

Average maps \( S_{\ell} \) were formed using Eq. (2) in Fig. 1 for all \( k_1 \in \{1, \ldots, N\}, k_2 \in \{1, \ldots, N\} \setminus \{k_1\}, m_1 \in \{1, \ldots, N_k\}, \) and \( m_2 \in \{1, \ldots, N_k\} \) resulting in N_{AVG} components, with N_{AVG} = \( \frac{1}{2} (N_{TOTAL}^2 - \sum_{k=1}^{N} (N_k)^2) \).

Each average map \( S_{\ell} \) for \( \ell = 1, \ldots, N_{AVG} \) was spatially correlated with all N_{TOTAL} component maps, and the map with the highest

![Fig. 1. Schematic diagram of data processing steps from single-subject fMRI raw data to group activity maps. For a detailed description of the component selection process we kindly refer the reader to Schöpf et al. (2010a).](image-url)
correlation was determined for each subject, defining an activity matrix \( \mathbf{A} \) of size \( N \times N_{\text{NAVG}} \) (see Eq. (3) in Fig. 2). A spatial equivalence level was introduced as a metric on the basis of which \( g \) spatially matching maps may be merged in order to avoid duplicating maps. Mean maps, corresponding t-maps and the sum of all absolute t-values were calculated. Mean maps of the activity map corresponding to the highest t-value sum were correlated with all other mean maps as described in detail in Schöpf et al. (2010a).

Single-subject component maps for the map with the highest t-value were analyzed in SPM using a one sample t-test. All steps in Stage 2 (see second stage in Fig. 1) were performed in Matlab (Matlab 6.5, Release 13, Mathworks Inc., Sherborn, MA, USA). Computations were performed on a CALLEO 321 Server equipped with two Quad-Core Intel Xeon E5450 3.0 GHz processors and 16 GB RAM running Ubuntu Linux 8.04 (Kernel version 2.6.24).

Comparison of FENICA and concatICA

In addition to analysis with FENICA, all studies were also analyzed using temporal concatenation group ICA (concatICA) introduced as probabilistic ICA (PICA) (Beckmann and Smith, 2004) as implemented in MELODIC (Multivariate Exploratory Linear Decomposition into Independent Components) version 3.0, a part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Before ICA was conducted, non-brain voxels were masked, and a voxel-wise de-meaning and normalization of the voxel-wise variance were performed. Pre-processed data were prewhitened and projected into a subspace using probabilistic Principal Component Analysis estimating the number of dimensions using the Minimum Description Length (MDL; Rissanen, 1978). MELODIC implements the algorithm FastICA which is based on the principle of maximizing higher-order statistics (i.e. negentropy) of the output to maximize the non-gaussianity of the estimated sources using fixed-point iterations. The optimum number of components to be estimated was determined. For the optimization of the non-gaussian sources contrast function and convergence threshold as suggested by Hyvärinen et al. (2001) were used. Estimated component maps were divided by the standard deviation of the residual noise and thresholded by fitting a mixture model to the intensity values histogram (Beckmann and Smith, 2004).

Comparison of FENICA and GLM analyses

In addition to analysis with FENICA, all studies were also analyzed with a GLM approach. For single-subject analysis, statistical parametric maps were calculated using SPM5 with regressors corresponding to the onset times of the ‘go’-events representing the motor condition (Study I), the individual TOL-tasks (Study II), and the \( \mathrm{CO}_2 \) events (Study III), respectively, convolved with the canonical HRF. A random effects analysis was performed over first level contrasts, and results were FDR corrected with \( p < 0.05 \) (Genovese et al., 2002).

A more detailed comparison of FENICA and GLM was conducted for Study III: for each supra-threshold cluster in the group results, the time course of the voxel with the highest t value was extracted for each subject. Group mean time courses were compared to the stimulus curve convolved with the HRF.

Results

The mean number of components obtained in single-subject ICAs was 25 ± 4 for Study I, 32 ± 24 for Study II and 29 ± 23 for Study III. As a direct result of the sample size (\( N \)) and the total number of independent components (\( N_{\text{TOTAL}} \)), values of \( N_{\text{NAVG}} \) given by the number of entries of the upper triangular matrix (CC) of size \( N_{\text{TOTAL}} \times N_{\text{TOTAL}} \) varied from 168,344 (for Study III) to 380,299 (for Study II).

Table 1 lists the mean number of components and standard deviations obtained with single-subject ICA for the three experiments, along with processing variables used in the FENICA analysis, including the total number of components \( N_{\text{TOTAL}} \) (which also indicates the size of the resulting correlation matrix, CC) and the number of average maps \( N_{\text{NAVG}} \).

Histogram plots of cross-correlation matrices show a decreasing occurrence of higher correlation coefficients (Fig. 2). For computational reasons, \( N_{\text{NAVG}} \) was limited to the 400 elements of the correlation coefficient matrix with the highest cc values. Although \( N_{\text{NAVG}} \) values varied across studies, cc ranges of the 400 highest elements of the correlation coefficient matrix were consistent. Ranges, mean values, and standard deviations of the 400 elements of each correlation coefficient matrix (cc range) are also given in Table 1. Not limiting \( N_{\text{NAVG}} \) increases calculation time unnecessarily, as low CC elements do not reflect consistent activation. The mean number of resulting activity networks, \( g \), varied for applied equivalence levels (nine levels between 0.1 and 0.9) for the three experiments: 7 networks (range ([3;12])) for Study I, 10 networks (range ([2;26])) for Study II, and 21 networks (range ([4;58])) for Study III. For each study, the activity map with the highest t-value sum was consistent over equivalence levels. For each experiment, the most consistent activity map (that with the highest t-value sum) corresponded to activation related to the task, and is described in more detail in the succeeding paragraphs.

Analysis using concatenated group ICA revealed 33 components for Study I, 29 components for Study II, and 37 components for Study III. Visual inspection was used to identify task-related activity maps.

A comparison of components detected with concatICA and FENICA is given in Table 2.

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**Fig. 2.** Histogram of correlation coefficient matrices (a) \( \mathrm{CC}_{\text{motor}} \) (698 × 698), (b) \( \mathrm{CC}_{\text{TOL}} \) (897 × 897), and (c) \( \mathrm{CC}_{\text{CO}_2} \) (628 × 628). All histogram plots of the cross-correlation matrices show decreasing occurrence of higher correlation coefficients. Therefore, \( N_{\text{NAVG}} \) (representing the elements of the coefficient matrix CC) was limited to 400, corresponding to the 400 highest elements, which ranged from 0.45 to 0.73 for Study I, from 0.42 to 0.66 for Study II, and from 0.37 to 0.58 for Study III (see Table 1). Intervals are marked with a red line.
Study I

Analysis using FENICA yielded, as the group component with the highest t-value sum, activation including pre-supplementary motor area (preSMA), supplementary motor area (SMA), right prefrontal regions, primary motor areas, pre-motor cortices, and occipital regions. A list of MNI co-ordinates of activation foci is given in Table 3.

Analysis using concatICA revealed two task-related components (≠ 10, ≠ 16) showing a splitting of motor-related activity into its lateral parts. Analysis using GLM yielded one predominately left lateralized motor processing network. A detailed visualization of significantly activated brain regions for all three methods is given in Fig. 3.

Study II

On average subjects completed 10.7 ± 3.0 TOL conditions in an average of 26.7 ± 16.4 s.

The most significant group component identified involved superior parietal regions, inferior parietal regions, dorsolateral prefrontal cortex, and inferior frontal regions. Activated foci are listed in Table 4. Group ICA revealed two task-related components (≠ 4, ≠ 7) showing two task-related activity maps splitting the large scale working memory network into its anterior and posterior parts. Analysis using GLM obtained mostly overlap with areas detected with both concatICA and FENICA. A detailed visualization of significantly activated brain regions for all three methods is given in Fig. 4.

Study III

Analysis of the chemosensory experiment using FENICA resulted in a map characterized by activity in regions including the thalamus, anterior cingulate cortex, insula, primary somatosensory cortices, precentral gyrus and brainstem. A detailed description of significantly activated brain regions is given in Table 5. ConcatICA detected one task-specific component (≠ 21) showing a great overlap to activated regions detected with FENICA. Significantly activated brain regions for all three analyses are visualized in Fig. 5.

Comparing FENICA and GLM analyses revealed four activity clusters: parts of the primary and secondary visual cortices (belonging to BA 17 and 18) and middle and inferior frontal areas (parts of BA 9, 10, and 45) which were detected with FENICA only (see Fig. 6).

Analysis of the temporal characteristics of these four ROIs in the group as well as in selected subjects showed activation to be delayed compared to the expected response (i.e., the stimulation periods convolved with a hemodynamic response).

Discussion

The aim of this study was to develop an exploratory analysis method for identifying activation in group data without the need for data-reduction, restriction of the number of components into which the data is deconstructed, common time courses amongst subjects, template matching or a priori knowledge of stimulus presentation or HRF characteristics. FENICA is a purely data-driven method based on single-subject ICA. We have tested this in three studies using motor, cognitive and chemosensory paradigms. In each case, the most consistent component identified with FENICA corresponded to activation related to the task.

The motor paradigm of Study I has been applied in a number of fMRI studies to examine motor planning and execution (for a meta-analysis of motor go/no-go paradigms see Simmonds et al. (2008)). FENICA yielded activity maps including all major areas known to be activated within the process of the successful preparing and executing of motor “go” stimuli (pre-SMA, SMA, motor cortices and occipital regions). This paradigm has been chosen to demonstrate the power of GLM as well as FENICA in a simple, regularly timed task design.

The Tower of London task (TOL) used in Study II has been widely used to assess executive functions such as planning and working memory (Baker et al., 1996; Berg and Byrd, 2002; Carder et al., 2004; Dagher et al., 1999; Morris et al., 1993; Owen et al., 1990; Shallice, 1982; Newman and Pittman, 2007; Newman et al., 2003; Utterrainer et al., 2003, 2004). The ability to plan involves strategy, coordination and sequencing of mental functions, and holding information in working memory. Problem solving tasks like the TOL have been shown to involve large-scale networks of cortical regions (Mesulam, 1990, 1998), including superior parietal regions (SPL) (BA 5 and 7), inferior parietal regions (IPL) (BA 40 and 39), dorsolateral prefrontal cortex (DLPFC) (BA 9, 10, 46, parts of BA 6 and 8), and inferior frontal regions (IFG) (BA 44, 45, and 47). An important part of the cortical network of planning ability seems to be the prefrontal cortex, as suggested by several studies using the TOL with patients suffering from frontal brain lesions (Owen et al., 1990; Shallice, 1982), frontal lobe dementia (Carlin et al., 2000) and schizophrenia (Morris et al., 1995; Pantelis et al., 1997; Schall et al., 1998). Using FENICA we were able to reproduce an activity map induced by the TOL task including all regions described in these studies to date.

Applying chemosensory stimuli in fMRI is challenging due to the strong connection to emotions and mnemonic processes, which leads to temporally unpredictable activation. This makes it a particular appropriate testing ground for an exploratory analysis method. Carbon dioxide (CO₂) stimulation of the nasal mucosa was chosen for Study III as a well established means of acute experimental

<table>
<thead>
<tr>
<th>Region</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>p</th>
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<td>11.76</td>
</tr>
<tr>
<td>R. precentral gyrus</td>
<td>48</td>
<td>12</td>
<td>54</td>
<td>11.67</td>
</tr>
<tr>
<td>L. postcentral gyrus (BA 4)</td>
<td>54</td>
<td>16</td>
<td>32</td>
<td>11.73</td>
</tr>
<tr>
<td>L. secondary visual cortex</td>
<td>4</td>
<td>80</td>
<td>12</td>
<td>3.56</td>
</tr>
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</table>

Table 2
Overview of concatICA and FENICA results. Number of estimated number of components using the Minimum Description Length criterion for concatICA, number of resulting activity related network and corresponding component numbers for concatICA and FENICA are provided.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Estimated comp. concatICA</th>
<th>Activity related NWs for concatICA</th>
<th>Comp. nb.</th>
<th>Activity related NWs for FENICA</th>
<th>Comp. nb.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study I</td>
<td>33</td>
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<td>1</td>
<td>≠ 1</td>
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<tr>
<td>Study II</td>
<td>29</td>
<td>≠ 4, ≠ 7</td>
<td>1</td>
<td>≠ 1</td>
<td></td>
</tr>
<tr>
<td>Study III</td>
<td>37</td>
<td>≠ 21</td>
<td>1</td>
<td>≠ 1</td>
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trigeminal pain (Kobal, 1985; Hari et al., 1997; Handwerker and Kobal, 1993).

Most odorants are known to affect both the trigeminal and the olfactory systems (Doty et al., 1978). Applying CO₂ to the nasal mucosa is known to activate areas involved in the processing of chemosensory signals as well as brain areas known to be engaged in handling painful stimuli (Iannilli et al., 2008; Boyle et al., 2007; Hummel et al., 2005, 2009).

Consistent with a meta-analysis of imaging studies using intranasal CO₂ stimulation by Albrecht et al. (2010), we were able to detect activation within the thalamus, anterior cingulate cortex, insula, primary and secondary somatosensory cortices, precentral gyrus and brainstem. Our results are in agreement with the hypothesis of CO₂ application being able to access the general pain processing network.

As GLM outcomes showed wide agreement in terms of activity maps for Studies I and II, direct comparison of activity clusters obtained with FENICA and a GLM analysis for Study III resulted in the detection of three frontal regions and one visual region with FENICA only. Several studies have applied chemosensory stimulation to evoke activity in regions related to the processing of other sensory stimuli such as vision and olfaction. Barry et al. (2001) showed that stimuli which were exclusively gustatory also were able to evoke activation in other sensory regions such as BA 17 and 18, part of the primary visual cortex. Frontal regions which were detected using FENICA are known to be part of a general pain processing network (for example see Kong et al. (2010)).

Analysis of the time courses underlying ROIs showed a temporal shift in activity, delayed with respect to the expected response, explaining why these areas were not identified in the GLM analysis. We were also able to show that the variation of stimulus-evoked responses was different for the selected ROIs within one subject.

Using the temporal concatenation group ICA generated a large number of group components (see Table 2), from which those reflecting activations have to be identified by visual inspection. In addition, task activity appeared in separate dorsal/ventral, and posterior/anterior components, indicating oversplitting of the data. Technical, physiological and motion artefacts dominated the results, appearing as components # 3, 5, 6, 8, 9, 14, 17, 18, 23, 24, 25, 28, 30, 32, and 33 for Study I, # 1, 7, 8, 10, 12, 13, 18, 19, 20, 21, 23, 25, 28, and 29 for Study II, and # 1, 2, 4, 6, 7, 10, 11, 12, 15, 20, 22, 24, 28, 29, 32, 35, 36, and 37 for Study III. No components reflecting significant noise or artefacts were detected using FENICA with the reported parameters. FENICA is based on the principle that activation is spatially consistent across subjects. Because artefactual sources (technical noise, respiration, movement, and cardiac action, for instance) are not generally consistent across subjects, these do not occur in FENICA results. In contrast, artefactual signals present in the data of a single subject (or a small number of subjects) in a concatICA tend to generate group components, as the criteria for identifying components are not consistency over the group (about which the concatICA approach has no information) but rather simply that the signal represents a significant source of fluctuation in the concatenated data set. Changing the equivalence level parameter as reported in Schöpf et al. (2010a) (the correlation coefficient at which networks are considered to originate from the same source) often result in a multiple detection of the same network of activation. If a number of data sets are affected by severe motor artefacts (e.g., speech or chin-motion paradigm), all group analysis methods (including FENICA) would be expected to detect significant group components as motion artefacts would be similar for all subjects.

Fig. 3. Activity maps of the motor experiment (Study I) analyzed with FENICA, concatICA, and GLM projected onto a standard template (FDR corrected, p<0.05). Shown are axial slices in Talairach space (z = -12, 28, 48, and 63) and a sagittal view indicating the position of the axial slices.

Table 4
Selected activity clusters and corresponding coordinates and corresponding T-values of the detected FENICA network activity map within Study II shown in Fig. 4 are reported (p<0.05 FDR-corrected, MNI coordinates in mm). Labeling of anatomical regions was performed using the SPM Anatomy Toolbox by Eickhoff et al. (2005).

<table>
<thead>
<tr>
<th>Region</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. superior parietal lobule</td>
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<td>-62</td>
<td>56</td>
<td>13.56</td>
</tr>
<tr>
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<td>-54</td>
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<td>8.85</td>
</tr>
<tr>
<td>R. middle temporal gyrus</td>
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<td>-60</td>
<td>-4</td>
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<tr>
<td>R. middle frontal gyrus (BA 6)</td>
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<td>10</td>
<td>66</td>
<td>8.20</td>
</tr>
<tr>
<td>R. inferior frontal gyrus (BA 45)</td>
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<td>36</td>
<td>22</td>
<td>7.86</td>
</tr>
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<td>L. anterior cingulate cortex</td>
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<td>32</td>
<td>24</td>
<td>6.32</td>
</tr>
<tr>
<td>L. supplementary motor area</td>
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<td>12</td>
<td>64</td>
<td>6.20</td>
</tr>
<tr>
<td>R. middle frontal gyrus (BA 10)</td>
<td>36</td>
<td>54</td>
<td>-4</td>
<td>4.80</td>
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</table>
Regression-based methods, such as the GLM implemented in AFNI (http://afni.nimh.nih.gov/afni), Brainvoyager (http://www.brainvoyager.com/), FSL (http://www.fmrib.ox.ac.uk/fsl/), and SPM (http://www.fil.ion.ucl.ac.uk/spm/), enable group analysis on the basis of single-subject analysis, taking into account variability across the group. We have described and illustrated the problems which arise when either neuronal or vascular responses are non-conform. FENICA is a group analysis method, based on ICA, which offers the opportunity to analyze fMRI data without model specification.

A number of group ICA methods already exist. The most widely adopted approach is to temporally concatenate single-subject data prior to performing an ICA (Beckmann and Smith, 2004; Calhoun et al., 2001). Computational challenges quickly arise due to the number of voxels and sample times included. For example, a standard volume of a subject consists of 517,845 (79×95×69) voxels, and if 450 volumes were acquired for 25 subjects, 517,845×450×25≃ 450 volumes were acquired for 25 subjects, 517,845×450×25 = 6·10¹⁰ data values would have to be analyzed at once. In temporal concatenation ICA therefore there is generally a need to radically reduce data before the ICA. This is either done by applying principal component analysis (PCA) or by downsampling the data.

A further challenge in gICA is the need to identify and evaluate group components. This can either be done by temporally correlating the model time course with the corresponding time courses of the group components or by template matching, which includes the spatial correlation of a predefined template with the group component maps. Template matching can also be performed on a single-subject level by selecting one component per subject based on the template and subsequently conducting a t-test on the derived components. Templates used are mostly defined through results of a previous study or, if patients are being compared to a control group, on the outcome of the control group. Though template matching tries to preserve the exploratory character of ICA, the resulting group components are strongly dependent on the template. If the components are evaluated based on the temporal correlation method, the resulting components are again coupled to a precise model definition.

Another form of group ICA is provided by TICA (Beckmann and Smith, 2005), by estimating a three-dimensional tensor representing group spatial maps, group time courses and subject-specific modes for each component. Though maintaining the multidimensionality and conserving the single-subject character, this method is constrained to use where identical stimulus timing exists for all subjects. Due to complex paradigm designs and stimuli provoking partly unpredictable neuronal activity this has become a major drawback, excluding its use in most sophisticated task presentation schemes and resting-state studies.

As well as requiring no model or HRF definition, uniquely amongst gICA methods FENICA requires no data reduction, even in high resolution studies of a large number of subjects. No template definition is needed, and it can be applied where stimulus timing is different for all subjects.

Limitations of FENICA are the unsolved problem of an implemented approach for group comparison which should be a topic of further research. Furthermore influences on resulting networks concerning sample size variability and model order changeability were not addressed in this study but will be investigated in future research.

Table 5
Selected activity clusters and corresponding coordinates and corresponding T-values of the detected FENICA activity map within Study III (shown in Fig. 5) are reported (p<0.05 FDR-corrected, MNI coordinates in mm). Labeling of anatomical regions was performed using the SPM Anatomy Toolbox by Eickhoff et al. (2005).

<table>
<thead>
<tr>
<th>Region</th>
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<th>y</th>
<th>z</th>
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</thead>
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</tr>
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<td>7.50</td>
<td></td>
</tr>
<tr>
<td>R. cingulate gyrus</td>
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</tr>
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<tr>
<td>R. piriform cortex</td>
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<td>16</td>
<td>4.55</td>
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<tr>
<td>L. brainstem</td>
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<td>4.33</td>
<td></td>
</tr>
<tr>
<td>R. primary somatosensory cortex</td>
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<td>3.54</td>
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<tr>
<td>R. precentral gyrus</td>
<td>44</td>
<td>16</td>
<td>3.00</td>
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</tbody>
</table>

Fig. 4. Activity map of the cognition experiment (Study II) analyzed with FENICA, concatICA, and GLM projected onto a standard template (FDR corrected, p<0.05). Shown are axial slices in Talairach space (z = -2, 8, 26, 38, 48, and 63) and a sagittal view indicating the position of the axial slices.
Conclusion

The new ICA method FENICA, recently presented in Schöpf et al. (2010a), is useful in the analysis of activation data as well as resting-state data. This novel, model free approach to the analysis of group fMRI data reliably identified activation in a wide variety of paradigms and stimuli types. Activation maps are in excellent agreement with those established in previous, model-based analyses. FENICA has the potential to become a valuable tool for group fMRI studies, eliminating a priori assumptions including model and HRF, and without the need to downsample data in large studies, define spatial templates or manually identify single-subject or group components. Using FENICA it is possible to analyze functional MRI data of experiments using complex stimulus design involving different modalities on a truly data-driven basis. FENICA is a single-subject based technique allowing for group statistics to be applied in a well-established framework and provides a truly exploratory, data-driven, operator independent and therefore an unbiased way of identifying common patterns of activation.

Acknowledgments

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