Investigation of fMRI neurofeedback of differential primary motor cortex activity using kinesthetic motor imagery

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A R T I C L E   I N F O

Article history:
Accepted 20 February 2012
Available online 3 March 2012

Keywords:
Real-time fMRI
Biofeedback
Neurofeedback
Motor imagery

A B S T R A C T

Functional MRI neurofeedback (fMRI NF) is an emerging technique that trains subjects to regulate their brain activity while they manipulate sensory stimulus representations of fMRI signals in “real-time”. Here we report an fMRI NF study of brain activity associated with kinesthetic motor imagery (kMI), analyzed using partial least squares (PLS), a multivariate analysis technique. Thirteen healthy young adult subjects performed kMI involving each hand separately, with NF training targeting regions of interest (ROIs) in the left and right primary motor cortex (M1). Throughout, subjects attempted to maximize a laterality index (LI) of brain activity—the difference in activity between the contralateral ROI (relative to the hand involved in kMI) and the ipsilateral M1 ROI—while receiving real-time updates on a visual display. Six of 13 subjects were successful in increasing the LI value, whereas the other 7 were not successful and performed similarly to 5 control subjects who received sham NF training. Ability to suppress activity in the ipsilateral M1 ROI was the primary driver of successful NF performance. Multiple PLS analyses depicted activated networks of brain regions involved with imagery, self-awareness, and feedback processing, and additionally showed that activation of the task positive network was correlated with task performance. These results indicate that fMRI NF of kMI is capable of modulating brain activity in primary motor regions in a subset of the population. In the future, such methods may be useful in the development of NF training methods for enhancing motor rehabilitation following stroke.

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Introduction

Functional magnetic resonance imaging (fMRI) has become a viable technique for investigating how individuals can regulate their brain activity. Real-time fMRI (rt-fMRI) enables measurements and analyses of blood oxygenation level dependent (BOLD) signals spatially localized on the scale of millimeters to centimeters (Cox et al., 1995; Voyvodic, 1999) with a temporal resolution of approximately seconds or less. These signals can then be presented in the form of sensory stimuli for the subject to regulate, in a biofeedback process that is often termed “neurofeedback” (NF). Although it is possible to derive feedback signals using whole brain analysis methods like support vector machine classification and other multivariate supervised learning approaches (Eklund et al., 2009; Hollmann et al., 2009; LaConte et al., 2007), most studies to date have involved NF of signals from localized regions of interest (ROIs). Work using ROI-based NF has targeted diverse brain regions involved in emotion (Caria et al., 2007; Hamilton et al., 2011; Johnston et al., 2010, 2011; Posse et al., 2003; Weiskopf et al., 2003), pain (deCharms et al., 2005), audition (Haller et al., 2010; Yoo et al., 2006, 2007), and language (Rota et al., 2009). In these reports, there have been overt behavioral manifestations of NF training, such as changes in emotional state or the sensation of pain, which have potential medical and therapeutic importance (deCharms, 2007).

There have also been a number of NF studies investigating the motor system, with overt hand movement (Yoo and Jolesz, 2002) and with motor imagery (Bray et al., 2007; deCharms et al., 2004; Johnson et al., 2012; Yoo et al., 2008). Neurofeedback methods using motor imagery may be potentially useful to enhance motor rehabilitation of stroke or other physical disabilities (Sharma et al., 2006). For example, one study has found evidence for regional enhancement of BOLD activation using motor imagery NF (deCharms et al., 2004), compared to a control group that received sham NF. Such an approach could potentially be used to enhance neuronal activity from damaged regions of the brain, improving stroke recovery. Others have improved motor imagery NF methodology with the addition of instrumental conditioning (Bray et al., 2007).
and selection of optimal feedback presentation schemes (Johnson et al., 2012) to maximize BOLD signals in the targeted regions after NF training. The longer-term effects of imagery-mediated fMRI NF have also been investigated (Yoo et al., 2008). Importantly, this study showed that a single session of fMRI NF involving motor imagery, followed by a two-week period of self-practice, enabled healthy individuals to consolidate and sustain elevated activity levels in brain regions associated with motor skill learning.

Despite the success of these NF studies, they should be viewed as preliminary. For example, brain and motor behavior changes observed in response to NF training need to be evaluated not only in the context of the individuals that respond to training, but also the non-responders. It has been commonly reported in past studies that not all participants were able to self-regulate successfully (Bray et al., 2007; Johnson et al., 2012; Yoo et al., 2008), although the neural differences between responders and non-responders remains poorly understood. In the absence of NF, motor imagery has been reported to elicit heterogenous brain responses across subjects (Stephan et al., 1995). Additionally, only one study has attempted to characterize the impact of motor imagery NF training in a behavioral test of overt motor function (Bray et al., 2007). As a last example, although the neural mechanisms underlying NF have been initially assessed and are thought to require a cortico-basal ganglia-thalamic circuit (Hinterberger et al., 2005; Yoo et al., 2008), different NF applications will likely engage slightly different networks of regions (deCharms, 2008), and a detailed investigation of the brain networks involved in successful NF of motor imagery signals still remains to be undertaken.

The primary focus of this paper is the exploration of the distributed networks and brain responses to NF involving kMI, through a post-hoc (offline) analysis of the fMRI data using the partial least squares (PLS) method (McIntosh et al., 1996). This multivariate approach is novel in the fMRI NF literature, as all previous ROI-based studies have mapped brain activity based on the general linear model (GLM) approach (Friston et al., 1995). The resulting networks of brain activity generated by PLS are expected to be consistent with the networks previously reported in the literature for NF (Hinterberger et al., 2005) and for NF with motor imagery (Bray et al., 2007; deCharms et al., 2004; Johnson et al., 2012; Yoo et al., 2008), while also accounting for response variability in kMI and NF across participants.

A secondary objective of this work is the characterization of inter-hemispheric interactions between the dominant and non-dominant M1 ROIs, while using a laterality index as the NF signal of interest. This is also novel in the NF literature and has relevance to the use of kMI for stroke recovery, considering cases in which the stroke lesion impacts either the dominant or non-dominant hand. Based on the small behavioral literature on kMI and lateralization (Stinear et al., 2006) it is of interest to explore the hypothesis that kMI NF relating to the non-dominant hand is less effective than that for the dominant hand in right-handed subjects, and how dominant/non-dominant M1 interactions modulate the NF signal.

**Methods**

**Experimental overview**

Subjects first performed a functional localizer task designed to identify ROIs over the left and right M1 regions involved in both overt and imagined movement. These ROIs served as the subsequent signal sources for kMI NF involving either the dominant or non-dominant hand. During the NF training, consisting of 4 experimental runs each lasting 9 min, subjects attempted to maximize a specific activation laterality (either left or right) based on fMRI signals from both ROIs during kMI of the hand, assisted by a real-time visual feedback display.

**Subjects**

Eighteen young, healthy, right-handed adult subjects participated with informed consent and with the approval of the Research Ethics Board at Baycrest Hospital, Toronto. To control for practice effects and assess responses to feedback information, subjects were split into two groups: 13 subjects in the experimental group (27 ± 3 years, 7 male) received “true” NF, whereas 5 subjects in the control group (24 ± 3 years, 2 male) received “sham” NF. The sham NF was yoked to feedback from a randomly chosen subject in the NF group. The control group treatment was identical to that of the NF group in every other respect, allowing for a direct comparison between subjects who received relevant feedback and those whose feedback was independent of their brain activity.

**Imaging**

Imaging was performed on a 3.0 T Magnetom TIM Trio system (VB15 software, Siemens Healthcare, Erlangen, Germany) using a 32-channel phased array head coil. Structural imaging was performed using a T1-weighted 3D MPRAGE sequence, at 1 × 1 × 1 mm³ isotropic resolution. All fMRI scans were performed using a modified 16 slice (5 mm) gradient echo T2*-weighted echo-planar imaging (EPI) acquisition, with 3 × 3 mm² in-plane resolution, 64 × 64 matrix, 30 ms TE and 1 s TR. Physiological recording of respiratory and cardiac signals was performed for all fMRI runs using a respiratory bellows and a pulse oximeter, respectively.

**Real-time setup**

The Siemens image reconstruction software was modified to provide real-time data transfer to a computer running AFNI (Cox, 1996) on Scientific Linux (www.scientificlinux.org) over a local network connection. The “AFNI computer” received one multi-slice, volumetric set of brain image data from the scanner immediately after each TR. In the initial functional localizer phase of the experiment, the AFNI computer was used to perform a rapid, scripted analysis using the general linear model (GLM) approach to produce statistical parametric maps (SPMs) identifying subject-specific regions of brain activity suitable for NF. The localizer experiment employed a simple blocked design alternating 15 s of rest, with 15 s of bilateral hand clenching, left hand (LH) imagery, and right hand (RH) imagery respectively, for a total of 300 s. The AFNI computer received the localized images immediately, and the scripted GLM analysis produced parametric maps identifying regions most active across both the overt movement and motor imagery tasks. Two 5 × 5 × 2 voxel (15 x 15 x 10 mm³) ROIs masks were manually defined over left and right M1 locations, centered on the M1 regions with the maximum t² value in the full F-statistic parametric maps. M1 was targeted due to its ease of location and principal role in motor action, and because potential NF applications in recovery of motor
function after stroke may target this region. The ROIs were made large enough to capture the entire hand motor area in all subjects.

During subsequent NF experiments, mean BOLD signal amplitudes were calculated for each ROI using the subject-specific masks defined from the localizer experiment. A separate “stimulus computer” running Windows XP received these calculated values from the AFNI computer with ~300 ms latency from acquisition, using a custom data server built with Python and real-time AFNI protocols. Stimuli were calculated and rendered graphically using the VisionEgg python library (Straw, 2008), then presented to the subjects using an LCD projector, projection screen, and a mirror mounted on the head coil.

**NF experiment**

All subjects performed 4 sequential NF training runs, each separated by 1–2 min of rest. Each run consisted of 12 NF trials: 6 RH trials, and 6 LH trials, pseudorandomly ordered. A diagram of an idealized NF trial is illustrated in Fig. 1. Each trial lasted 45 s, consisting of: 20 s rest, cued with a fractal image and text; 5 s of NF cue represented by one of two fractal images; 15 s of real-time feedback; and 5 s of trial results. The NF cue was displayed for 5 s to accommodate the associated hemodynamic response, so that NF display began after BOLD onset. Cue images were balanced across subjects, representing RH and LH trials respectively, and remained constant throughout the experiment for each subject.

Subjects were carefully instructed prior to the onset of NF experiments. They were asked not to make any overt hand movements throughout, except when specifically instructed to do so (see Reaction time tests, below). When performing imagery, they were specifically instructed to avoid vMI techniques (seeing in the “mind’s eye”), and to focus only on kMI by imagining the execution and feeling of hand movements. Subjects all started with imagined hand clenching, although they were free to use any kMI strategy to increase the NF signal as long as the strategy was restricted to single hand movements. They were asked to perform kMI immediately on display of the cue and to sustain their performance throughout the NF period. They were not told which cue was associated with which type of trial; rather, they were asked to determine the correct associations based on NF. The instructions were identical for both experimental and control subjects.

During the real-time feedback, subjects viewed a horizontal arrow. The length of the arrow was scaled for every TR in proportion to the laterality index (LI), calculated as:

\[
LI = \frac{k \times (IROI_{\text{left}} - rIROI_{\text{right}})}{IROI_{\text{left, rest}} - rIROI_{\text{right, rest}}},
\]

(1)

where the integer \( k = +1 \) for a RH trial, and \(-1\) for a LH trial, \( IROI_{\text{left}} \) and \( rIROI_{\text{right}} \) indicate the BOLD signal amplitude in the left and right ROIs in the \( n \)th TR interval during kMI, respectively; and \( IROI_{\text{left, rest}} \) and \( rIROI_{\text{right, rest}} \) are the mean BOLD signal amplitude during rest, respectively. This NF signal (a fractional signal difference) was used to control for global or non-specific activation changes, as well as to explore laterality-specific interactions. An additional vertical line was overlaid on the display, to provide subjects with a cumulative, time-averaged representation of the laterality index over the duration of the trial:

\[
LI = -\frac{1}{N} \sum_{n=1}^{N} LI_n.
\]

(2)

![Fig. 1](image_url)

**Fig. 1.** Example diagram of NF experiment following the functional localizer. Each subject performed four 9 min NF training runs and a pre- and post-reaction time test. Each NF run consists of twelve 45 s blocks, (6 RH and 6 LH trials each), pseudorandomly ordered. The 45 s block is made up of a 20 s rest period, a 5 s cue, a 15 s period of real-time NF and motor imagery, and a 5 s result (reward) display. The subjects earned 1 point for each successful trial, and earned nothing for each failed trial. The two reaction time tests consisted of 20 jittered trials, 5 for each combination of cue 1 × cue 2. Cue 1 was one of the 2 fractal cue images used during NF, and cue 2 indicated either R or L denoting a RH or LH finger response.
where \( n \) is the number of TR intervals from the beginning of the trial, and \( L_{1_n} \) is the instantaneous value of LI calculated from Eq. (1). The direction of LI polarity (positive or negative) was visually presented such that the indicators moved right when RH activity was detected, and vice versa.

Subjects were asked to maximize the length of the instantaneous NF arrow once they had determined the cue associations, and trial performance was evaluated based on the average LI over the entire trial duration. The NF training employed an operant conditioning strategy, where trial success was dependent on the LI surpassing a threshold \( T \) that was invisible to the subject. The value of \( T \) was changed according to a conditioning schedule (Fig. 2), where 4 consecutive successful trials increased \( T \) to the smallest value from the previous 4 trials. After 4 consecutive failed trials, \( T \) decreased to the previous \( T \) value, to a minimum of 0. Subjects received points for each successful trial, and were asked to attain as many points possible. This measure of success was used solely for the purpose of accruing reward points in the conditioning procedure, and was not used as a measure of NF performance ability. The LI data were subsequently analyzed using a 3-factor mixed factorial ANOVA, with run (1–4) and hand (LH or RH) as the within-subject factors, and group as the between-subjects factor.

**Reaction time tests**

Two reaction time assessments were performed in the scanner, immediately pre- and post-NF to consider the impact of the NF conditioning cue associations, and the potential effect of the NF training on a simple overt motor behavior test. Given a logistical requirement to conduct the experiment within a 1 h time frame, the reaction time assessments were each undertaken in short, 3 min. intervals. The experiments were identical, each consisting of 20 time-jittered and pseudorandomly ordered trials of visually cued button press responses performed with the index finger. Two cues were used to prompt the subjects for each trial: an image cue appeared for 1.75 s, and a reaction cue displaying the letter “R” or “L” was displayed immediately after for 0.25 s, prompting the subjects to respond with their right or left hand respectively. Each test was composed of: 5 RH congruent, 5 RH incongruent, 5 LH congruent, and 5 LH incongruent trials. For congruent trials, the image cue was identical to the cue used in the NF training to perform kMI for the associated hand. For incongruent trials, the image cue opposed the NF association. Reaction times were measured using an 8-button fiber-optic response box system (Current Designs, Philadelphia, PA), recording button responses from the index finger of each hand timed from the onset of the reaction cue.

The reaction time data were analyzed using a 4-factor mixed factorial ANOVA, with time (pre- or post-training), hand (LH or RH), and cue-type (congruent or incongruent) as within-subject factors, and group (NF or control) as the between-subjects factor. Data were normalized using a log transform to meet normality conditions for parametric statistical testing.

**Electromyography**

Electromyography (EMG) was recorded for 9 subjects in the NF group to verify that no overt muscle activity occurred during imagery. The recordings were performed with an fMRI-compatible EEG system (BrainAmp ExG MR, Brain Products, Munich, Germany), using differential electrodes placed on the extensor carpi radialis longus muscle of the right forearm. Recordings from 4 subjects in the NF group and the subjects in the control group were not possible due to equipment availability. All subjects were also asked to report any overt movement that occurred during NF. The EMG time courses were analyzed by comparing the RMS voltage values for the rest periods and the NF periods using planned paired t-tests, after MRI gradient artifact removal using the BrainAmp Analyzer 2 software (version 2.01, Brain Products, Munich, Germany).

**Post-experiment**

Immediately after the fMRI session, each subject was debriefed regarding their ability determine the NF conditioning cue associations, and were asked to report the degree of engagement they felt with the feedback signal during NF trials. Control subjects were also asked whether they perceived that the NF signal was a sham. Several months after the experiment, subjects were assessed using the MIQ-RS questionnaire for motor imagery (Gregg et al., 2010) to assess baseline ability to perform kMI.

**fMRI data analysis**

The fMRI data were pre-processed using the AFNI software package (Cox, 1996) to perform rigid body motion correction, physiological noise correction, and spatial smoothing (6 mm Gaussian kernel). The individual subject data were then spatially normalized to the Montreal Neurological Institute (MNI) template for group comparison.

Brain activation maps were generated using PLS, a multivariate method for determining spatial patterns or networks in brain activity that maximizes covariances between neuroimaging data and external measures. The external measures can include task design contrasts (task-PLS), external behavioral measures (behavioral-PLS), or fMRI signals from “seed” voxels (seed-PLS) (McIntosh et al., 1996). Latent variables (LVs) are output from a singular value decomposition of the covariance matrix created by cross-covarying the imaging data with the external measures. These LVs are similar to parametric map output from traditional GLM analysis, except that voxel saliences or weights replace model estimates, and bootstrap ratios replace t-values or z-scores. The LVs capture networks of activity spread across the entire brain. Often the PLS output contains a large number of LVs, and only the most interesting and most significant are retained for analysis. Statistical significance and stability are assessed using permutation tests and bootstrap resampling. Bootstrap ratios for each voxel are
calculated as the voxel salience divided by the standard error estimated from the bootstrap resampling.

In the present work, both task- and behavioral-PLS were used to analyze the fMRI data. Task-PLS was used to examine various groups and task contrasts, and behavioral-PLS was used to explore whether consistent brain patterns were correlated with task performance. Brain scores were used to assess the degree of expression of a particular LV pattern by comparing the inner product of the salience values with the mean BOLD data across conditions. In all analyses, 500 permutations of condition labels were used, and 80–100 bootstrap resamples, depending on the group sizes. Activation maps were thresholded at an absolute bootstrap ratio greater than 3, corresponding approximately to 95% confidence intervals. A 4 s response lag was used in the experimental onset timing to accommodate for the hemodynamic response delay. The PLS data matrices were created using the block design approach, where each 45 s trial was split into 4 (of 6 possible) conditions: rest, cue (LH or RH), NF (LH or RH) and result, with their respective durations outlined above. The data were averaged within each condition and across all trials to produce mean values for each condition, which were then averaged across subjects in each group and mean-centered (task-PLS) or correlated with a performance measure (behavioral-PLS) prior to the SVD decomposition.

Results

EMG

No significant difference in muscle activity was found between rest and NF conditions across all tested subjects: rest = 52.0±38.7 μV, NF = 78.0±74.2 μV (mean ± standard deviation, p > 0.3). Manual inspection of the EMG data revealed 5/9 subjects showed no visible muscle activity during NF periods, while 4 subjects showed some visible evidence of sparse “spikes” during at least one NF period, defined as any signal > 200 μV in amplitude. None of these subjects showed more than 6 spikes over the 4 runs and none lasted more than 500 ms in duration. The spikes were likely due to accidental movement, as no subjects reported any overt hand movement during the NF period. In comparison, the average EMG voltage recorded during the reaction time tests was 158.3±127.5 μV, significantly greater (p < 0.05, corrected) than the recorded rest or NF EMG signal amplitudes.

ROI masks

Fig. 3 shows an axial slice through the group-averaged ROI mask regions transformed to MNI space. The regions of maximum overlap across subjects were determined by transforming the rectangular ROIs to MNI space. The regions of maximum overlap were 2–18 subjects each and are thresholded to show only regions that overlap between 2 and 18 subjects (% overlap respectively). ROIs appear to be well localized to the hand-motor regions on the pre-central gyrus.

Reaction-time

The results of the 4-factor ANOVA using the experimental and control group showed only a significant main effect of the hand factor (F1,16 =8.99, p < 0.01), with the RH responses faster than the LH responses. No other main effects or interactions achieved significance, including any effects with the time or cue-type factors.

NF behavior, LIIs, and thresholds

All 13 NF group subjects and 4 of 5 control subjects were able to identify the correct cue–trial type relationships. One subject in the control group was unsure, due to the perceived erratic behavior of the feedback display, and none of the control subjects were aware that they received sham feedback during the experiment. In post-hoc assessment of baseline motor imagery ability using the MIQ-RS, attrition led to responses from only 6 out of 13 NF group subjects and 3 out of 5 control subjects. From the data available, the kinesthetic scores were similar within and across groups: 5.52±0.72 (mean ± standard deviation) for the NF group, and 5.62±0.44 for the control group. No significant relationships were found between NF performance (see below) and MIQ-RS scores, nor was any relationship found between NF performance and subject engagement.

Linear regression tests on the NF threshold T vs. trial number in the NF group showed significant positive slopes for LH trials (r = 0.27, p < 0.01) and RH trials (r = 0.18, p < 0.01) and no significant findings for the control group (p > 0.05) (Fig. 4). Additionally, linear regressions were performed on the mean number of rewarded trials in the NF group by run for RH trials (3.2, 2.8, 3.2, 2.5) and for LH trials (3.8, 3.3, 3.4, 3.2). No significant change was measured in the number of RH rewarded trials (p = 0.37) or LH rewarded trials (p = 0.11). These analyses indicate that T increased without a drop in the number of rewards (i.e., overall, subjects performed better over time).

A 3-factor mixed factorial ANOVA for the LI values showed a significant interaction of group x run number (F3,48 = 3.59, p = 0.02), suggesting the presence of a training effect with NF and not with sham NF. No other significant main effects (group, run, hand) or interactions were found.

These results were subsequently explored further to assess inter-subject variability. All trials from each subject were tested for individual significance using an independent samples t-test (p < 0.05) of the BOLD signals from each ROI during the 15 s of NF against the corresponding 20 s of rest data. The NF performance for a trial was deemed “good” if one of the following three scenarios occurred: the target ROI mean was significantly increased without any change in the opposite ROI; the opposite ROI significantly decreased without any change in the target ROI; or if both the target ROI increased and the opposite ROI decreased simultaneously. Trials that did not meet the above conditions were labeled “poor”. Fig. 5 is a scatter plot of the number of good LH trials against good RH trials, indicating considerable variability in NF performance across all subjects. A threshold was set at 25% LH or RH trials to separate the NF group into “good NF” and “poor NF” responders based on the upper limit of performance for the control subjects. This resulted in 5 good responders in LH trials, and 4 good responders in RH trials, with 3 subjects above both LH and RH thresholds.

Fig. 6 shows the LI values split by the new groupings, averaged across subject by runs, for RH and LH trials separately. The results of
the 3-factor ANOVA showed 2 strong significant effects: a main effect of group (good NF, poor NF, control) \( (F_{2,12} = 19.42, p < 0.01) \); and an interaction of group \( \times \) run \( (F_{6,36} = 3.41, p < 0.01) \). The group \( \times \) run interaction indicates that only the good responder group showed increased LI over time, whereas the poor and control subject data consistently showed LI values close to 0. No main effect or interaction involving the hand factor achieved significance \( (p > 0.10) \), although LI values for poor responders in RH trials show a slight trend that decreased over time. Planned t-contrasts of the LI values of the good NF group against the poor NF and control groups indicate that the good NF group LI values were higher than those for the poor NF and control groups in all the RH runs and runs 2 and 3 of the LH trials, at a significance level of \( p < 0.05 \) (Bonferroni corrected). Additionally, a simple comparison of the num-
ber of rewards per group indicated a significant difference for RH trials \( (p < 0.01) \), although no significant difference was found in LH trials \( (p > 0.2) \), and no significant changes were found in the number of rewards across runs.

A breakdown of BOLD activity in the left and right ROIs for the good and poor responder NF groups is shown in Fig. 7. For the good responder group, it is evident that the largely bilateral symmetry in activation between the left and right ROIs in run 1 gave way to more unilateral activation asymmetry between ROIs in runs 2–4. In RH trials, negative activation or suppression of the ipsilateral left ROI appeared to be the driving effect of the positive LI in runs 2–4, and vice versa for LH trials. No such pattern was observed in the data for the poor responder group, where BOLD percent signal change was mildly suppressed or near 0 bilaterally across runs. A 3-factor mixed factorial ANOVA with factors of group, run and side (dominant vs. non-dominant) and for RH and LH trials separately indicated that significant interactions of group \( \times \) side \( (p < 0.01) \) and group \( \times \) side \( \times \) run \( (p < 0.05) \) were present in RH trials, and just missed significance in LH trials \( (p = 0.081 \) and \( p = 0.057 \) respectively). A post-hoc assessment of activity in other motor regions such as the supplementary motor area (SMA) and left and right premotor areas for NF related changes yielded no consistent patterns of activation change, indicating that these results are specific to M1.

**Behavioral PLS**

To investigate the brain activation mediating the distinct performance differences between good and poor responders, behavioral PLS was performed using the number of good trials as the measure of NF performance. Analyses were performed for the entire NF group without including the control group, considering the performance effects shown...
in Figs. 4–7, and that behavioral PLS of only the control group showed no behavioral LVs (bLVs) covarying significantly with the behavioral measure \((p > 0.4)\). In the NF group, 1 significant bLV was found (bLV1, \(p = 0.02, 66.6\%\) cross-block covariance), and both LH and RH conditions were significantly correlated with NF performance \((r = 0.42\) and \(r = 0.81\), respectively). The behavioral PLS validated the behavioral classification undertaken in Fig. 5, confirming that there are distinct brain differences in NF conditions across the performance spectrum. The activation map and brain score scatter plots for bLV1 are shown in Fig. 8, showing regions including the bilateral posterior insula, basal ganglia, thalamus, right anterior insula, anterior cingulate (AC) and cingulate gyrus, left precentral gyrus, bilateral middle frontal gyrus (MFG), right supramarginal gyrus, left superior parietal lobule and bilateral superior frontal gyrus (SFG).

### Task PLS

To investigate these differences further with the new group classification, task PLS was performed on the data across all conditions, separating the subjects into 3 groups as above: 6 good NF responders, 7 poor NF responders, and 5 control subjects. Although the group sizes for this analysis were small, it produced a number of interesting contrasts.

Three significant task LVs (tLVs) were found: tLV1 \((p < 0.001, 66.5\%\) cross-block covariance) highlighting NF vs. Rest contrast, tLV2 \((p < 0.001, 17.0\%\) cross-block covariance) highlighting a Cue vs. Rest contrast, and tLV3 \((p = 0.034, 6.3\%\) cross-block covariance) showing a group contrast between the good responders and the control group during NF conditions (poor responders did not express tLV3, within near zero brain scores). In the context of the present work, tLV1 and tLV3 are of primary interest.

The spatial activation map for the most predominant and pertinent latent variable, tLV1, is shown in Fig. 9, with bilateral activation of the anterior insula, medial frontal gyrus, bilateral middle temporal gyrus (MTG), thalamus, basal ganglia (putamen, caudate, pallidum), bilateral premotor, SMA, left SFG and bilateral inferior and superior parietal lobules (IPL/SPL). Significant clusters for tLV1 and tLV3 are listed in Table 1. In comparison to tLV1, very few brain regions were associated with tLV3: left AC and left superior frontal gyrus were more active for good NF responders, whereas right middle frontal gyrus was more active for controls. For brevity, tLV3 activation maps and design scores are not shown.

### Discussion

This study investigated the brain and behavioral responses to fMRI NF training using kMI over a training period of 40 min. The study,
which reports findings that are largely consistent with the previous literature, is the first to explore brain activity in relation to variable inter-subject response during NF training, and to explore how differential feedback signals from the dominant and non-dominant M1 behave in response to kMI and NF.

A key outcome of the study was that the statistically significant effects of fMRI NF, found on average between the NF and control groups, were driven by a subset of the NF group based on both performance and brain activity. It is evident from the behavioral PLS results that a network of brain regions was engaged in proportion to NF performance (number of good trials), with approximately half of the NF group showing poor performance (0 to 6 good trials), and half showing a range of performance enhancement resulting from the NF training (approximately 7 to 20 good trials, out of a maximum of 24). The results of the behavioral PLS justify the approach initially undertaken, based on behavior, to classify individuals in the NF group into two categories (good and poor responders), from which categorized BOLD characteristics and task-PLS results naturally followed. Although the classification thresholds were chosen on visual inspection, they were conservatively chosen from the standpoint that all poor responders in the NF group were identified as having performance similar to that of the controls. It is unlikely that slight readjustment of the classification thresholds would markedly change interpretation of the study results.

There is much evidence in the previous literature for a heterogeneous response to motor imagery NF in young healthy subjects: Bray et al. (2007) reported only 17/26 subjects meeting a performance
In a post-hoc analysis, deCharms et al. (2004) found no statistically significant change in activation of an ipsilateral M1 ROI, although the ipsilateral ROI was not directly included in the NF. Bray et al. however, observed signal decreases in the non-rewarded ROIs (foot ROI during hand trials and vice versa), where the signal difference between the ROIs was directly acted upon by subjects. This is consistent with the results presented here, and lends support to the claim that use of a laterality signal for differential NF engages additional covert associative learning mechanisms responsible for signal suppression (Bray et al., 2007). Other evidence indicates that suppression of ipsilateral M1 enhances motor skill learning (Kobayashi et al., 2009). Collectively, these findings support continued investigation of differential ROI feedback signals for enacting brain and behavioral changes.

An effect of lateralization was expected to be observed with poorer NF performance for LH kMI, given the right handed subject group. There were some trends visible in the resulting data, although no statistically significant results: the L1 values averaged slightly higher in RH trials than for LH trials in the good responder group, and the ROI data suggested that stronger ipsilateral suppression in RH trials drove this increase. One report suggests that suppression of ipsilateral M1 is stronger in RH movement than LH movement for right-handed subjects (Newton et al., 2005), supporting the observed trends. The lack of statistically significant findings in the present study either result from subject response variability masking the underlying effect, or from no effect being present. If the latter scenario is correct, then important implications exist for future application of NF to stroke rehabilitation. Without a lateralization constraint, potential candidates for NF training can be selected regardless of whether the stroke occurred near the dominant or non-dominant motor cortex. If handedness does modulate NF ability, however, patients with strokes occurring near the dominant hand motor cortex would potentially be preferential candidates for NF training. Further study clearly needs to be done to address this question, particularly with a left-handed subject group to fully characterize the effects of handedness.

Looking at brain activation results extending beyond the M1 ROIs, the first LV of the task PLS (tLV1) produced a “NF vs. Rest” contrast, and showed an expected pattern of activity in motor control regions and self-awareness regions elicited by the motor imagery NF task. Activation of basal ganglia structures, thalamus, and cortical motor regions suggest engagement of the cortico-basal ganglia-thalamic circuits previously reported to be important in successful NF (Hinterberger et al., 2005). Strong engagement of parietal cortical, premotor and SMA regions are indicative of kMI (Guillot et al., 2009), whereas activation of the right anterior insula and AC have been linked to interoception, or awareness of internal state (Crichtley et al., 2004). The regions more active during rest likely reflect the default mode network, including the posterior cingulate, lateral parietal (angular gyrus) and superior frontal activations (Fox et al., 2005).

Interestingly, statistically significant activity in the M1 ROI regions was not observed in tLV1. There is evidence in the literature that M1 activity can be much less relative to SMA and pre-motor activity during motor imagery (Lotze et al., 1999; Sharma et al., 2006), although the literature is conflicting in this respect (Porro et al., 1996). Given that evidence of M1 suppression was observed in the ROI data analysis, one might have expected to observe M1 ROIs more active during rest. This may not have been observed for tLV1 because of the variability in subject response, as discussed above, where M1 ROI activity was inconsistently activated (or suppressed) across subjects and trials. The ROI results and the behavioral PLS results, which classified performance based on significance measures derived from the M1 ROI signals, leave little doubt that both M1 regions were involved in the NF task, even if they were not depicted in tLV1.

The behavioral PLS results identified brain regions that were significantly correlated with performance during the NF task. As might be expected, the activation map for tLV1 substantially overlapped bilaterally with that for tLV2, particularly in the insula, basal ganglia, thalamus, MPF and premotor cortex. The overlap confirms that NF success was mediated through engagement of regions already involved in the NF task, and not a separate mediating network. Additionally, several regions expressed in bLV1 coincide with the task positive network (TPN), a common set of regions activated during tasks requiring

Table 1

<table>
<thead>
<tr>
<th>Region</th>
<th>MNI-x</th>
<th>MNI-y</th>
<th>MNI-z</th>
<th>Peak BSR</th>
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<tbody>
<tr>
<td>tLV1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>More active during NF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle frontal gyrus-R</td>
<td>39</td>
<td>37</td>
<td>31</td>
<td>-9.6</td>
</tr>
<tr>
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<td>-27</td>
<td>-7</td>
<td>61</td>
<td>-9.6</td>
</tr>
<tr>
<td>Premotor cortex-R</td>
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<td>-8</td>
<td>59</td>
<td>-9.4</td>
</tr>
<tr>
<td>Supplementary motor area</td>
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<td>0</td>
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<td>24</td>
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<td>-13.1</td>
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<td>-1</td>
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</tr>
<tr>
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<td>-51</td>
<td>40</td>
<td>-10.7</td>
</tr>
<tr>
<td>Superior parietal lobule-L</td>
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<td>-58</td>
<td>55</td>
<td>-12.6</td>
</tr>
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<td>7</td>
<td>34</td>
<td>-10.0</td>
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<tr>
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</tr>
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<tr>
<td>Putamen-R</td>
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<td>15</td>
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<td>-8.0</td>
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<td></td>
<td></td>
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<tr>
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<td>12.5</td>
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<td>8.9</td>
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<td>Angular gyrus-L</td>
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<td>-66</td>
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<td>tLV2</td>
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<tr>
<td>More active during NF for good responders</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Anterior cingulate-L</td>
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<td>8</td>
<td>26</td>
<td>-3.1</td>
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<tr>
<td>Superior frontal gyrus-L</td>
<td>-19</td>
<td>42</td>
<td>21</td>
<td>-5.6</td>
</tr>
<tr>
<td>More active during NF for controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle temporal gyrus-R</td>
<td>53</td>
<td>-58</td>
<td>-4</td>
<td>3.8</td>
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</tbody>
</table>
attention modulation (Fox et al., 2005). These regions include the frontal eye fields (premotor), insula and dorsolateral prefrontal areas. This observation suggests that expression of the TPN is positively correlated with successful NF, and that expression of the TPN may reflect subject engagement in the NF training. Empirically, TPN expression represents a strong marker for NF performance in RH trials with 65.6% explained variance, and to a lesser extent in LH trials with 17.6% explained variance. Although this study did not control for the effects of attention, evidence for co-expression of attentional networks during NF possibly indicates that attentional processes are a major factor mediating NF performance. This is supported by the fact that the difference in the number of rewards given between good and poor responders for RH trials is constant with time, and evident even in the first run, indicating that success is not a learning effect and may be better explained as an attentional effect. Because no significant differences were found in the number of rewards given between groups in LH trials, however, the behavior of all good responders cannot be explained solely by an attentional effect of having more rewards. Future work could be undertaken to assess the underlying causal linkage of TPN expression and NF performance, and a deeper exploration into other factors contributing to performance variation.

The tLV1 results account for much less of the explained covariance (6.3% versus 66.5% for tLV1), but are of interest nevertheless because they highlight brain regions that were significantly different in the good responder group compared to the control group during the NF trials. To date, only two studies employing sham feedback control groups have looked at group differences in brain activity, both using a GLM analysis (Johnson et al., 2012; Yoo et al., 2008). In the present work, left lateralized AC and SFG were found to be significantly more active in the good responder group, compared to increased right MTG activation in the control group. Both the AC and SFG have been implicated in studies of general feedback processing (Mies et al., 2011), with both responding more to positive feedback, and the left SFG also sensitive to feedback validity. Response variability, particularly across good responders in the NF group, may account for the sparse pattern of brain activity observed in tLV1.

In comparison, Yoo et al. (2008) found left pre-central gyrus, bilateral post-central gyrus, right MTG and right parahippocampal gyrus activation in an experimental>control contrast, and right medial frontal gyrus, right inferior parietal lobule, left cuneus and bilateral precuneus in the control>experimental contrast. Interestingly, Johnson et al. (2012) did not find any significant activation in an experimental>control contrast, but did find activation in the control>experimental contrast, including the right MTG reported here. The lack of strong consensus between these studies and the tLV1 results of the present study may be due to differences in experiment design and the particular aspect of brain activity chosen for NF training, and in the details of the training procedure.

In the simple reaction time analysis, the significant main effect of hand (indicating subjects reacted faster in RH trials compared to LH trials) is an expected result given that subjects were all RH dominant. The lack of any significant main or interaction effects for the group factor suggests that the NF training did not affect visuomotor response times over the duration tested, but it should also be acknowledged that the number of reaction time trials was rather low in this experiment (5 per condition per hand, 40 total), limiting statistical power to detect changes. Further studies specifically investigating the effect of NF training on overt motor performance will be necessary to provide some more conclusive picture of NF training effects.

Future directions for NF investigation include analyses to determine predictors for NF success, toward improving NF technology for responders and the exclusion of non-responders. One potential target for the selection process could involve expression of the TPN as found in this work. Adoption of whole brain classification approaches (LaConte, 2011) may prove advantageous in targeting entire networks of regions, and for enhancing overall NF efficacy. Future work may also include investigation of NF ROIs in non-primary motor regions involved in motor learning and skill acquisition (Ungerleider et al., 2002), or use of more sophisticated methods for calculating real-time BOLD estimates (Hinds et al., 2011), which may lead to more sensitive and robust feedback measures. Alternative feedback signals may also be of interest, including targeting BOLD signal variance measures (Garrett et al., 2010) instead of the commonly employed mean BOLD measures. Lastly, NF might potentially be used in the long term as an aid in learning to perform kMi more effectively for use in imagery therapy to promote stroke recovery (Braun et al., 2006; de Vries and Mulder, 2007; Sharma et al., 2006). For example, NF could potentially be used to train individuals to perform kMi over vMi by rewarding increased activity in primary motor regions and decreased activity in primary visual areas. Alternatively, NF training could be used potentially to identify those who can perform kMi well, distinguishing them as candidates for imagery-based therapeutic intervention. We anticipate that some of the above issues will be topics of future investigation in our laboratory.

Acknowledgments

The authors would like to thank the anonymous reviewers for their insightful and helpful comments. This research was funded by the Canadian Institutes of Health Research (CIHR) and the Heart and Stroke Foundation of Ontario (HSFO).

References


