PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE

ESCUELA DE INGENIERIA

HOW FEEDBACK, MOTOR IMAGERY AND REWARD INFLUENCE BRAIN SELF-REGULATION USING REAL-TIME FMRI

PRADYUMNA SEPÚLVEDA DELGADO

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Master of Science in Engineering

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SERGIO RUIZ

Santiago de Chile, December, 2015

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To Amrta, Matsya y Saci

I owe you everything

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ABSTRACT

Real-time fMRI (rtfMRI) neurofeedback (NF) enables the volitional control of brain metabolic activity, potentially leading to behavioral changes. The learning process involved in achieving self-regulation of brain hemodynamics is presumed to be related to several factors such as type and contingency of feedback, reward, the use of mental imagery, duration or length of training, among others. Explicitly instructing participants to use mental imagery is a common practice in rtfMRI-NF experiments, under the assumption that this strategy will improve and accelerate the learning process. Likewise, giving monetary reward according to performance is assumed to reinforce and improve brain self-regulation. However, how to set an optimal strategy for improving volitional control remain unclear. To evaluate the differential effect of these factors on achieving brain self-regulation we assessed feedback, explicit instructions and monetary reward while training healthy individuals to up-regulate the blood-oxygen-level dependent (BOLD) signal in the Supplementary Motor Area (SMA). Four groups were trained in a two-day rtfMRI-NF protocol: G_F with NF only, $G_{F,I}$ with NF+explicit instructions (motor imagery), $G_{F,R}$ with NF+monetary reward, and $G_{F,I,R}$ with NF+explicit instructions (motor imagery)+monetary reward. Our results showed that G_F increased significantly their BOLD self- regulation from day-1 to day-2. $G_{F,R}$ showed the highest BOLD signal amplitude in SMA during the training, but it did not show significant change from day-1 to day-2. $G_{F,I}$ and $G_{F,I,R}$ did not show BOLD signal amplitudes significantly higher than G_F or significant change between the two days. Whole brain univariate analysis showed similar activations among the four training groups. Similarly, functional connectivity in the bilateral motor cortical and prefrontal regions of the brain showed common patterns among the four groups. On the other hand, the variation of functional connectivity during the training showed distinct patterns among the groups, representing the varied influences of feedback, reward and instructions on the brain.

Key words: Neurofeedback, real-time fMRI, learning, reward, mental strategy, motor imagery.

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RESUMEN

El uso de *Neurofeedback* (NF) con resonancia magnética funcional en tiempo real (*Real-time fMRI neurofeedback*, rtfMRI) permite la auto-regulación de la actividad metabólica del cerebro. El control de la actividad cerebral puede generar cambios conductuales, lo cual abre nuevas oportunidades para potenciales aplicaciones tanto terapéuticas como de investigación en neurociencias. El proceso de aprendizaje involucrado en alcanzar auto-regulación de la actividad hemodinámica del cerebro se cree que está relacionado a factores como el tipo y contingencia del *feedback*, recompensa, el uso de imaginería mental, la duración del entrenamiento, entre otros. Instruir explícitamente a los participantes a usar imaginería mental es una práctica común en experimentos de rtfMRI-NF, bajo el supuesto de que esta estrategia mejorará y acelerará el proceso de aprendizaje. Del mismo modo, se asume que la entrega de recompensa monetaria según desempeño refuerza y mejora la auto-regulación cerebral. Sin embargo, la estrategia óptima para mejorar el control voluntario de la actividad cerebral aún no está clara. Para evaluar el efecto de estos factores en la auto-regulación, el presente estudio consideró la influencia de *feedback*, instrucciones explícitas y recompensa monetaria en el proceso de entrenamiento de sujetos sanos para aumentar la señal BOLD (*Blood Oxygen Level Dependent*, Dependiente del Nivel de Oxígeno Sanguíneo) en el Área Motora Suplementaria. Cuatro grupos fueron entrenados en un protocolo de rtfMRI-NF durante dos días: G_F sólo con NF, $G_{F,I}$ con NF + instrucciones explícitas (imaginería motora), $G_{F,R}$ con NF + recompensa monetaria, y $G_{F,I,R}$ con NF+ instrucciones explícitas (imaginería motora) + recompensa monetaria. Nuestros resultados muestran que G_F aumentó significativamente su auto-regulación de la actividad BOLD entre el día 1 y el día 2. $G_{F,R}$ mostró la mayor amplitud en la señal BOLD en la región objetivo durante el entrenamiento, pero no mostró un cambio significante entre el día 1 y día 2. Tanto $G_{F,I}$ como $G_{F,I,R}$ (grupos *con* imaginería motora) no mostraron una amplitud de la señal BOLD significativamente mayor que G_F ni tampoco cambios significativos entre el día 1 y día 2. El análisis univariado del cerebro reveló activaciones similares entre los cuatro

grupos de entrenamiento. Igualmente, la conectividad funcional en regiones corticales motoras bilaterales y prefrontales mostró patrones comunes entre los cuatro grupos. Por otro lado, la variación de la conectividad funcional durante el entrenamiento presentó patrones distintos entre los grupos, representando las variadas influencias en el cerebro del *feedback*, recompensa e instrucciones.

Palabras claves: ***Neurofeedback, real-time fMRI,* aprendizaje, recompensa, estrategia mental, imaginería motora.**

CONTEXT

Brain computer interface (BCI) is a system that measures activity in the Central Nervous System (CNS), in circumscribed or extended regions, to interpret, process and transform it into an output signal to improve or replace the natural output from CNS (Wolpaw & Winter-Wolpaw, 2012). BCI has a wide spectrum of applications: prosthetic limb control, word-spellers for locked-in patients, videogames, emotional detectors, among others (Morimoto & Kawato, 2015; Birbaumer and Cohen, 2007; Haynes & Rees, 2006; Sitaram et al., 2011; Pfurtscheller et al., 2008). Neurofeedback (NF) is a core part of BCI that enables subjects to interact with their self-generated functional brain signal in real time through sensory input such as visual, auditory, etc. The objective is to develop voluntary control or self-regulation of the brain activity, generating changes based in plasticity and brain functional reorganization after a training period. In this way, NF appears as a potential alternative to standard options in rehabilitation therapy and treatment of neurological/psychiatric conditions, usually related to dysfunctional brain activations, avoiding invasive approaches such as pharmacological therapies or surgical intervention.

Electroencephalography (EEG) is the most common way to acquire brain electrical signals for NF due to its non-invasive nature, comparatively cheap equipment and high temporal resolution. However, EEG lacks of spatial resolution and has only cortical coverage. Alternative non-invasive techniques have been used to overcome some of these constraints. Functional magnetic resonance imaging (fMRI) is a technique that measures brain hemodynamic/metabolic changes, i.e. fluctuations in the blood oxygenation level dependent (BOLD) signal contrasting magnetic properties of hemoglobin in blood (Fox and Raichle, 1986; Ogawa et al., 1990; Kwong et al., 1992). When hemoglobin has oxygen saturation (oxyhemoglobin) it behaves as a diamagnetic substance, in turn, low oxygen attached to hemoglobin molecule (deoxyhemoglobin) shows paramagnetic properties. Therefore, MRI signal is more intense in those voxels with higher concentration of oxyhemoglobin. Correlation between neuronal electric

activity and hemodynamic changes has been demonstrated (Logothetis, 2008). If neural activity in a particular brain region rises, it is observed an increment in oxygenated blood flow bigger than the oxygen metabolic consumption due to neuron activity, increasing signal intensity in those regions. To measure these changes, fMRI acquires brain volumes with 'voxels' that represent BOLD signal observed in part of the brain tissue during a particular time period (usually in the range of seconds). Sequences of brain volumes are acquired producing time series of brain activity. Thus, fMRI is a technique that indirectly obtains neuronal signals with higher spatial resolution, whole brain coverage, although with lower temporal resolution than EEG. Despite this temporal drawback, real time fMRI (rtfMRI) has been implemented for NF training.

It has been shown that particular brain regions get activated in response to executive or cognitive processes, such as emotional, visual, auditory, etc. Using rtfMRI-NF training, metabolic brain signals (BOLD signals) in these areas can be self-regulated in healthy and pathological populations (Sulzer et al., 2013a). As an external sign of brain self-regulation, behavioral changes can be generated after successful modulation of brain activity (as demonstrated in studies of patients with schizophrenia, Parkinson's disease, chronic pain and depression; Ruiz et al., 2014). For instance, Parkinson patients can be trained to increase activity in their motor cortex using rtfMRI-NF, leading to an improved performance in a finger-tapping test (Subramanian et al., 2011). rtfMRI-NF appears as a promising therapeutic technique, however, many aspects in the design of training protocols are still unclear.

In rtfMRI-NF experiments, self-regulation is measured comparing the BOLD signal between an "active" self-regulation period (can be up or down-regulation depending of the purpose of the experiment) and an "inactive" baseline period (participant are asked to relax). In "active" periods participants have to voluntarily increase or decrease their brain activity guided by contingent feedback (often visual) that shows the instantaneous change of BOLD signal. At the beginning of the training process, it is expected that participants may not be able to generate differences ("active" vs. "inactive") in BOLD

signal. After getting familiar with the "feedback control" and practicing they may be able to obtain higher changes in BOLD signal, usually with the help of some kind of internal mechanism (i.e. achieving a calm state, remembering old memories, concentrating, imagining, etc.; Sulzer et al., 2013a). Therefore, the magnitude of the BOLD change can be used to rate the degree of successfulness of brain self-regulation.

In the present study, additional factors, i.e. monetary reward and explicit mental imagery, have been included in NF experiments. These factors have been proposed in NF literature as alternatives to improve brain self-regulation: giving money incentives may increment the rewarding value of obtaining "good" feedback (desirable brain activation) and mental imagery related to its proposed function may help to activate the region of interest (e.g. the insula cortex, an "emotional" region, may get activated with autobiographical with happy memories). However, the effectiveness of such factors has not been demonstrated yet.

Another open question in rtfMRI-NF is the neural substrates or brain regions (or networks) needed to achieve brain self-regulation (Sulzer et al. 2013a). Based on that purpose some studies have looked for complementary regions that activate when participants try to control their brain activity (Caria et al., 2007; Linden et al., 2012; Subramanian et al., 2011; Yoo et al., 2008) or connectivity patterns that could be related to brain self-regulation (Rota et al., 2011; Ruiz et al., 2013a; Zotev et al., 2011).

INTRODUCTION

During the last decade, several studies have demonstrated that brain's metabolic signals can be voluntarily controlled by healthy individuals and patients by means of neurofeedback (NF; Birbaumer et al., 2007a; Birbaumer et al., 2013). In the NF studies using the Blood Oxygen-level Dependent (BOLD) signal, real-time Near Infrared Spectroscopy (rtfNIRS; Mihara et al., 2013; Naseer et al., 2015; Sitaram et al., 2007b) and Functional Magnetic Resonance (rtfMRI) have been used; the latter being the most informative because of its higher spatial resolution and whole-brain coverage (Caria et al., 2007; deCharms et al., 2005; Haller et al., 2013; Hamilton et al., 2011; Lawrence et al., 2013; Yoo et al., 2008; Young et al., 2014; Zotev et al., 2011). Volitional control of brain metabolism can lead to behavioral changes (deCharms et al., 2005; Linden et al., 2012; Ruiz et al., 2013a; Subramanian et al., 2011; Young et al., 2014), therefore opening new opportunities for potential therapeutic and research applications (Lee et al., 2011; Ruiz et al., 2013b; Sitaram et al. 2007a; Sitaram et al. 2011; Sitaram et al. 2014; Weiskopf et al., 2012).

The magnitude of self-regulation typically expressed as percentage difference in the hemodynamic signal between the regulation and baseline trials, and the learning effect, expressed as change in the magnitude of self-regulation over time during neurofeedback training (deCharms et al., 2005), are two important measures of a participant's performance during neurofeedback training. Both the magnitude of self-regulation and the learning effect can be influenced by several factors such as type of feedback, reward, instructed mental strategies, session duration, among others (Schwartz and Andrasik, 2003; Sulzer et al., 2013a). Volitional control depends on contingent feedback, as has been demonstrated by the inclusion of control conditions, such as, non-contingent feedback (sham-feedback) and mental imagery in the absence of feedback (Caria et al., 2007; Caria et al., 2012; deCharms et al., 2005, Hamilton et al., 2011; Rota et al., 2009; Zotev et al., 2011). In the majority of rtfMRI-NF studies so far participants were instructed to control the feedback related to the BOLD signal extracted from the region

of interest (Caria et al., 2007; deCharms et al., 2005; Lawrence et al., 2014; Scheinost et al., 2013; Sokunbi et al., 2014). In these cases, contingent (visual) stimuli may intrinsically represent a reward or reinforcement since it guides the desired response based on the internal motivation of the experimental subjects (Fetz et al., 2006; Ruiz et al. 2014; Strehl, 2014).

Furthermore, instructing participants to use certain kind of mental imagery is a common practice, employed with the aim to enhance the efficiency of the learning process (Caria et al., 2007; Hwang et al., 2009; Lawrence et al., 2013; Ray et al., 2015; Rota et al., 2009; Scharnoswski et al., 2012; Sitaram et al., 2011; Subramanian et al., 2011; Sulzer et al., 2013b; Yoo et al., 2008; Young et al., 2014; Zilverstand et al., 2015).

However, opposing views have been raised about the importance of these strategies, particularly in electroencephalography NF (EEG-NF; Kober et al., 2013; Strehl et al., 2014) and rtfMRI-NF studies (Shibata et al., 2011; Sulzer et al., 2013a; Birbaumer et al., 2013). In fact, learning to self-regulate brain activity has been proposed as a process of operant conditioning (Birbaumer et al., 2013) since the early reports of brain signal control in non-human animals (Carmena et al., 2003; Fetz, 1969; Fetz and Finocchio, 1971; Koralek, 2012; Philippens and Vanwersch, 2010; Schafer and Moore, 2011; Shinkman et al., 1974; Sterman et al., 1978). Based on this outlook, explicit and conscious strategies may not be necessary and may even hinder efficient learning (Kober et al., 2013; Witte et al., 2013). Recently, human studies using rtfMRI-NF have also started to consider this aspect in their experimental design, increasing the relevance of reward in the training process. Monetary reward, proportional to the desired change in the brain signal, has been used in studies as another factor to reinforce learning (Buyukturkoglu et al. 2015; Bray et al. 2007; Shibata et al. 2011; Megumi et al., 2015), often with protocols in which the participants are not informed or aware of the meaning of the feedback signal (Bray et al., 2007; Megumi et al.,2015; Shibata et al.,2011)

Unravelling the underlying psychobiological process of learning of self-regulation is one of the most important open issues in the field of neurofeedback and Brain-Computer

Interfaces (Emert et al., 2015; Sulzer et al. 2013a; Ruiz et al., 2014). Finding an optimal strategy to enhance brain self-regulation is of fundamental importance for the development of neurofeedback for clinical interventions.

For this purpose, we focused our study on testing and comparing three factors presumed to influence learning brain self-regulation: contingent feedback, explicit instructions related to the activity of the region of interest (ROI), i.e. motor imagery, and monetary reward. The first aim of the current study was to compare the efficiency of motor imagery and monetary reward as complementary strategies to contingent feedback.

We trained four groups of healthy individuals using different combinations of these factors, to achieve volitional control of supplementary motor area (SMA). We chose SMA as the ROI, as it has an important role in planning and execution of motor activity and its dysfunction has been related to motor deficits observed post stroke and movement disorders, such as, Parkinson's disease (Jahanshahi et al., 1995; Nachev et al., 2008; Radman et al., 2013; Roland et al., 1980). Additionally, the function of SMA has been extensively studied during motor imagery (Gerardin et al., 2000; Guillot et al., 2012; Kasess et al., 2008; Lafleur et al., 2002; Lotze & Halsband, 2006) and also through fMRI-NF studies (Scharnowski et al. 2015), particularly as a potential intervention for treating Parkinson's disease (Buyukturkoglu et al., 2013; Subramanian et al., 2011).

We evaluated the *magnitude of self-regulation* (r_{SMA}), expressed as percentage difference in the hemodynamic signal between the regulation and baseline trials, and the *learning effect (*Δr_{SMA}), expressed as change in the magnitude of self-regulation over time during neurofeedback training (deCharms et al., 2005). Further, we explored changes in the brain across the different experimental groups with both univariate analysis and functional connectivity (FC) analysis. First, we used univariate analysis by Statistical Parametric Mapping (SPM) to find differences in brain activations in the four experimental groups. Additionally, FC analysis was performed to compare the functional connectivity changes due feedback, motor imagery and monetary reward.

MATERIALS AND METHODS

1. **Participants**

Twenty male, right handed volunteers, aged 18-35 years (22.75 ± 1.6) and without any history of previous psychiatric or neurological disorders were included in the study. Exclusion criteria included the presence of electronic or ferromagnetic body implants and prior history of claustrophobia or panic attacks. All participants in the study were naïve to NF and fMRI experiments. The experimental protocol was approved by the ethics committee of Pontificia Universidad Católica de Chile. Each participant signed a written informed consent on each day of the study.

Participants underwent two days of NF training to achieve volitional control of SMA (ROI 1). To evaluate the effects of the different factors, namely, feedback, motor imagery and reward, on NF training, participants were randomly distributed in 4 groups of equal size (n=5), matched by age.

The following were the four groups of participants (figure 1):

Group G_F: Participants of this group received only contingent feedback (F) from SMA. No further instructions or strategies to self-regulate were given.

Group $G_{F,I}$: In addition to contingent feedback, participants of this group were instructed that feedback was proportional to the activity of a movement related area of the brain, and hence participants could use mental imagery of movement (i.e., motor imagery, I) without performing actual movement, for self-regulation.

Group $G_{F,R}$: Participants were given contingent feedback and monetary reward (R) proportional to the increase in the BOLD signal in the SMA at the end of each up-regulation block.

Group $G_{F,I,R}$: Participants were given contingent feedback, monetary reward and instructions for motor imagery.

Every group received contingent visual feedback from SMA by means of a graphical thermometer. The bars presented in the thermometer reflected the BOLD signal-level in SMA. The thermometer was regularly updated at intervals of 1.5 s. All participants were instructed to observe the thermometer display and to increase the thermometer bars knowing that it was related to their brain activity. Participants were additionally informed to consider the delay in the feedback signal due to the tardiness of the brain hemodynamic response as well as due to restrictions imposed by data acquisition and processing.

2. **MR acquisition**

The rtfMRI system was implemented using a Philips Achieva 1.5T MR scanner (Philips Heathcare, Best, The Netherlands) at the Biomedical Imaging Center of the Pontificia Universidad Católica de Chile. A standard 8-channel head coil was used. Functional image acquisition used FFE-EPI sequence with TR/TE=1500/45 ms, matrix size=64x64, flip angle α=70°, FOV: RL = 210 mm; AP = 210 mm; FH = 79 mm. Sixteen slices (voxel size=3.2x3.3x4 mm^3, gap= 1 mm) oriented with AC/PC alignment and 150 scans (10 dummy scans) were used in each run. Anatomical T1-weighted brain volumes were acquired each training day using T1W-3D TFE sequence with TR/TE=7.4/3.4 ms, matrix size =208x227, α=8°, 317 partitions, voxels size=1.1x1.1x0.6 mm^3, TI=868.7ms. To prevent discomfort during MRI sessions, pads and air cushions were used to fix the head.

3. **Real-time fMRI system**

To implement the rtfMRI system a typical setup used in rtfMRI-NF experiments was assembled (Caria et al., 2012; Ruiz et al.,2014; Weiskopf et al. 2004; figure S2 and S12). At the beginning of each measurement, participants were positioned in the scanner and reference scans were acquired. Later, using an EPI sequence (see MR acquisition) functional brain volumes were generated. During image acquisition, brain volumes were transferred in real-time directly from the scanner's image reconstruction system using the

Direct Reconstructor Interface (DRIN) application (Philips Heathcare, Best, The Netherlands) to a PC (BCI PC) which had the necessary software to analyze it in real time. No modifications were done to the scanner's image reconstruction system, as described by Sitaram et al. (2011).

A standard PC running Turbo Brain Voyager 3.0 (TBV-PC) rtfMRI software (Brain Innovations, The Netherlands) read the incoming ANALYZE (.img/.hdr) brain volumes to perform real-time 3D motion correction and statistical analysis (Weiskopf et al., 2003). TBV parameters were set to match parameters of the EPI acquisition and to obtain BOLD signal information coming from the two selected ROIs after each repetition time (TR) of the scans. Custom MATLAB scripts used ROI information to compute the feedback (thermometer bars) by comparing between up-regulation and baseline blocks (details below). The feedback output was stored in a shared file (text file) in the TBV-PC to be accessed from another computer (Presentation-PC) in the local network. Presentation® 17.1 software (Neurobehavioral Systems, USA) read the feedback file continuously and refreshed the images on the screen, corresponding to the calculation of the thermometer bars, on a MR-compatible visual display system (NordicNeuroLab AS, Norway).

4. **Experimental protocol**

Table 1. Experimental Protocol

Day 1	Explanation and instructions to participants
	Training Session 1
	ROI localizer run
	Neurofeedback training runs (4)
	Transfer run
	Anatomical MRI acquisition
Day 2	Training Session 2
	ROI localizer run
	Neurofeedback training runs (4)
	Transfer run
	Anatomical MRI acquisition
Day 3	Debriefing

4.1 **Training sessions**

Each training session consisted of a functional localizer, 4 training runs and a transfer run. Training was conducted in 2 days, with at least one day of gap (with no training) between days 1 and 2 (figure S3).

4.1.**1 Region of interest (ROI) localizer trials**

SMA (ROI 1) was delineated using both a functional localizer and anatomical references. During the functional localizer, participants performed overt motor execution. To ensure that G_F and $G_{F,R}$ (groups without motor imagery) do not get any

hint that the self-regulation task that follows the localizer might be related to movement or motor activity, the functional localizer was implemented as a 2-back task (Conway et al., 2003; Kirchner, 1958) in which participants were presented a sequence of stimuli, and the required task was to report (with a button) if the current stimulus and the stimulus observed '2' steps earlier in the sequence were the same. Although, typically the n-back tasks are used to evaluate working memory, in this experiment we used the task to ensure frequent button presses to elicit activations in the motor areas. The localizer run included four baseline blocks and three 2-back blocks, so that each block was presented for 30 s. For anatomical reference, the superior part of the posterior frontal lobe around the perpendicular line to anterior commissure (AC; y=0) was used. A volume inside Broadmann Area 6 was selected, whose location was later validated in an offline analysis. ROI 1 was delineated as two contiguous slices, each one of 4x4 voxels in a transversal brain section. The reference ROI (ROI 2), delineated as a single slice around anterior part of the third ventricle, was selected to cancel effects of global activation (figure S4).

4.1.2 **Neurofeedback training runs**

Eight training runs were equally distributed in two scanning days (four runs per day). Each run of 150 brain volumes included first 10 dummy scans (15 s) to reach T1 steady state (which were later discarded), followed by alternating baseline (4) and up-regulation (3) blocks (20 volumes, 30 s per block). During baseline blocks, volunteers were asked to remain in rest, and the image of the thermometer remained static. During up-regulation blocks, contingent feedback was provided. Groups $G_{F,R}$ and $G_{F,I,R}$ were visually presented the value of their monetary reward, in the last 3 seconds (2 volumes) of the block, using an image indicating the amount of money earned corresponding to the increase in the BOLD signal in the previous up-regulation block.

4.1.3. **Feedback calculation**

As in other previous studies (Caria et al. 2007; Lawrence et al. 2013; Ninaus et al., 2013; Ruiz et al., 2013a), graphical depiction of a thermometer was used as visual feedback of BOLD signal changes, with moving bars showing the increments (red bars rising over middle point) or decrements (blue bars under the middle point) of the BOLD signal in SMA, in comparison to the immediately preceding baseline block, using the following formula:

$$F = (BOLD_{Upreg} – BOLD_{Base})_{ROI1} - (BOLD_{Upreg} – BOLD_{Base})_{ROI2} \quad (1),$$

where F is the feedback value, $BOLD_{Upreg}$ is the BOLD signal in ROI1 or ROI2 during a moving average calculated from the last 3 scans during the up-regulation block, and $BOLD_{Base}$ the average BOLD signal during the entire immediately preceding baseline block (figure S5). Subsequently, F was rounded-off to the closest integer. In case of abrupt changes (considered if greater than 8 points in this study) in the F-value, potentially due to movement artifacts, swallowing, etc., an online correction was applied by replacing the spurious value by the F-value from the previous TR.

In groups with monetary reward ($G_{F,R}$ and $G_{F,I,R}$), the amount of money given to each participant was calculated in proportion to the mean increase in BOLD in the up-regulation block in comparison to that of the baseline block. To ensure correspondence between real-time feedback (thermometer bars) and monetary reward, the equation (2) was used for calculating the amount of monetary rewarded:

$$R = F' * M \quad (2),$$

where R is the reward value and M the monetary units per 1 unit of F'. F' (equation 2) was computed using the same formula as F (equation 1), but considering the percentage BOLD increase in all the up-regulation block compared to the previous baseline block. The maximum permissible reward for each block was 3 USD. The total reward given to participant was the sum of the amount of money earned in each reward

block with a total maximum of 60 USD for the whole training. If the calculation of the monetary reward resulted in a negative number, the value was fixed to zero.

5. **Self-reports and subjective ratings**

At the end of the two training days, participants were asked about their comfort during training, subjective appreciation about the level of control over the feedback signal and descriptions of mental strategies used to control the thermometer. The questionnaire responses were assessed using a five-point Likert scale (1: completely disagree/difficult; 5: fully agree/easy).

6. **Transfer run**

At the end of both training days, an additional run was included during which participants were instructed to perform the same up-regulation/baseline paradigm described previously, but without receiving feedback. The aim of transfer runs was to test if participants can maintain volitional control of the BOLD signal after training. A further aim is to test if magnitude of self-regulation can be maintained in a context different from a NF experiment. The number and duration of blocks was the same as that of the NF training runs, with the only difference that the thermometer (feedback) was not shown. Unlike previous studies with transfer runs performed only once and during the last day (Caria et al., 2007; Ruiz et al., 2013a; Zotev et al., 2011), here a transfer run was included at the end of each day. The objective was to familiarize the participant with the transfer, avoiding novelty effect over the results. For the final analysis, only the transfer run of the second day was used.

7. **Offline processing**

7.1. **Preprocessing**

For brain imagining analysis, a spatial pre-processing step using SPM 8 (Wellcome Department of Imaging Neuroscience, London, UK) was performed, involving motion correction, realignment and slice-timing correction. Functional EPI images were coregistered with the anatomical images of the same day. Normalization to Montreal Neurological Institute (MNI) space was done in anatomical and functional EPI volumes. Smoothing with a Gaussian kernel of FWHM of 8x8x8 was applied over all functional volumes.

To execute 1st level analysis, general linear model (GLM) was defined to evaluate regions responsive to self-regulation, considering two conditions (up-regulation and baseline). Convolution of the regressor with canonical hemodynamic response function (HRF) was performed. Six generated motion confounds were added to the model. After estimating the 1st level model, whole brain analysis was performed at group level to find other areas responsive to up-regulation (defined contrast = 1 -1, i.e., up-regulation - baseline) using 2nd level analysis in SPM. One-sample t-test was performed for each group taking data from the second day only, in order to find significant brain activation of learned self-regulation and the different factors. Whole brain map (figure 4) shows significant t-values (threshold of $p<0.001$ and FDR $p<0.01$, cluster size = 10) and are visualized using the xjView toolbox (http://www.alivelearn.net/xjview). Brain regions defined in the AAL atlas (Tzourio-Mazoyer et al., 2002) were used to find the location of activation. Repeated measures ANOVA was used to determine regions particularly activated for each one of the tested factors (explicit instructions (motor imagery) vs monetary reward).

7.2 **SMA-ROI self-regulation progress**

To determine the effect of BOLD self-regulation in the target region, smoothed normalized brain volumes were used. The analysis was performed in an 8 mm^3 ROI in the SMA (MNI coordinate limits: x: -8, 8; y: -8, 8; z: 52, 68). This region was selected comprising a wide area in the SMA, including the regions named in the literature as pre-SMA and SMA proper (Mayka et al., 2006). Using mean BOLD values across each run (including training and transfer runs) the Percentage BOLD (r_{SMA}) was computed as below:

$$r_{SMA} = 100 * \frac{\mathrm{Mean}\left(\mathrm{BOLD}_{\mathrm{UpReg}}\right) - \mathrm{Mean}(\mathrm{BOLD}_{\mathrm{Base}})}{\mathrm{Mean}(\mathrm{BOLD}_{\mathrm{Base}})} \quad (3)$$

where $BOLD_{UpReg}$ and $BOLD_{Base}$ are vectors with the mean BOLD signal time series in the SMA-ROI during up-regulation and baseline blocks. Group comparison of self-regulation levels was done using one-way ANOVA. To measure the participant's learning effect (Δr_{SMA}) in terms of his improvement in increasing the BOLD signal in the up-regulation condition compared to the baseline condition over the two days of training, we considered the difference of the mean r_{SMA} in the 4 runs of second day minus the mean r_{SMA} of the 4 runs of first day. All data was checked for normality and non-parametrical tests were used when appropriate.

Additionally, to determine if inter-subject r_{SMA} variability can be affected by the inclusion of training factors (i.e. feedback, motor imagery and monetary reward) standard deviation (SD) of the group r_{SMA} for each run was calculated. In this case, Kruskal-Wallis test was used to check for group effect, U Mann-Whitney test was used as *post-hoc* and Bonferroni correction was considered. Intra-subject variability was also analyzed by group. For this purpose, r_{SMA} for each up-regulation TR was calculated for each subject and the variance of r_{SMA} (using SD) was calculated for each run. With SD values for each run and participant, repeated measures ANOVA test was calculated. Additionally, significant run effect was tested in each group using Friedman test. A

measure of functional SNR, defined as the ratio of the signal difference between the experimental conditions to their combined noise (Huettel, Song & McCarthy, 2014), was calculated using the Fisher Score (FS; Bishop, 1995; Lal et al., 2004; Ruiz et al., 2013a) as follows:

$$FS = \frac{\left[\mathrm{Mean}(\mathrm{BOLD}_{\mathrm{UpReg}}) - \mathrm{Mean}(\mathrm{BOLD}_{\mathrm{Base}})\right]^2}{\mathrm{Var}(\mathrm{BOLD}_{\mathrm{UpReg}}) + \mathrm{Var}(\mathrm{BOLD}_{\mathrm{Base}})} \quad (4)$$

FS was calculated for each subject, run and block. Repeated measures ANOVA was used to compare the FS across groups, runs and blocks. Significant run effect was tested in each group using Friedman test.

The relationship between intra-subject variability and delivered monetary reward was also evaluated using linear regression from the $G_{F,R}$ data.

In all cases, Spearman correlation coefficient was used to test dependence of variability/FS and run progress.

7.3 **Functional connectivity analysis**

FC analysis was performed to recognize network changes during up-regulation in SMA in different experimental groups. For this purpose, correlation coefficients were computed to measure the linear relationship between BOLD activity in different voxels or regions (Friston, 2011). To perform ROI to ROI FC analysis, the CONN toolbox was used (Whitfield-Gabrieli & Nieto-Castanon, 2012) after the following pre-processing steps were performed: denoising using bandpass-filtering (0.008Hz-0.09Hz), inclusion of estimated head motion parameters, white matter and CSF as covariates, and linear detrending and despiking before calculating regression. Regions inside the field of view were selected from the AAL atlas (Tzourio-Mazoyer et al., 2002) (please see supplementary table S1) for grouping the brain voxels inside these areas to calculate the ROI to ROI BOLD signal correlations. Additionally, a customized ROI of Nucleus

Accumbens was included in the analysis due its relevance in reward processing (Ikemoto et al., 1999; Knutson et al., 2001).

ROI to ROI bivariate correlations were calculated for up-regulation blocks. Each ROI pair (seed-target) was considered as independent from other pairs (i.e. calculation of the correlation coefficient (*r*) in isolation). Correlation coefficient was calculated according to the formula (Whitfield-Gabrieli & Nieto-Castanon, 2012):

$$r = (x^t x)^{-\frac{1}{2}} (x^t y)(y^t y)^{-\frac{1}{2}} \qquad (5)$$

where x and y are vectors of the BOLD time-series for seed ROI and target ROI.

CONN analysis produced one FC Z matrix (51x51 in this case) for each study group (4) and run (8). FC Z matrix contained Fisher-transformed correlation coefficients z_{FC} (i,j) (z_{FC}(i,j)= atan(r(i,j))) between all the i and j ROI pairs. In our analysis, z_{FC} was used to report the FC values between ROIs.

To assess the similarities and differences of FC in the brain among the groups, FC patterns in different groups were compared with the FC pattern of the group G_F (reference group). In the following sections, two different ways of selecting the ROIs for the above analysis are described. In the two cases, brain regions in G_F are sorted according to two different criteria, namely, 1) Mean pairwise correlation coefficients of functional connectivity (mean z_{FC} values), from all the training runs, between the top 6 regions, and 2) Rate of change, slope, of the the z_{FC} values between the top 6 regions over the course of neurofeedback training. Additionally, 4 extra regions were selected for each of the two criteria described above but with restriction of SMA as the seed region (i.e. the selected region needs to be connected with SMA). Finally, right and left SMAs were also included in the analysis for both cases. Therefore, the complete analysis considered 12 regions for each criterion.

7.3.1 **Mean FC changes among groups**

As the first criterion, *mean z_{FC} values across runs* were considered to unveil functional connections that could be relevant during the NF training process. Considering the FC data of the reference group, the 3 pairs of regions with the top *mean z_{FC}* values taking in consideration all regions in FC Z matrix (hereafter called the "whole-brain connectivity matrix") were selected. To give relevance to SMA as the target region in our NF training, the 4 regions with top mean z_{FC} values and (right or left) SMA as seed area were additionally selected. Left and right SMAs were also included in the analysis. In total, mean z_{FC} analysis included 12 regions (top mean whole-brain connectivity matrix (n=6) + top mean SMA-seeded (n=4) + R&L-SMA (n=2)).

Plots were generated (figure 5) considering the 12 selected regions for each group, by presenting significant functional connections ($|z_{FC}| \geq 0.26$, $p<0.001$) among them. For the purpose of visualization, the thickness of the lines connecting the ROIs is represented to be proportional to the magnitude of $z_{FC,}$ and the lines are drawn in red color for positive and blue for negative z_{FC} values.

7.3.2 **Slope FC changes among groups**

As a second criterion for analysis we used the *rate of change of z_{FC} values* across the NF training process, i.e. we considered the slope of z_{FC} curve across the 8 training runs. Similar to the previous method used in the analysis of *mean FC changes among groups* (with G_F as the reference), 3 pairs of regions with the top z_{FC} slope from the whole-brain connectivity matrix were selected. Four additional regions with the top z_{FC} slope, considering SMA as the seed region, were selected. Again, left and right SMAs were included in the analysis. In total, this analysis included 12 regions (top slope whole-brain connectivity matrix (n=6) + top slope SMA-seeded (n=4) + R&L-SMA (n=2)).

The 12 selected regions and the changes between mean z_{FC} of day 2 minus the mean z_{FC} of day 1 ($\Delta z_{FC} = |z_{FC,2} - z_{FC,1}| > 0.15$) for each group are presented here (figure 6).

In this case, the thickness of line is proportional to the magnitude of Δz_{FC}. The red colour of the line was chosen for positive Δz_{FC} values and the blue colour for negative Δz_{FC} values.

Additionally, a correlation change index (CCI) was calculated considering the 12 selected regions (a connectivity matrix with only these regions was generated) and the "whole-brain connectivity matrix". CCI summarizes in one value how the brain's functional connectivity changed in the selected network.

$$CCI_{incr} = \frac{\sum_i \sum_j |z_{FC,2}(i,j) - z_{FC,1}(i,j)|}{N_{total}} \text{ with } \left(z_{FC,2}(i,j) - z_{FC,1}(i,j)\right) > 0 \quad (6)$$

$$CCI_{decr} = \frac{\sum_i \sum_j |z_{FC,2}(i,j) - z_{FC,1}(i,j)|}{N_{total}} \text{ with } \left(z_{FC,2}(i,j) - z_{FC,1}(i,j)\right) < 0 \quad (7)$$

With $z_{FC,X}(i,j)$ being the mean value of z_{FC} between regions i and j during day X, and N_{total} being the total number of possible bi-regional functional connections (66 for the selected 12 regions, and 1275 for whole-brain connectivity matrix). Since the FC Z matrix is symmetrical, and to avoid unnecessary duplication of the calculus, only one permutation of the pair of ROI i-j was considered in the summation (see equations 5 and 6). Only half of FC Z matrix was considered to calculate CCI. Consequently, CCI_{incr} and CCI_{decr} express the mean z_{FC} increase and decrease (between training days 1 and 2), respectively, observed in the network. CCI_{incr} and CCI_{decr} were calculated for each group.

RESULTS

1. Strategies for self-regulation and self-reported performance

During participants' self-reports the strategies or methods that generated the best control of feedback signal were collected. Participants of groups $\mathbf{G_{F,I}}$ and $\mathbf{G_{F,I,R}}$ used motor imagery as expected, including running, moving hands, dancing, among others. In the case of Groups $\mathbf{G_F}$ and $\mathbf{G_{F,R}}$, although participants were not instructed to use mental imagery at all, during the debriefing at the end of all the training runs, they reported the use of a variety of mental strategies for self-regulation (meditation, relaxation, sequential thinking, focusing, etc.) but quite different from motor imagery. Please see table 2.

Similar levels of comfort were reported across groups (Kruskal Wallis non parametric test, day 1: $H(3) = 1.09$, $p > 0.05$, ns; day 2: $H(3) = 3.33$, $p > 0.05$, ns). Participants were asked to rate a Likert scale (5 top performance) their perceived success in controlling the feedback signal (i.e., thermometer) during days 1 and 2 of training. Taking all groups together, a significant increase in ratings was observed during the second day (reported day 1 Mdn=3; day 2 Mdn=4, paired Wilcoxon signed rank test, $Z = -2.98$, $p < 0.01$). No significant differences between groups were found in self-report of performance (Kruskal Wallis non parametric test, day 1: $H(3) = 6.55$, $p > 0.05$; day 2: $H(3) = 2.39$, $p > 0.05$, ns).

Table 2. Reported strategies during the rtfMRI-NF training sessions.

	Strategy reported
$\mathbf{G_F}$ (Feedback)	Relaxing (nature environment related to rest: beach, forest, vacation)
	Remembering (trying to remember details of friends and trips)
	Meditation (concentration in one body point free of other thoughts)
	Positive mood (encouraging himself to increase the thermometer bars)
	Focusing (heat from a fire, movement of a flame, concentration on a white background)
$\mathbf{G_{F,I}}$ (Feedback + instructed imagery)	Motor imagery (a very active rock concert)
	Motor imagery (aggressive movements to get released from the scanner)
	Motor imagery (running, fast movements in scanner)
	Motor imagery (fast and intense movements while playing basketball)
	Motor imagery (playing piano and rugby)
$\mathbf{G_{F,R}}$ (Feedback + Monetary Reward)	Recalling (remembering topics and linking them to new ones)
	Sequences (repeat 3 words sequences, chosen at the moment, not necessarily related)
	"Speaking in his brain" (inner speaking) and recalling important autobiographical memories
	Concentrating on increasing the bars of the feedback thermometer. Thinking about videogames
	Relaxing and focusing on increasing the bars of the feedback thermometer
$\mathbf{G_{F,I,R}}$ (Feedback + Monetary Reward + Instructed Imagery)	Motor imagery (funk dancing)
	Motor imagery (pumping activity or repetitive movement)
	Motor imagery (simple first person actions, i.e. move right hand to touch left elbow)
	Motor imagery (skate tricks)
	Motor imagery (swimming, running, boxing)

2. **SMA-ROI self-regulation**

2.1 **SMA BOLD activation levels**

First, we compared self-regulation of the BOLD signal of the SMA in the four groups of participants across the two days of training. Percent BOLD change (r_{SMA}) during a feedback run was used as an indicator of the amplitude of self-regulation. All groups had a significant mean increase in BOLD activity for each day, (one-sample t-test, compared to zero; $G_{F\ d1}$: M=0.10, t(19)=2.87, p=0.01; $G_{F\ d2}$: M=0.15, t(19)=4.31, p<0.001; $G_{F,I\ d1}$: M=0.11, t(19)=2.49, p<0.05; $G_{F,I\ d2}$: M=0.19, t(19)=3.52, p<0.01; $G_{F,R\ d1}$: M=0.30, t(19)=5.03, p<0.001; $G_{F,R\ d2}$: M=0.29, t(19)=4.75, p<0.001; $G_{F,I,R\ d1}$: M=0.22, t(19)=4.62, p<0.001; $G_{F,I,R\ d2}$: M=0.20, t(19)=3.37, p<0.01). Figure 1 (figure S6 and supplementary table S2) shows the values for up-regulation during the training runs of days 1 and 2. Group differences were tested using one-way ANOVA among the total number of training runs in each one of the 4 groups (40 total runs by group). A significant group factor appears in this case ($F_{3\text{-}156}$=4.643; p<0.01). In Games-Howell *post-hoc* test for multiple comparisons $G_{F,R}$ showed a significant mean difference with groups G_F and $G_{F,I}$ (p<0.01 & p<0.05; respectively). Analysis by day also showed a significant group effect ($F_{7\text{-}152}$ = 2.205; p<0.05), although in the *post-hoc* analysis no significant difference was found between the groups.

To test if subjective perception of feedback control and magnitude of SMA self-regulation were correlated, a comparison of self-report ratings of day 1 and day 2 with the BOLD signal difference (r_{SMA}) were performed. A significant positive correlation was found between r_{SMA} and self-report rating for each day (day 1: r(18)=.48, p<0.05; day 2: r(18)=. 53, p<0.05).

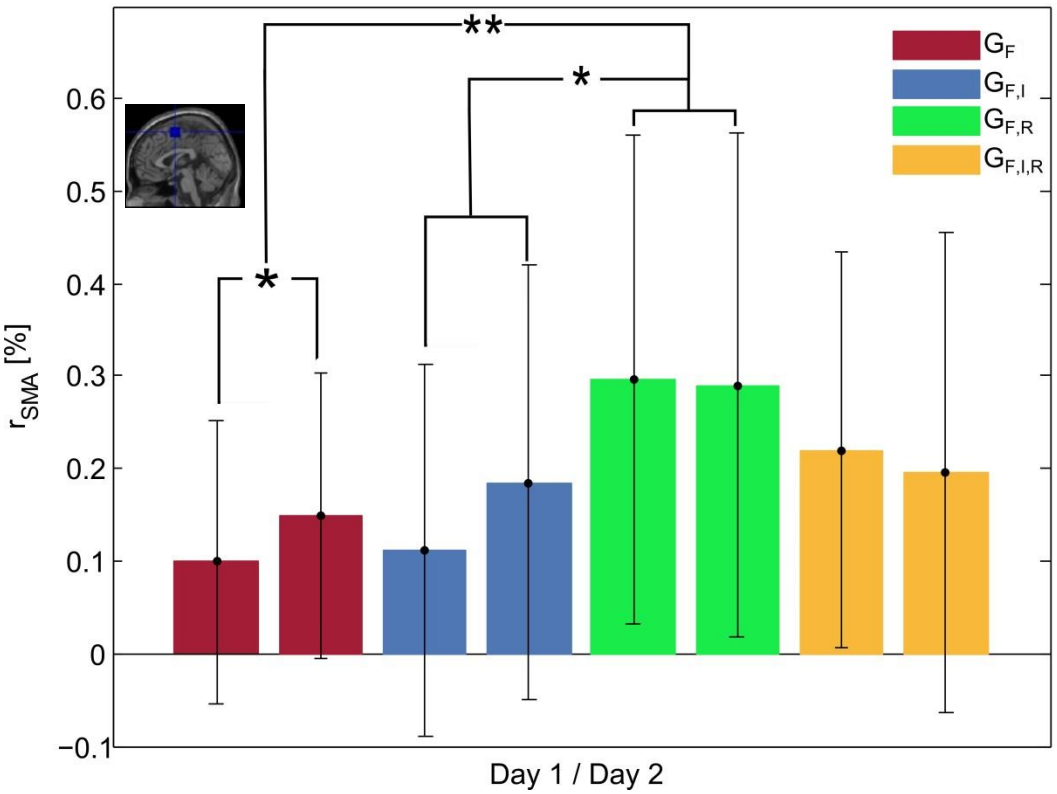


Figure 1. Mean BOLD signal change ($\mathbf{r_{SMA}}$) for the first and second day training runs in the SMA-ROI (MNI x=0, y=0, z=60) for each group. A significant difference was found between days 1 and 2 for Group G_F. From ANOVA analysis, Group G_R was significantly different from 1 and 2. Standard deviation bars and SMA-ROI are shown (** = p<0.01; * = p<0.05).

2.2 **Self-regulation learning**

The learning effect in SMA self-regulation throughout the training days was analysed as the difference between mean values of r_{SMA} in days 1 and 2 (Δr_{SMA}). Δr_{SMA} was defined as our measure of learning self-regulation of the SMA (deCharms et al 2005). A significant difference between day 1 and day 2 was found only for G_F (Δr_{SMA}, five values one-sample Wilcoxon signed-rank test for median difference from 0, G_F: Δr_{SMA}

Mdn=0.064, Z=-2.023, p<0.05; $G_{F,I}$: Δr_{SMA} Mdn=0.025, Z=-0.674, p>0.05, ns; $G_{F,R}$:Δr_{SMA} Mdn=0.016, Z=-0.135, p>0.05,ns; $G_{F,I,R}$: Δr_{SMA} Mdn=-0.091, Z=-0.674, p>0.05,ns). Similarly, observing the progress of the mean value of r_{SMA} run by run in each group, a positive linear tendency (increased magnitude of self-regulation through training runs) was found for G_F (r_s=.55; p=0.16; supplementary figure S1). For group comparisons of Δr_{SMA}, non-significant differences were found (Kruskal Wallis non-parametric test, Mdn: G1=0.064, G2=0.025, G3=0.016, G4=-0.091; H(3)=-0.58, p>0.05, n.s.).

2.3 **Transfer Runs**

Whether participants maintained the capability to self-regulate after training was evaluated in the transfer runs, in which discriminative stimuli for up-regulation and baseline were presented as in the feedback training runs but no feedback of the ROI signal was provided. Day 2 transfer run was used in the analysis to avoid the novelty effect during the transfer run of day 1 (figure 2). One-sample Wilcoxon signed rank test was performed to verify that participants could up-regulate their activity during the transfer run. All groups presented a magnitude of self-regulation (r_{SMA}) significantly different from zero during the transfer runs (one-sample Wilcoxon signed-rank test for median difference from 0; G_F: Mdn= 0.269, $G_{F,I}$: Mdn= 0.234, $G_{F,R}$: Mdn= 0.10, $G_{F,I,R}$: Mdn= 0.15; Z=-2.023, p<0.05 in all groups). No significant differences in transfer r_{SMA} were found among groups (Kruskal Wallis non parametric test, H(3)= 2.109,p>0.1, ns). The magnitude of self-regulation (r_{SMA}) in the transfer run was not significantly different from training day 2 in the 4 groups (Wilcoxon signed-rank test, G_F: Z=-1.753, p>0.05 (p=0.08), ns, $G_{F,I}$: Z=-0.944, p>0.05,ns, $G_{F,R}$: Z=-1.753, p>0.05 (p=0.08), ns, $G_{F,I,R}$: Z=-0.135, p>0.05,ns).

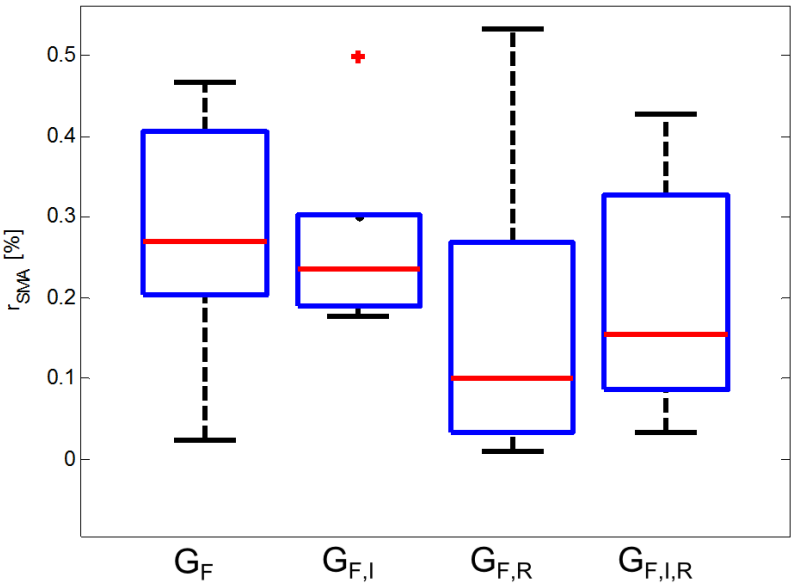


Figure 2. Box plot showing results of the transfer run for all groups on day 2. The y-axis shows r_{SMA} values presented in the brain region centered at MNI: x=0, y=0, z=60, and the x-axis indicates the 4 groups. No significant differences between the groups were found. All groups presented significant increases in SMA BOLD during the up-regulation blocks of the transfer runs.

2.4 **Variability analysis**

High level of variability in the data was observed in all the groups. To analyze if variability can be related to the experimental factors, namely, feedback, motor imagery or reward, we compared the inter-subject variability using the standard deviation (SD) of the signals among the groups (figure 3). Kruskal-Wallis test reported a significant group effect among the inter-subject SD of r_{SMA} in the training runs (Group SD G_F: Mdn=0.157; $G_{F,I}$: Mdn=0.224; $G_{F,R}$: Mdn=0.263; $G_{F,I,R}$: Mdn=0.270; H(3)=21.463,p=.006). Subsequent *post-hoc* test found significant differences, after applying Bonferroni correction, between G_F and $G_{F,R}$ (corrected α=0.5/6=0.0083; G_F vs $G_{F,I}$: Z=-2.310 ,p=0.021, ns; G_F vs $G_{F,R}$: Z=-3.151, p = 0.002 ; G_F vs $G_{F,I,R}$: Z=-2.415, p = 0.016, ns; $G_{F,I}$ vs $G_{F,R}$: Z= -1.365, p > 0.1, ns; $G_{F,I}$ vs $G_{F,I,R}$: Z= -1.155 , p > 0.1, ns;

$G_{F,R}$ vs $G_{F,I,R}$: Z= -0.525, p> 0.1, ns). Linear regression of group inter-subject variability across runs showed no significant linear trend across runs (G_F: -0,0001x + 0,1634, r_s=-.071, p>0.1, ns ; $G_{F,I}$: y = 0,0025x + 0,2052, r_s=.024, p>0.1, n.s.; $G_{F,R}$: -0,0088x + 0,321, r_s=-.357, p>0.1, ns; $G_{F,I,R}$: y = 0,0111x + 0,1974, r_s=.262, p>0.1, ns). Analyses of intra-subject variability vs functional SNR and intra-subject variability vs reward, both by group and feedback run, showed no significant effects (please refer to figures S7, S8, S9, S10 and S11).

3. **Whole brain univariate analysis**

To examine activations in other brain regions during the up-regulation blocks, group level, univariate, whole-brain analysis was performed using Statistical Parametric Mapping (SPM) (figure 4). Only the data from the second day's training was used to focus our analysis on brain activations resulting on late stages of training. The calculations were done considering the contrasts up-regulation > baseline blocks ([1 -1]). The results showed that SMA activation was present in all groups. Other brain regions that were consistently activated in all study groups were bilateral precentral gyrus, insula and supramarginal gyrus. From the 2-way ANOVA (considering the factors effects, groups and runs) no major differences in activations were found except some clusters of scattered activations (e.g. group $G_{F,R}$ has only a significantly increased cluster of k_E=19 at right precentral gyrus after applying FWE a p<0.05) (please refer to supplementary table S3).

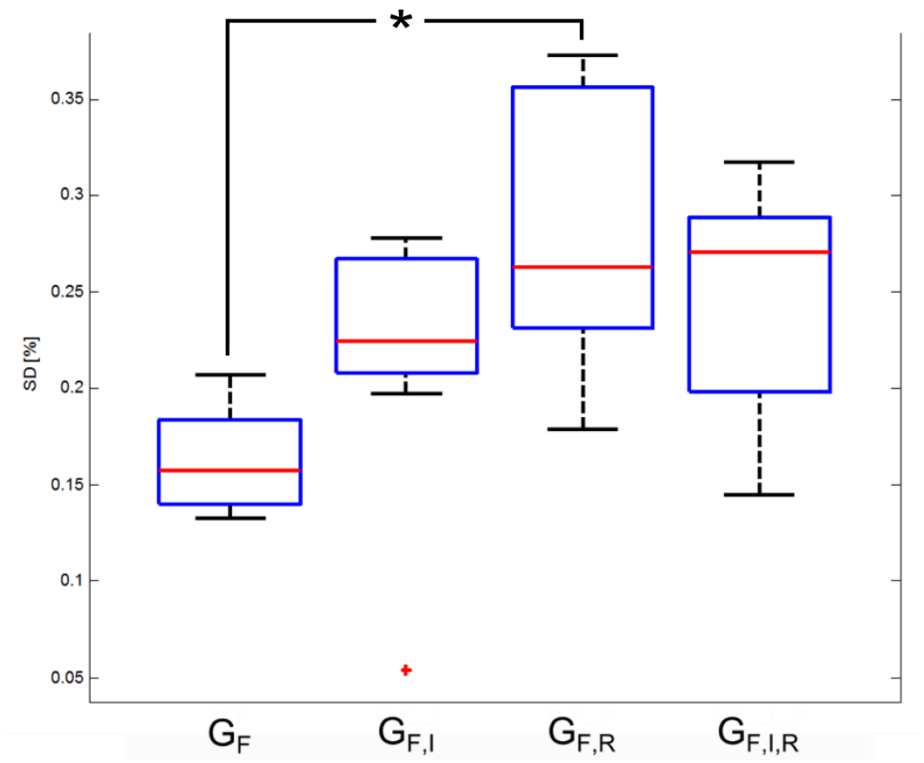


Figure 3. Box plot showing the inter-subject variability by groups. The presented distribution considers standard deviation of the mean (SD group r_{SMA}) for each one of the 8 NF training runs by group.

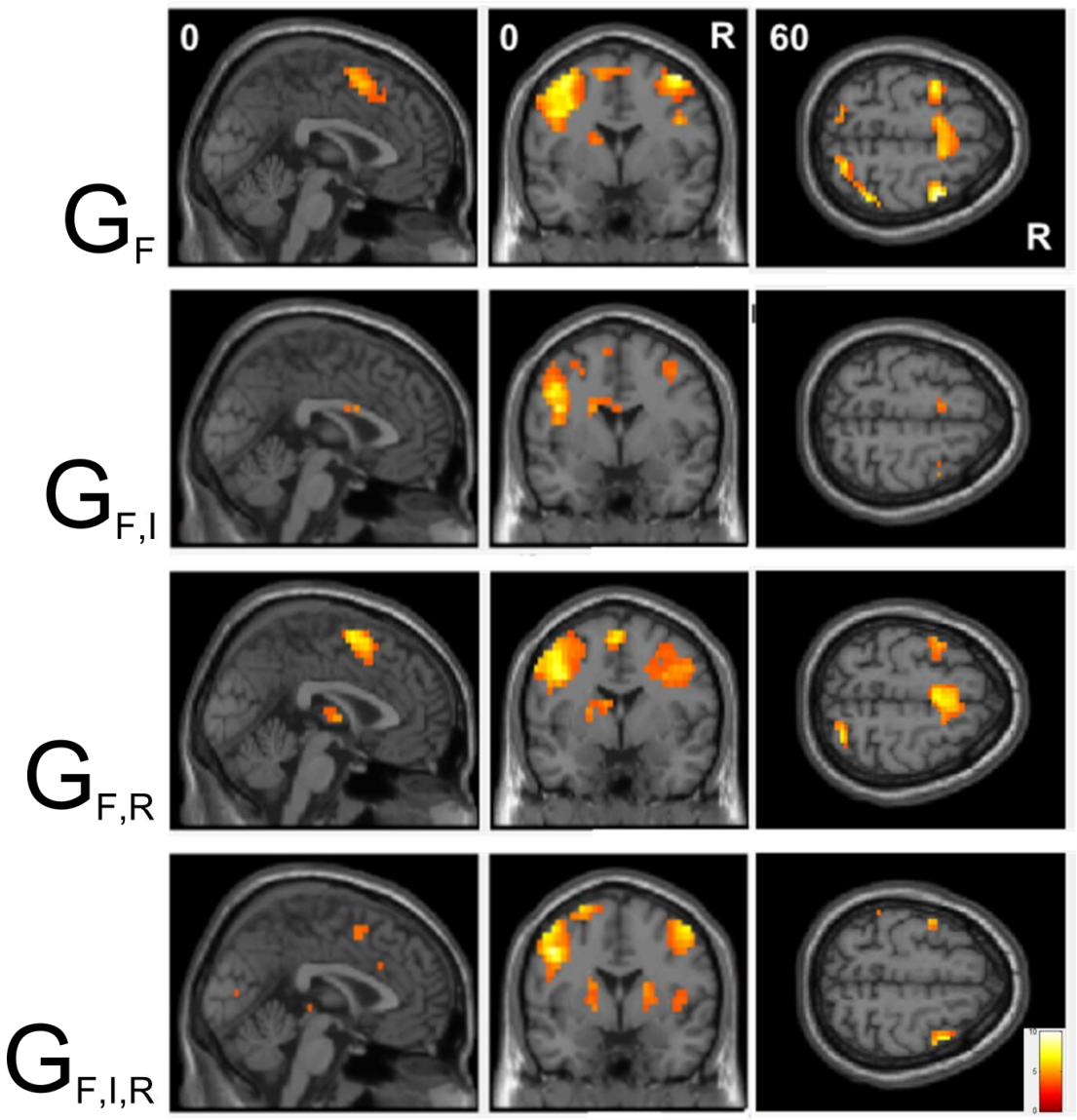


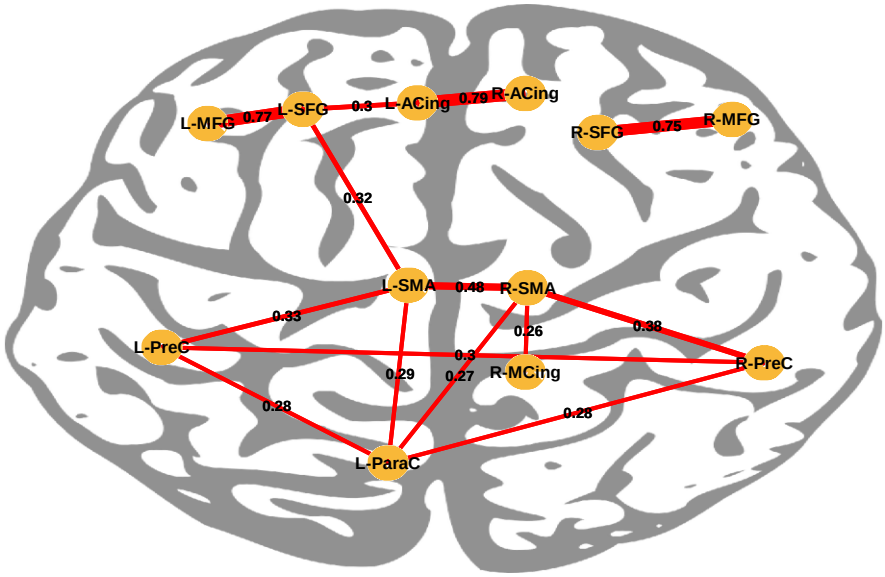
Figure 4. Activation maps during the up-regulation of SMA obtained from whole-brain statistical parametric mapping (SPM) during day 2 with one-sample t-test, (FDR $p<0.01$, cluster size = 10). SMA activity is present in all groups.

4. **Functional connectivity analysis**

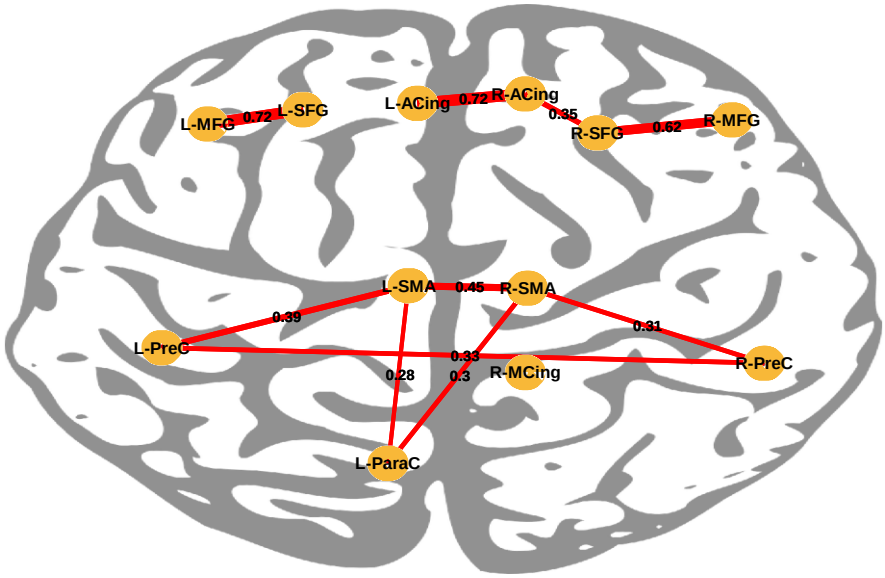
4.1 **Comparison of mean FC changes among groups**

The 12 selected regions of this analysis, based on the AAL atlas (Tzourio-Mazoyer et al., 2002), were: Precentral (precentral gyrus) L, Precentral R, Frontal Sup (superior frontal gyrus) L, Frontal Sup R , Frontal Mid (middle frontal gyrus) L, Frontal Mid R, Supp Motor Area (SMA) L, Supp Motor Area R, Cingulum Ant (anterior cingulate cortex) L, Cingulum Ant R, Cingulum Mid R, and Paracentral Lobule L. FC patterns for the 12 selected regions by group are presented in figure 5. Despite the slight differences observed in FC patterns, a consistent pattern of correlations was found across all groups. Regions that appear with $z_{FC,T}$ (mean z_{FC} during the 8 training runs, $z_{FC,T}$) are: middle frontal gyrus and superior frontal gyrus in right and left hemispheres, left and right anterior cingulate gyrus, left and right SMA, left and right precentral gyrus, left SMA and precentral gyrus (supplementary table S4). In general, two zones of correlated regions can be observed, one frontal and a posterior-motor functional network. Only for Group G_F a path between these two zones (left superior frontal gyrus and SMA) appears with higher correlation.

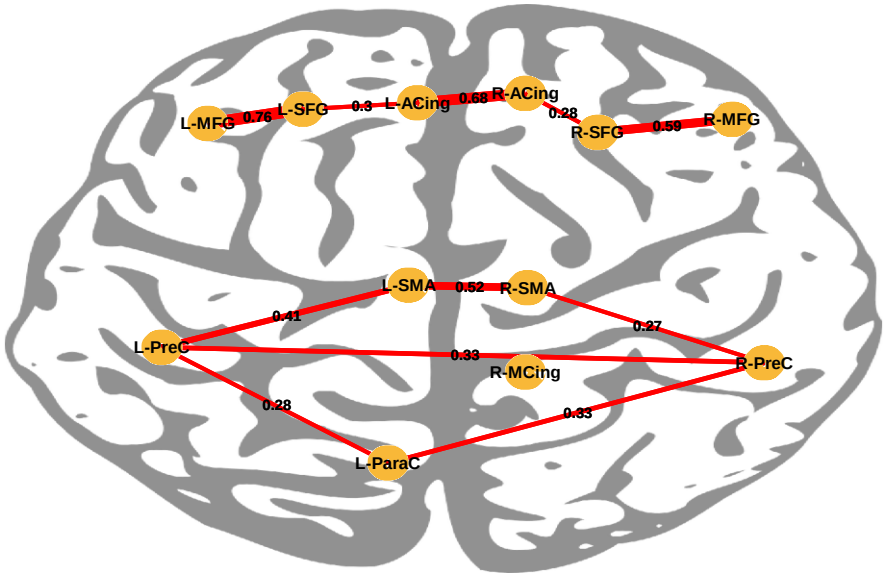
G_F: Contingent NF



$G_{F,I}$: Contingent NF + Motor Imagery



$G_{F,R}$: Contingent NF + Monetary Reward



$G_{F,I,R}$: Contingent NF + Motor Imagery + Monetary Reward

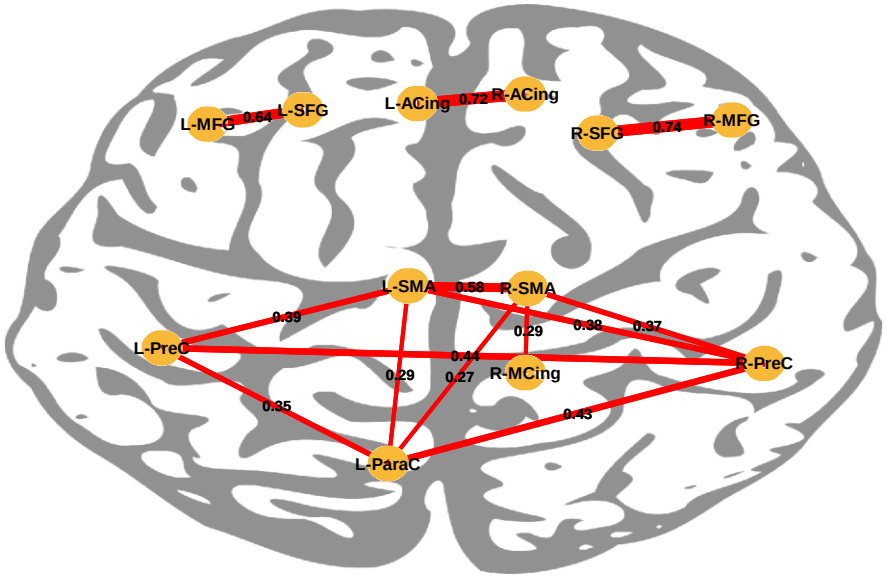


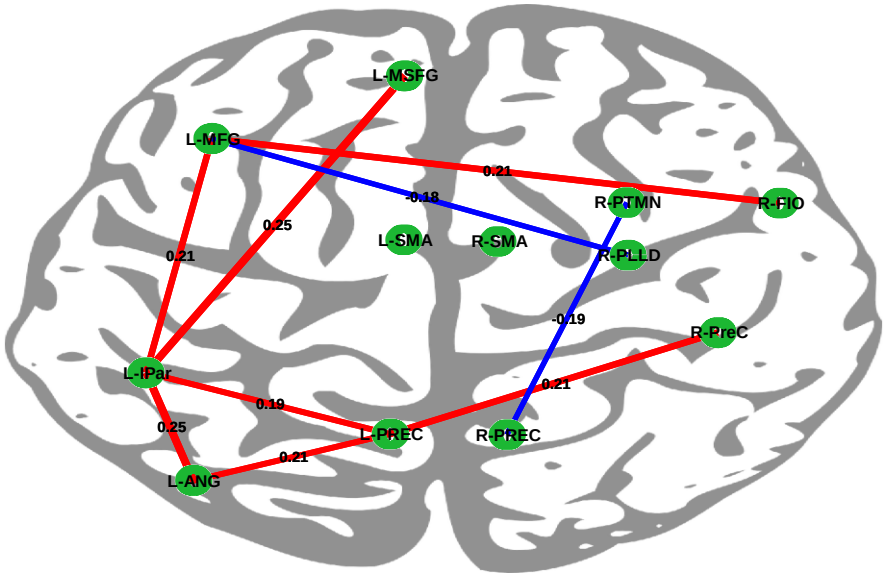
Figure 5. FC values for the selected 12 brain regions with the highest mean correlation values across all the NF training runs. The effect of the different experimental factors (feedback, motor imagery and reward) on FC patterns was analysed in comparison to the

group $\mathbf{G_F}$ as the reference group (see section 7.3 for details on the method). The thickness of lines is proportional to z_{FC} (z_{FC} values shown on the line). FC patterns across groups were found to be similar to each other. (Precentral L = L-PreC; Precentral R = R-PreC; Frontal Sup L = L-SFG; Frontal Sup R = R-SFG; Frontal Mid L = L-MFG; Frontal Mid R = R-MFG; Supp Motor Area L = L-SMA; Supp Motor Area R = R-SMA; Cingulum Ant L = L-ACing; Cingulum Ant R = R-ACing; Cingulum Mid R = R-MCing; Paracentral Lobule L = L-ParaC).

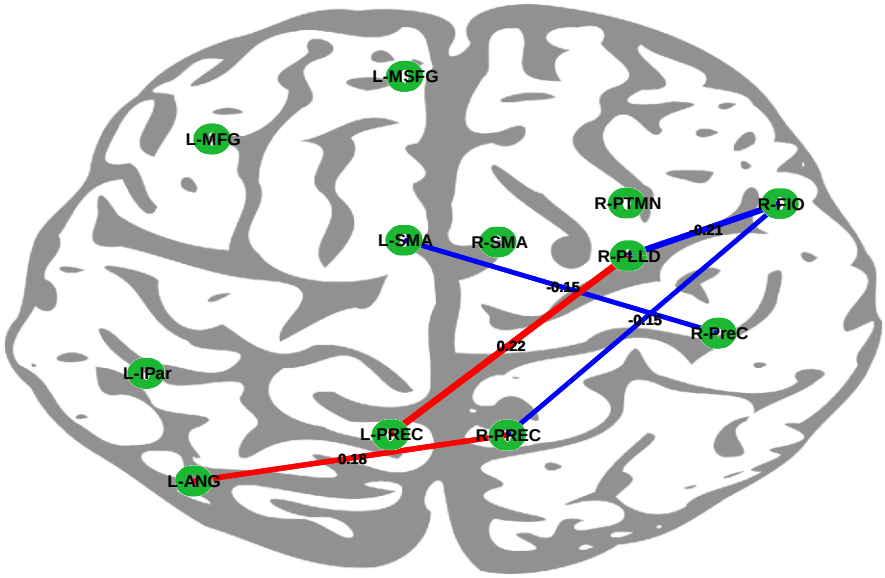
4.2 **Comparison of slopes of FC changes among groups**

The selected 12 regions with the highest slope values were Supp Motor Area R, Supp Motor Area L, Precentral R, Frontal Mid L, Frontal Inf Oper R (inferior frontal gyrus par opercularis, Broca area BA 44), Frontal Sup Medial L (medial superior frontal gyrus, MFG), Parietal Inf L (inferior parietal excluding supramarginal and angular gyrus), Angular L (angular gyrus), Precuneus L, Precuneus R, Putamen R and Pallidum R. Figure 6 presents the increases and decreases (red and blue respectively) in z_{FC} between days 1 and 2. Inspection of the figure 6 indicates that Groups $\mathbf{G_F}$ and $\mathbf{G_{F,R}}$ have higher number of regions that increased their FC between days 1 and 2. On the other hand, groups $\mathbf{G_{F,I}}$ and $\mathbf{G_{F,I,R}}$ have less regions with increases in FC values and more regions that display decreases in FC values. Additionally, bilateral precuneus in $\mathbf{G_{F,R}}$ increased its connections across training (Δz_{FC} (L-precuneus, L-MFG)= .23, Δz_{FC} (R-precuneus, L-MFG)= .34 ; z_{FC}(L-precuneus, L-MFG)=0.38 during last training run).

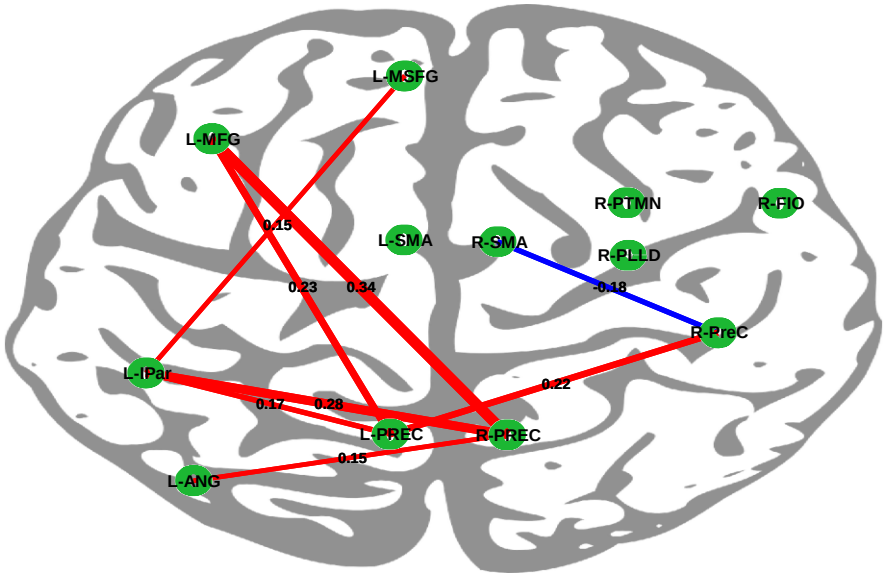
G_F: Contingent NF



$G_{F,I}$: Contingent NF + Motor Imagery



$G_{F,R}$: Contingent NF + Monetary Reward



$G_{F,I,R}$: Contingent NF + Motor Imagery + Monetary Reward

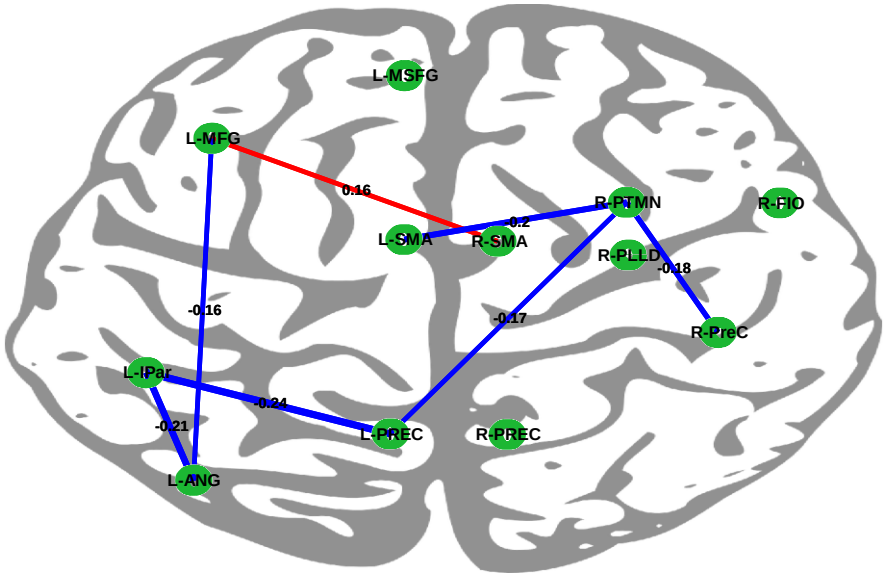


Figure 6. FC increases/decreases between 12 brain regions with the highest change rate (slope) across NF. The thickness of each line is proportional to the corresponding Δz_{FC} for the connection (increases with red lines, decreases with blue and z_{FC} values shown on the line). Groups without motor imagery presented higher FC increases than imagery

groups (Supp Motor Area R = R-SMA; Supp Motor Area L = L-SMA; Precentral R = R-PreC; Frontal Mid L = L-MFG; Frontal Inf Oper R = R-FIO; Frontal Sup Medial L = L-MSFG; Parietal Inf L = L-IPar; Angular L = L-ANG; Precuneus L = L-PREC; Precuneus R = R-PREC; Putamen R = R-PTMN; Pallidum R = R-PLLD).

Furthermore, CCI (mean FC increases (CCI_{incr}) or decreases (CCI_{decr}) in a network from day 1 to day 2) was used as complementary information to FC slope plots to express the changes in FC from day 1 to day 2. Hence, for each group CCI_{incr} and CCI_{decr} were calculated from the connectivity matrix of the 12 selected regions (supplementary table S5) and the "whole-brain connectivity matrix" (supplementary table S6). As can be inferred from the connectivity plots, Group $\mathbf{G_F}$ and $\mathbf{G_{F,R}}$ have higher increases in z_{FC} (higher CCI_{incr} values) and lower decreases in z_{FC} (lower CCI_{decr} values) compared to other groups, i.e. these groups showed more increments and less decrements in correlation values on the second day of training compared to the first day. However, considering "whole-brain connectivity matrix", $\mathbf{G_{F,R}}$ alone had the greatest increase in correlation coefficients (higher CCI_{incr}) during the second day of training.

DISCUSSION

The first aim of the current study was to compare the effects of three different factors that are expected to influence the capability of learning volitional control of brain activity, i.e. contingent feedback, motor imagery and monetary reward. For this purpose, we trained four experimental groups using a combination of these 3 factors in a rtfMRI NF experiment.

We used two measures to study self-regulation proficiency in the ROI: 1) r_{SMA}, which expresses the magnitude of self-regulation of SMA during the NF training, and 2) Δr_{SMA} that indicates the improvement in the magnitude of self-regulation, namely, the learning effect, through the training process.

In most of the NF studies so far participants were provided instructions to use mental imagery to control brain activity, in addition to contingent feedback (Caria et al., 2007; Hwang et al., 2009; Lawrence et al., 2013; Rota et al., 2009; Scharnoswski et al., 2012; Sitaram et al., 2011; Subramanian et al., 2011; Yoo et al., 2008; Young et al., 2014; Zilverstand et al., 2015). Although monetary reward has not been used extensively, successful self-regulation in rtfMRI without instructing mental imagery have also been recently reported (Bray et al. 2007; Buyukturkoglu et al. 2015; Shibata et al. 2011; Megumi et al., 2015). In our study, all the groups, irrespective of whether they were given instructions or not, were able to up-regulate the BOLD signal in the SMA throughout the experiment. Interestingly, when the magnitude of up-regulation in SMA was compared among the groups, the group in which monetary reward was given showed the highest amplitude of self-regulation during the training period, in comparison with no-reward groups (G_F and $G_{F,I}$). Most significantly, the groups in which motor imagery ($G_{F,I}$ and $G_{F,I,R}$) was used showed reduced magnitude of self-regulation compared with the group that was solely provided reward (G_R), as well as reduced learning effect compared with the group that only used contingent feedback alone (G_F). Furthermore, the detrimental effect of motor imagery can be seen when comparing the

group that received contingent feedback and reward ($G_{F,R}$) with the group that received feedback, reward and instruction to perform motor imagery ($G_{F,I,R}$). Results suggest that the inclusion of motor imagery degrades the magnitude of up-regulation in spite of the contingent presentation of reward.

While evaluating the learning effect across training days (Δr_{SMA}), it is apparent that the only group in which learning was observed was the group that was given only contingent feedback ($\mathbf{G_F}$). Considering that group $\mathbf{G_{F,R}}$ had the highest level of SMA up-regulation already on day 1, it is possible that the lack of learning effect in this group could be due to a ceiling effect (achievement of a very high-level up-regulation already on day 1). An alternate explanation is that the learning curve for this group is more gradual and cannot be recognized clearly in two training days.

The results indicating that the inclusion of explicit instructions to perform motor imagery do not improve up-regulation might be counterintuitive considering the widespread use of such instructions in NF experiments (Caria et al., 2007; Lawrence et al., 2013; Rota et al., 2009; Scharnoswski et al., 2012; Sitaram et al., 2011; Subramanian et al., 2011; Sulzer et al., 2013b; Yoo et al., 2008; Young et al., 2014; Zilverstand et al., 2015). For this reason, we emphasize the need to examine the mechanisms involved in learning brain self-regulation, which are still far from being totally elucidated (Scharnowski et al., 2012). Our results may also be construed as supporting the proposal that operant conditioning can play an important role for successful learning of brain hemodynamics control (Birbaumer et al., 2013). In operant conditioning, desirable responses are positively reinforced and negative ones discouraged leading finally to an automatized skill achieved through a "trial-and-error" process (Strehl, 2014). In the present experiment, the desirable response, i.e. BOLD signal increase in SMA, is reinforced by the rising bars of the thermometer during the training runs, assuming that participants assign reward values to the thermometer bars.

However, the self-reports of the participants at the end of NF training indicate that even when participants were not instructed any motor imagery (in groups G_F and $G_{F,R}$) they

did indeed use some form of mental imagery although not always related to motor imagery. This opens an important point that NF training in humans even in the absence of explicit instructions can induce participants to incorporate some form of mental strategy to learn volitional control of their brain signals. In the group $G_{F,R}$, an additional factor, namely monetary reward was provided to the already existing feedback information, generating a stronger reinforcement with the consequential rise in brain activations.

One of the major features in this kind of learning is the secondary place of the conscious involvement of the participant in performing the requested task, i.e., moving the thermometer bars (Birbaumer et al. 2013). In fact, similar to our experiment (for groups G_F and $G_{F,R}$) , Bray et al. (2007) and Shibata et al. (2011) did not inform the participants about the exact meaning or the contingency of the delivered feedback signal, yet demonstrated learned volitional control. Some studies from EEG-NF also support this view, leading to a speculation that use of mental imagery and conscious brain resources thereof can impair an efficient mechanism of brain control (Kober et al., 2013; Witte et al., 2013).

The “Dual process theory” proposed by Lacroix (1986) states that both "feedforward” and “feedback" processes are involved in the control of the desired signal. Feedforward processes are active when verbal instructions enable participants to retrieve existing behavioral programs to effectively perform the task, e.g. a motor imagery program of moving the right hand. The aim of the NF training then is to find the program (or a combination of programs) that generates the best control of the feedback signal. On the other hand, "feedback processes" are active when participants do not receive verbal instructions about the bodily signal they have to control, and consequently, need to construct a new behavioral program through determination of the properties of the system (interoception) by trial and error, based on contingent feedback. Therefore, the dual-process theory suggests that giving explicit verbal information about the potentially relevant behavioral programs that control the selected body signal can help participants

to reduce the time needed for constructing a new program. In other words, verbal instruction to use mental imagery can be seen as a "shortcut" for helping to achieve self-regulation. Through NF training, further refinements of the selected behavioral program is achieved using the feedback signal to reach an optimal response. However, learning to self-regulate brain signals can be impaired when: 1) the behavioral program to perform self-regulation is not retrieved because it simply does not exist, i.e. it is not in the subject's behavioral repertoire; 2) participants may possess behavioral programs that work only partially, therefore maintaining the use of an ill-fitted strategy through the course of NF training. In these scenarios, the theory proposes that subjects end up relying on feedback processes to control the signal, presumably through operant conditioning processes.

Previous biofeedback studies evaluated the effects of reward in addition to contingent feedback (Bennet et al., 1978; Blanchard et al., 1974; Bouchard & Granger, 1980). Blanchard et al. (1974) studied the additive effects of monetary reward and feedback to train voluntary increase of heart rate. A non-consistent advantage of delivering monetary reward in comparison to using a feedback-only scheme was found. In contrast, Bennet et al. (1978) found an increase in heart rate score in the groups with reward in comparison to the non-reward group. Additionally, Bennet et al. (1978) also studied the effect of the cognitive strategies on learning to increase heart rate. Various mental strategies (e.g. frightening or sexual thoughts) were reported, indicating that a wide variety of imagery can be used to control heart rate.

In the present study, successful up-regulation of SMA was achieved in participants of the groups $\mathbf{G_F}$ and $\mathbf{G_{F,R}}$ despite not using motor imagery (as usually instructed in the previous rtfMRI-NF studies). However, the participants of the above two groups used other mental strategies even when they were not instructed (See Table 2). This outcome could be explained by the role of SMA on non-movement related brain activity (Chung et al., 2005; Nachev et al., 2008) that might have been used by the participants included in the non-imagery groups. Further, there was a noticeable lack of learning effect in the

groups which were provided explicit instructions of motor imagery ($\mathbf{G_{F,I}}$ and $\mathbf{G_{F,I,R}}$). This outcome could be due to the sub-optimal levels of BOLD increase due to the use of motor imagery while more flexible exploration of other forms of mental imagery could have produced greater signal increases in SMA. Alternately, the above outcome may also be due to the inability of the current real-time fMRI approach to precisely localize the SMA sub-clusters pertaining to movement of a specific body part. Future progress in fMRI signal acquisition (e.g., Multiplexed EPI sequences for sub-second whole brain fMRI, Feinberg et al 2011) and real-time pattern classification (e.g., Cox et al., 2003; Rana et al., 2013; Sitaram et al., 2011; Zheng et al., 2013) may allow precise feedback of the brain activity pertaining to a specific brain function that is being addressed.

In the transfer runs, when no contingent feedback was presented, all the four experimental groups were able to up-regulate SMA. However, it is interesting to note that the performance of $\mathbf{G_{F,R}}$ in transfer runs was similar to the other groups, and this group did not show the greater increase in self-regulation magnitude in the transfer runs as was earlier observed during the training runs. Hence, although the use of monetary reward in NF training has a positive effect on learning, the removal of the reward signal during the transfer runs reduced the magnitude of self-regulation leading to an extinction of the learning effect. Further work should carefully assess the effect of different reward schedules on learning and its extinction with time.

The large variability we observe in r_{SMA} values during the training runs in all the groups may be due the small group sizes in our study, but can also be explained by the large intra- and inter-subject variability that has been generally observed in fMRI studies (Gaxiola-Valdez et al., 2012; Kannurpati et al., 2010; Lund et al., 2005). Alternatively, it is possible that the large variability in the data may represent the exploratory trial and error process in which participants use different types of mental imagery to achieve self-regulation (Galea et al., 2013; Pekny et al., 2015; Wolpert et al., 2011).

We evaluated whether the variability in the SMA signal during NF training could have been generated due to the differential effects of feedback, motor imagery and reward.

We found a significant increase in inter-subject variability in the group $\mathbf{G_{F,R}}$ in comparison to group $\mathbf{G_F}$**.** The inclusion of reward as an additional factor tended to increase both the amplitude and the variability of the magnitude of self-regulation (r_{SMA}). A possibility is that the inclusion of monetary reward amplifies the desired response (r_{SMA}), as observed in our results, and consequently enhances the already high inter-subject variability. Previous studies on operant conditioning have shown that when the value or strength of the reinforcement is increased (e.g. giving more food pellets to a rat), the desired response tends to increase the magnitude or the speed in reaching the asymptote of the learning curve (Bower & Trapold, 1959; Bower & Miller, 1960). However, it was also reported that learning is dependent on the maintenance of the reward and is prone to extinction when the reinforcer is taken out. This supports our observation of a noticeable decrease in the SMA up-regulation in the transfer runs in comparison to the training runs in the reward group. Finally, the presentation of reward in $\mathbf{G_{F,R}}$ could have introduced an additional source of variability to the problem due to the individual differences in participants' response towards reward (Cohen et al., 2005; Peters & Büchel, 2011).

In our experiment, we assume that when participants receive only feedback ($\mathbf{G_F}$), they have an intrinsic motivation to achieve volitional control. On the other hand, with the inclusion of the monetary incentives, the extrinsic reward starts to have a prominent role as a motivational factor for learning self-regulation. Previous studies have found that although extrinsic reward may generate better results initially, in the long term, the intrinsic motivation is undermined and the initial, positive results can diminish when the explicit reward is retired (Birch et al., 1984; Deci et al., 1971; Deci et al., 1999). Therefore, reinforcing intrinsic motivations can be a more reliable approach for long-term training. The inclusion of reward can be helpful, particularly during the initial stages of NF training, but the maintenance of this factor for longer duration should be carefully evaluated to maintain the beneficial effects in brain self-regulation.

The second aim of our study was to explore neural substrates of brain hemodynamic control. For this purpose we conducted univariate analysis and FC analysis of the whole brain. The analysis of magnitude of self-regulation showed differences between groups. However, this difference did not persist when we looked at the spatial brain activation elicited during up-regulation. The results of univariate, whole brain analysis showed that brain activations were strikingly similar across the groups. Similarly, from the comparison of mean FC changes among groups, we found a core network of connections that is observed in the all four groups. This similarity in brain activations and FC patterns among the groups can be due the fact that contingent feedback was given to all the groups.

Our results indicating the common activations in insula, left supramarginal gyrus and precentral gyrus have been reported in previous NF studies. Ninaus et al. (2013) asked participants to control the feedback signal (a thermometer) during a covert sham-feedback experiment in fMRI. The reported regions of significant activation, when participants tried to get control of thermometer bars in contrast to only watching the moving bars, were the insula, supramarginal gyrus, precentral gyrus, anterior cingulate gyrus, middle frontal gyrus, thalamus and SMA. Another study, a meta-analysis of 12 rtfMRI studies by Emmert et al. (2015) also found similar activations, particularly, in the anterior insula and tempo-parietal areas along prefrontal cortex (dorsolateral and ventrolateral).

The active regions during the up-regulation blocks in our study have been previously linked to different brain processes. Insula has been related to driving attention to inner states (Haller et al., 2013; Ninaus et al., 2013) in NF tasks. Supramarginal gyrus has been reported to participate in inner speech and language production (Geva et al., 2011; Hartwigsen et al., 2015). SMA as part of the motor network has well documented connections with precentral gyrus (Kasess et al., 2008; Solodkin et al., 2004).

The analysis of mean FC values shows that the FC patterns were similar among groups, and can be roughly divided in two spatial groups: frontal and motor areas. However, this

separation could be partially favored by the method used to select the regions involved in the analysis: we selected "whole-brain connectivity matrix" and "SMA-seeded" regions. From the FC analysis, we found a strong frontal network with connections between superior frontal gyrus and middle frontal gyrus across groups. Prefrontal involvement has been previously reported in NF studies (Emmert et al., 2015). Superior and middle frontal regions have also been associated with attentional processes (Corbetta & Schulman, 2002) and motor imagery (Halder et al., 2011).

In contrast, from the analysis of *changes (or slope)* in FC through the training, we found that different factors, namely, feedback, motor imagery and reward, had different effects on the functional connections. Groups that were instructed to use motor imagery tended to have relatively less enhancement of the connection strengths (correlation coefficients) from day 1 to day 2, due to training, in comparison to groups without it (table S5, figure 6). FC increases were also found between precuneus and parietal regions (inferior parietal and angular gyrus) particularly in groups with contingent feedback and monetary reward (groups G_F and $G_{F,R}$). Furthermore, the highest increase in FC was observed between precuneus and middle frontal gyrus in group $G_{F,R}$. Precuneus has been linked with autobiographical memory (Eustache et al., 2004; Rauchs et al., 2013), imagery and self-processing operations (Cavanna & Trimble, 2006).

Our study has a few technical and scientific limitations. High inter- and intra-subject variability (in terms of standard deviation of the BOLD signal in the SMA) due to small group size and limited training period (2 days of 4 training runs per day) are two major limitations. It is still an open question as to how many days of training is required for successful learning, especially in the context of inter-subject variability (Sulzer et al., 2013a). Due to restrictions of scanning time and cost, it is difficult to incorporate extensive training in order to attain clear asymptotic levels of BOLD self-regulation. In the present study, it is conceivable that significant learning effect was not attained in the group of contingent NF and reward, because of the short period of training. However, it should be also noted that previous NF studies have performed training with similar

durations of training (Caria et al., 2007; Hamilton et al., 2011; Lawrence et al., 2013; Rota et al., 2011; Young et al., 2014) and reported successful brain self-regulation. Future work should investigate the effect of training time on changes in the brain and behavior.

CONCLUSION

The present study provides first evidence for the differential effects of three factors, namely feedback, motor imagery and monetary reward, on learning brain self-regulation of the SMA. The results indicate that the explicit instruction to the participants to use motor imagery may not necessarily have a beneficial effect on learning. In contrast, the presentation of contingent feedback alone produced a significant learning effect. Further, when monetary reward was provided to the participants in proportion to their performance, a tendency for higher magnitudes of self-regulation was observed, although no learning effect was noticed during the course of the training. Results of the univariate and functional connectivity analyses show a remarkable similarity in brain activations and functional connectivity across all groups, indicating that similar neural processes may be involved in self-regulation despite differences in the way participants were trained. However, differences in the mean functional connectivity values and their change over time (slope) in the groups also indicate differences in the effect of feedback, motor imagery and reward on the dynamic changes in brain during the training period.

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APENDIX

APPENDIX A. SUPPLEMENTARY INFORMATION

Table S1. Included brain regions for functional connectivity analysis (AAL atlas references

001-Precentral L
002-Precentral R
003-Frontal Sup L
004-Frontal Sup R
007-Frontal Mid L
008-Frontal Mid R
011-Frontal Inf Oper L
012-Frontal Inf Oper R
013-Frontal Inf Tri L
014-Frontal Inf Tri R
017-Rolandic Oper L
018-Rolandic Oper R
019-Supp Motor Area L
020-Supp Motor Area R
023-Frontal Sup Medial L
024-Frontal Sup Medial R
029-Insula L
030-Insula R
031-Cingulum Ant L
032-Cingulum Ant R
033-Cingulum Mid L
034-Cingulum Mid R
035-Cingulum Post L
036-Cingulum Post R
057-Postcentral L
058-Postcentral R
059-Parietal Sup L
060-Parietal Sup R
061-Parietal Inf L
062-Parietal Inf R
063-SupraMarginal L
064-SupraMarginal R
065-Angular L
066-Angular R
067-Precuneus L
068-Precuneus R
069-Paracentral Lobule L
070-Paracentral Lobule R
071-Caudate L
072-Caudate R
073-Putamen L
074-Putamen R
075-Pallidum L
076-Pallidum R
077-Thalamus L
078-Thalamus R
079-Heschl L
080-Heschl R
081-Temporal Sup L
082-Temporal Sup R
117-N Accumbens (custom)

Table S2. BOLD changes expressed as r_{SMA} [%] across SMA self-regulation training, values plotted in figure above.

	Total		Day 1		Day 2	
	Mean [%]	SD	Mean [%]	SD	Mean [%]	SD
G_F	0,124	0,140	0,099	0,154	0,148	0,137
$G_{F,I}$	0,148	0,188	0,111	0,174	0,185	0,215
$G_{F,R}$	0,294	0,249	0,297	0,260	0,291	0,269
$G_{F,I,R}$	0,208	0,192	0,220	0,154	0,195	0,242

Table S3. Significantly activated regions in the whole brain, univariate analysis, considering all groups. FWE correction was used. Two-way ANOVA using 4 groups and 4 runs of the second day as factors.

	peak	peak	cluster	cluster			
AAL region	T	p(FWE-corr)	equivk	P (FWE-corr)	x,y,z {mm}	x,y,z {mm}	x,y,z {mm}
Precentral L	9.83	1.4801E-10	208	6.6613E-16	-45.20	-0.48	40.00
Frontal Inf Oper L	4.93	0.03197132			-58.32	15.92	25.00
Frontal Inf Oper R	8.27	7.7242E-08	336	0	53.20	12.64	20.00
Precentral R	8.00	2.2827E-07			46.64	6.08	30.00
Insula R	7.54	1.4684E-06			33.52	22.48	5.00
SupraMarginal R	7.60	1.1735E-06	127	5.2534E-12	59.76	-30.00	45.00
SupraMarginal R	6.59	6.9798E-05			59.76	-33.28	30.00
Parietal Inf R	6.31	0.00021555			33.52	-43.12	45.00
SMA R	6.86	2.3838E-05	102	1.1986E-10	4.00	6.08	60.00
SMA L	6.81	2.909E-05			-2.56	2.80	65.00
SupraMarginal L	6.05	0.00059491	21	5.0183E-05	-55.04	-39.84	25.00
Insula R	5.95	0.00090142	32	5.3075E-06	-35.36	22.48	0.00
Insula L	5.31	0.0094958			-28.80	19.20	10.00
Parietal Inf L	5.41	0.00687267	7	0.00183168	-61.60	-36.56	45.00
Frontal Mid R	5.32	0.00902333	12	0.00043411	46.64	42.16	15.00

Table S4. Regions with high mean FC during NF training across the groups. $z_{FC,T}$ values presented with standard deviation in the parentheses (Precentral L = L-PreC; Precentral R = R-PreC; Frontal Sup L = L-SFG; Frontal Sup R = R-SFG; Frontal Mid L = L-MFG; Frontal Mid R = R-MFG; Supp Motor Area L = L-SMA; Supp Motor Area R = R-SMA; Cingulum Ant L = L-ACing; Cingulum Ant R = R-ACing; Cingulum Mid R = R-MCing; Paracentral Lobule L = L-ParaC).

	$z_{FC,T}$			
ROI 1 / ROI 2	G_F	$G_{F,I}$	$G_{F,R}$	$G_{F,I,R}$
R-MFG/R-SFG	.75 (0.14)	.62 (0.10)	.59 (0.07)	.74 (0.12)
L-MFG/L-SFG	.77 (0.13)	.72 (0.16)	.76 (0.13)	.64 (0.16)
L-Acing/R-ACing	.79 (0.09)	.72 (0.13)	.68 (0.12)	.72 (0.13)
L-SMA/R-SMA	.48 (0.15)	.45 (0.20)	.52 (0.10)	.58 (0.12)
L-PreC/R-PreC	.30 (0.12)	.33 (0.10)	.33 (0.08)	.44 (0.19)
L-PreC/L-SMA	.33 (0.12)	.39 (0.12)	.41 (0.12)	.39 (0.16)

Table S5. CCI values calculated from the connectivity matrix for FC slope comparison

	G_F	**$G_{F,I}$**	**$G_{F,R}$**	**$G_{F,I,R}$**
CCI_{incr}	0,0573	0,0363	0,0527	0,0246
CCI_{decr}	0,0267	0,0365	0,0230	0,0475

Tables S6. CCI values calculated from the original connectivity matrix (" whole-brain connectivity matrix ")

	G_F	**$G_{F,I}$**	**$G_{F,R}$**	**$G_{F,I,R}$**
CCI_{incr}	0,0338	0,0326	0,0442	0,0333
CCI_{decr}	0,0461	0,0459	0,0307	0,0459

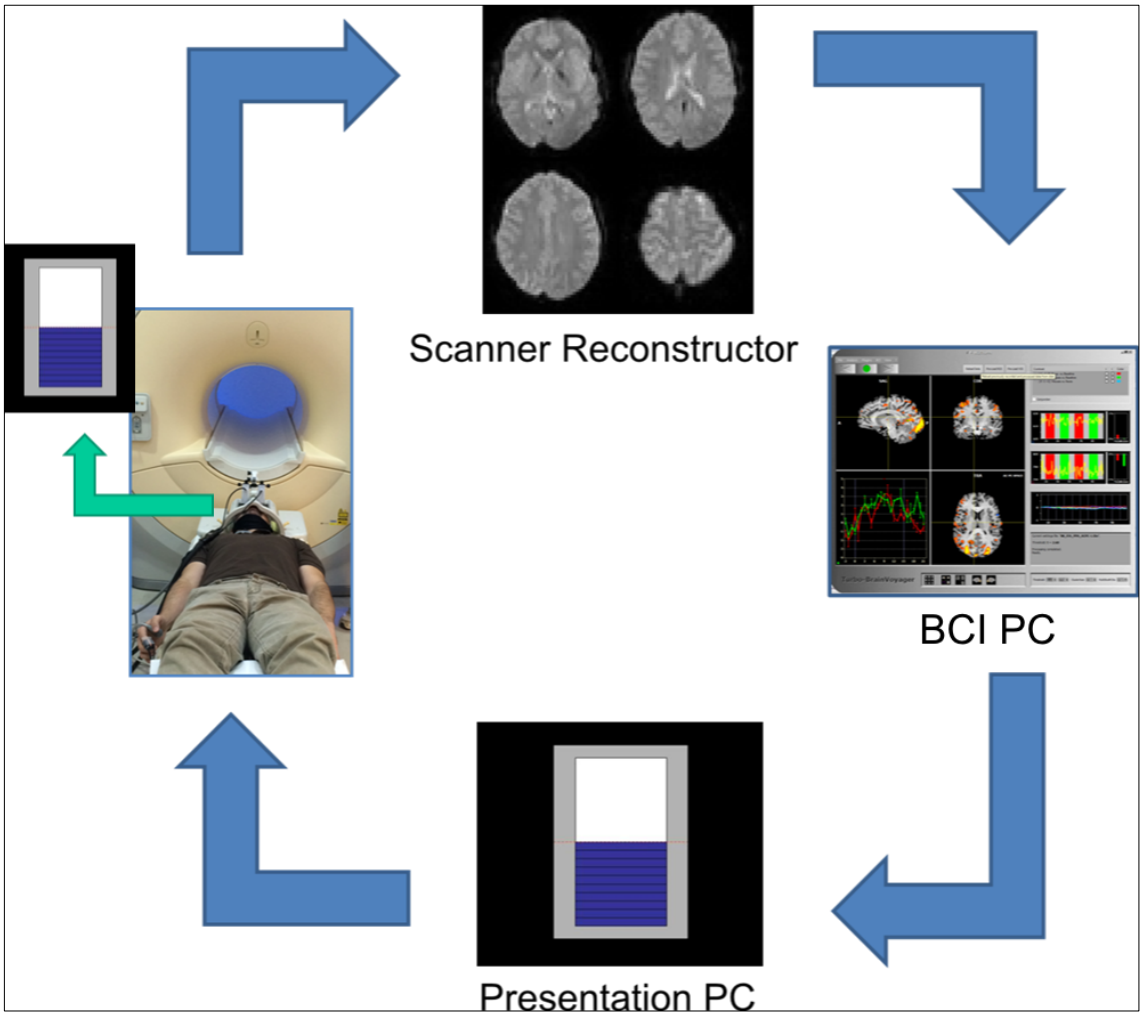


Figure S1. rtfMRI setup

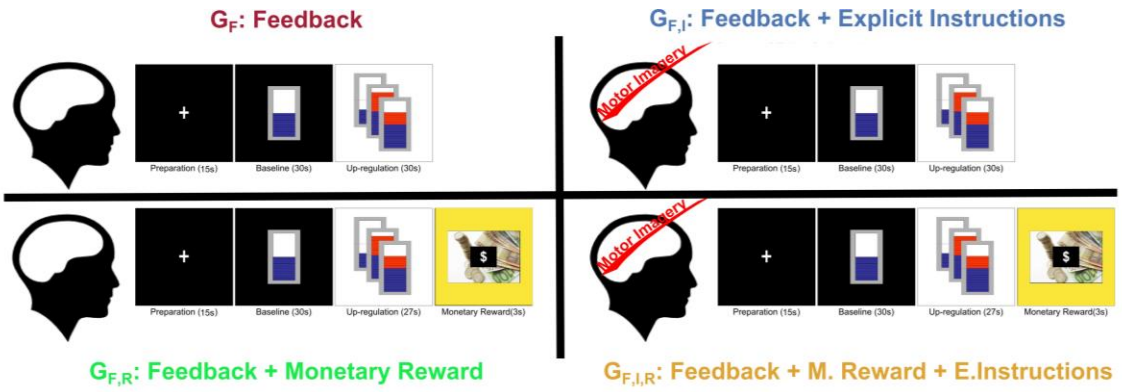
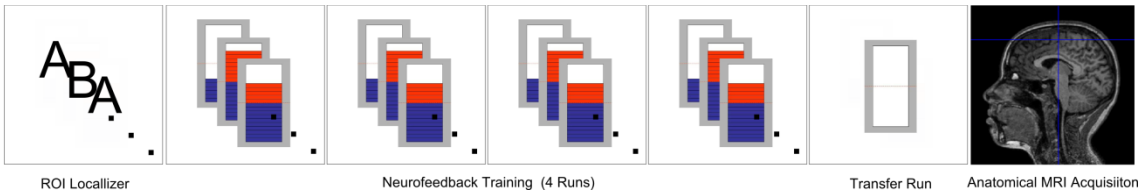


Figure S2. Four groups of participants were included in this study for comparing the influence of the three following factors on volitional regulation of SMA, namely, contingent feedback, verbal instruction to perform mental imagery, and monetary reward. The experimental protocol consisted of alternating baseline (rest) and up-regulation blocks. Group G_F was provided only with contingent visual feedback. $G_{F,I}$ was provided with verbal instructions to use motor imagery of one's choice. $G_{F,R}$ received monetary reward proportional to the increase in the BOLD signal during the up-regulation period in comparison to the baseline period (yellow blocks show the amount of money awarded). $G_{F,I,R}$ was provided with all three factors: contingent feedback, verbal instruction and monetary reward.

a)



b)

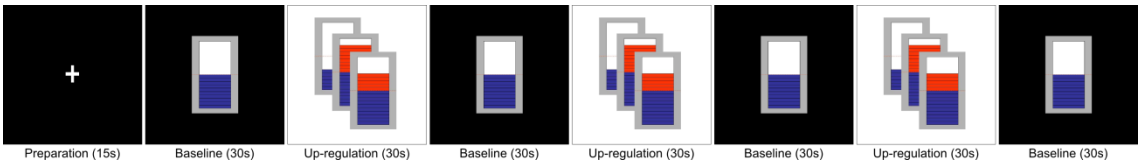


Figure S3. Training sesion (a). Training each day considers a ROI localizer test followed by 4 training runs and 1 transfer runs. Anatomical T1-weighted volume is acquired at the end of the session. Total training involves two training sessions. Training run (b) considers a preparation window (15 s) which appears during the acquisition of the dummy scans. Later, 4 baseline and 3 up-regulation blocks are repeated interleaved. Voluntaries are check for their comfort into the MR scanner and strategies/methods to control their brain signal during the time-out between runs.

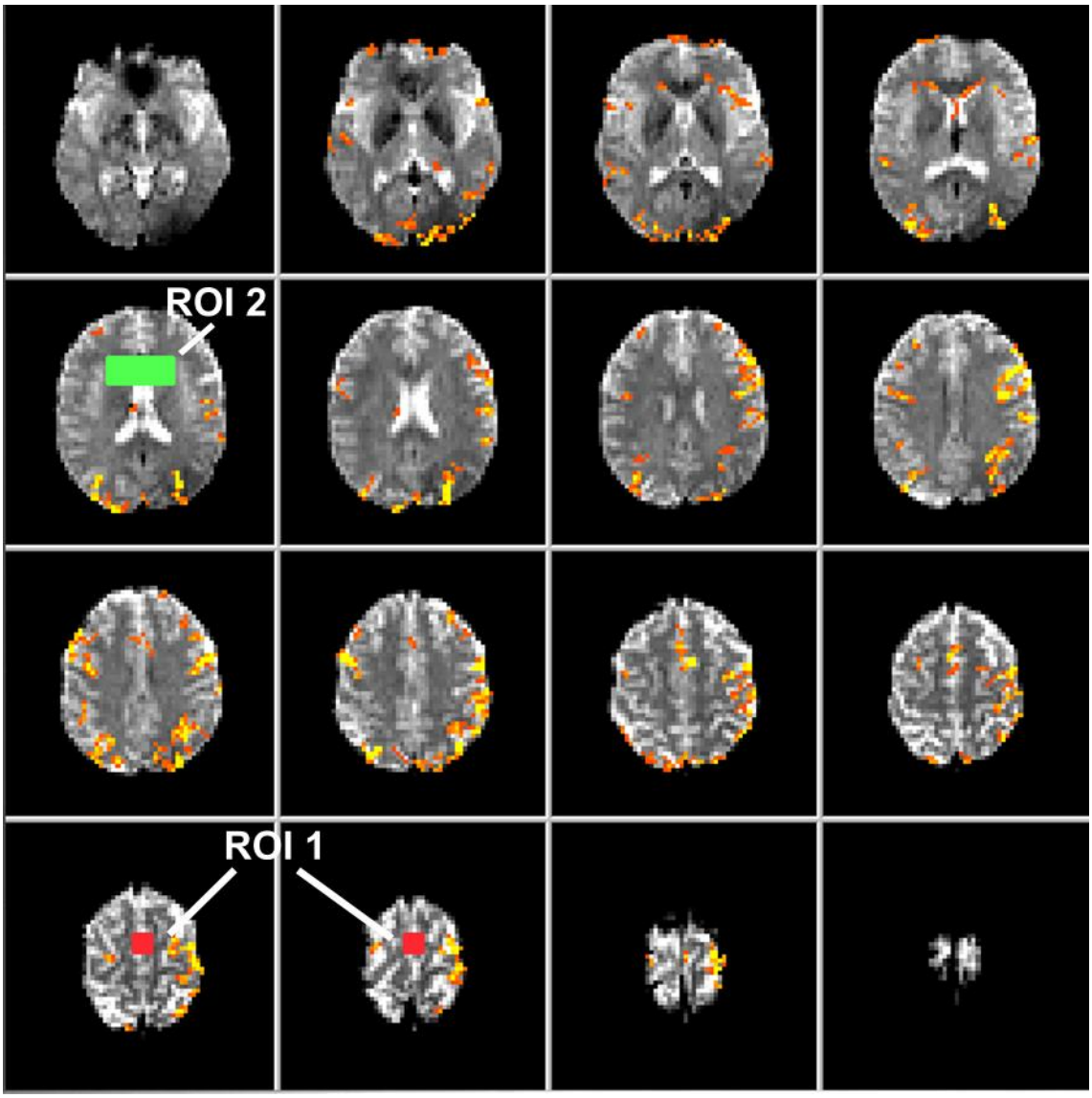


Figure S4. SMA ROI localization using Turbo Brain Voyager. In red, selected area comprising SMA is depicted (ROI 1). In green, reference region is selected around an area no responding to motor action pointing to pick general changes in brain BOLD signal (ROI 2).

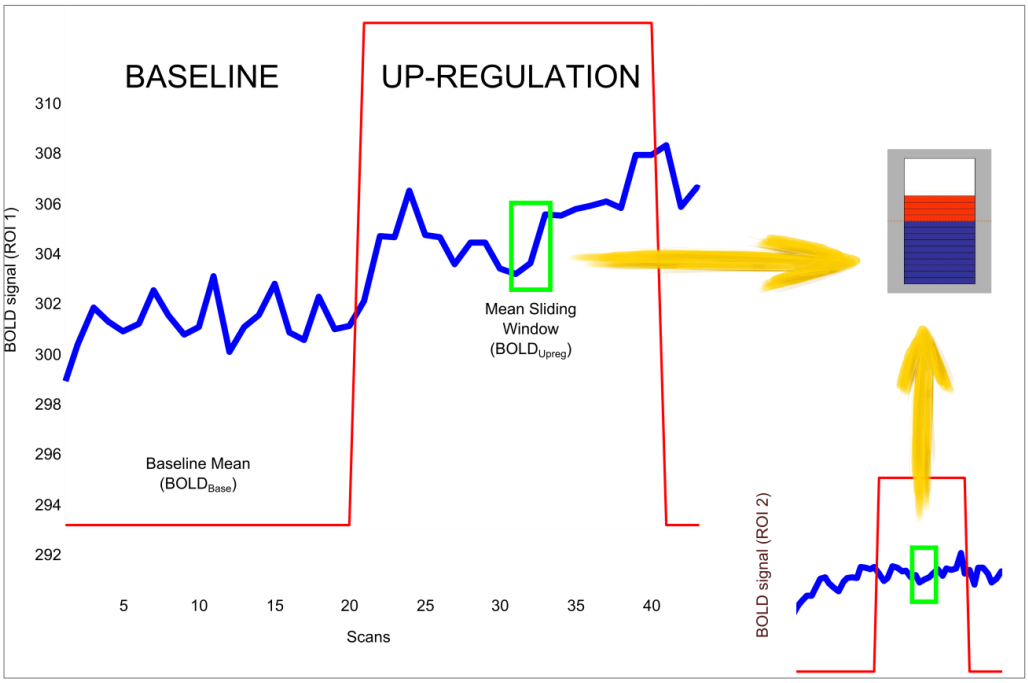


Figure S5. Feedback calculation. Information of ROI 1 (SMA) and ROI 2 (reference) are integrated to deliver feedback information (number of bars in the thermometer). Mean sliding window moving during up-regulation blocks ($BOLD_{Upreg}$) and mean of baseline during the previous baseline block ($BOLD_{Base}$) are used in equation 1 to calculate the feedback (F).

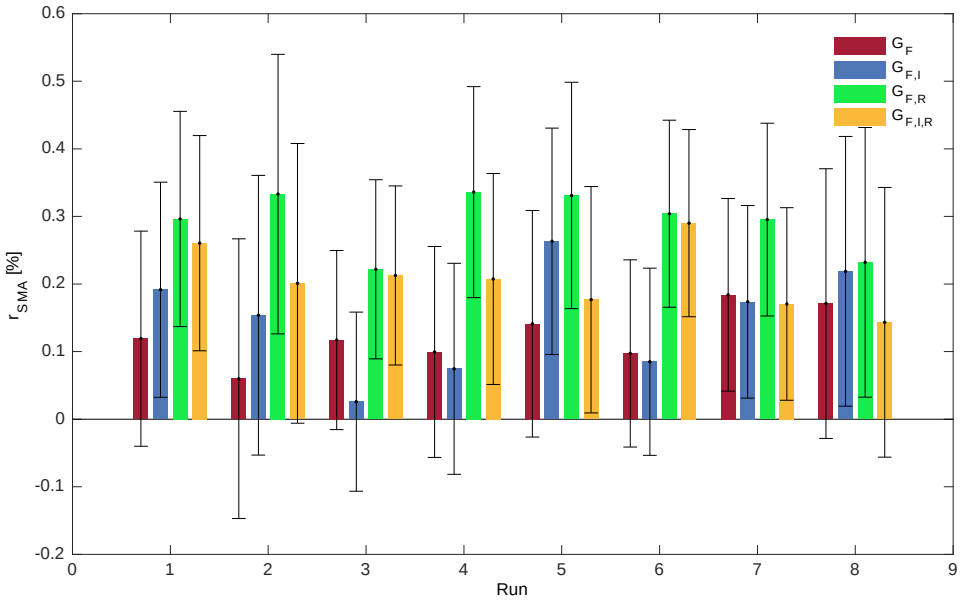


Figure S6. Mean BOLD signal change ($\mathbf{r_{SMA}}$) for each training run in SMA-ROI (MNI x=0, y=0, z=60) and group. No significant linear trend was found, although a positive trend (increased magnitude of self-regulation through training runs) was found for G_F (r_s=.55; p=0.16) and negative trend for $G_{F,I,R}$ (r_s=-.57; p=0.13). Standard deviation bars are shown.

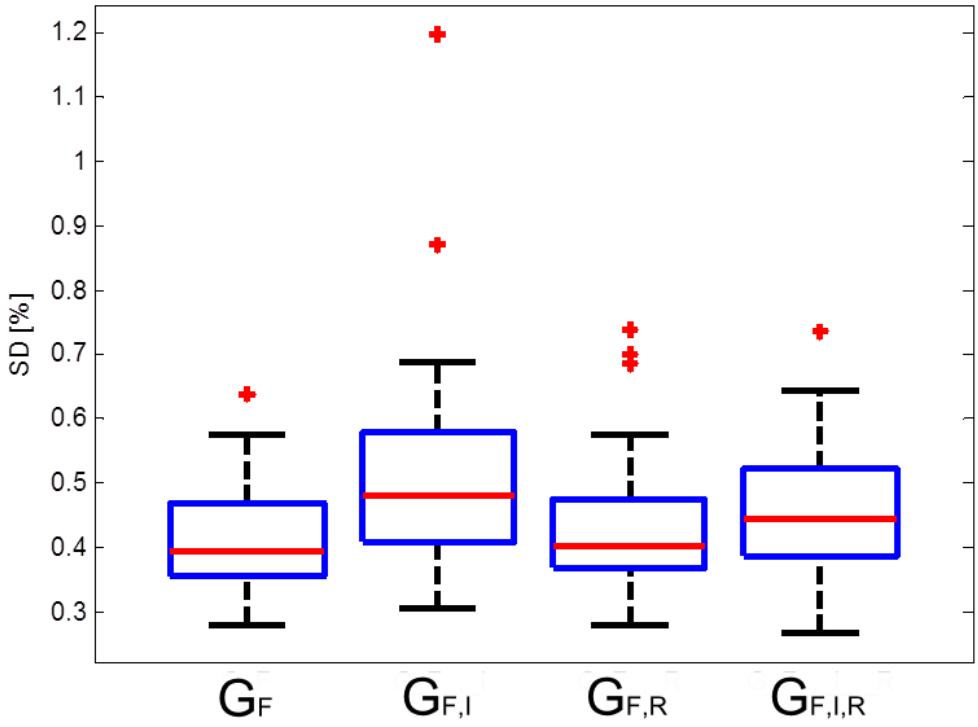


Figure S7. **Intra-subject variability.** Box plot showing the intra-subject variability by groups. The presented distribution considers group r_{SMA}'s standard deviation of the mean (SD) for each one of the 8 NF training runs by subject. To compare the groups we used repeated measures ANOVA. The results show that study groups do not present significant differences at intra-subject variability level, $F_{3,16} = 2.743$, p=0.077 (p>0.05).

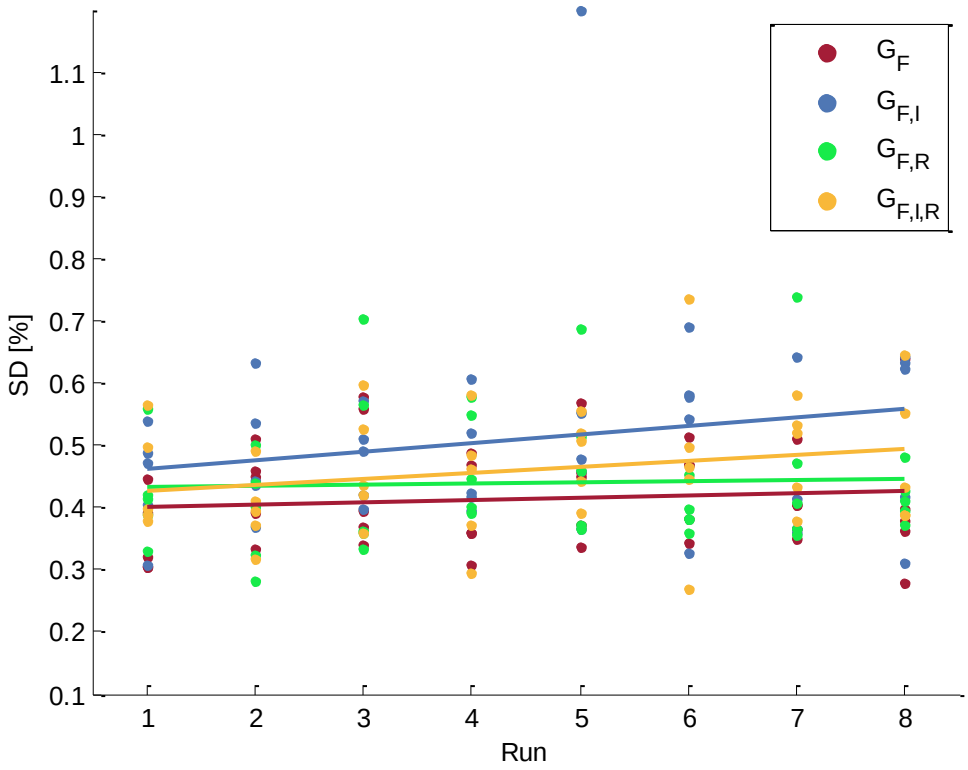


Figure S8. Intra-subject variability. Linear regression of intra-subject variability (SD) through the training runs, by group. Each dot corresponds to the SD in a run of an individual subject. No significant linear correlation was found for the variability during the training (G_F: 0.0036x + 0.398,r_s=.127, p=0.436 ; $G_{F,I}$: y = 0,0138x + 0,448 , r_s=.184, p=0.25 ; $G_{F,R}$: 0,0018x + 0,4301, r_s=.058, p=0.724 ; $G_{F,I,R}$: y = 0,0095x + 0,417 , r_s=.206, p=0.202). To test if there is a significant influence of runs (significant change in SD along the training session) we tested the run effect by group using Friedman test. No significant run effect appeared in any of the groups (G_F: $\chi^2(7)$= 9.667, p=0.208; $G_{F,I}$: $\chi^2(7)$=1.467, p=0.983; $G_{F,R}$: $\chi^2(7)$= 3.933, p=0.787; $G_{F,I,R}$: $\chi^2(7)$= 3.467, p=0.839).

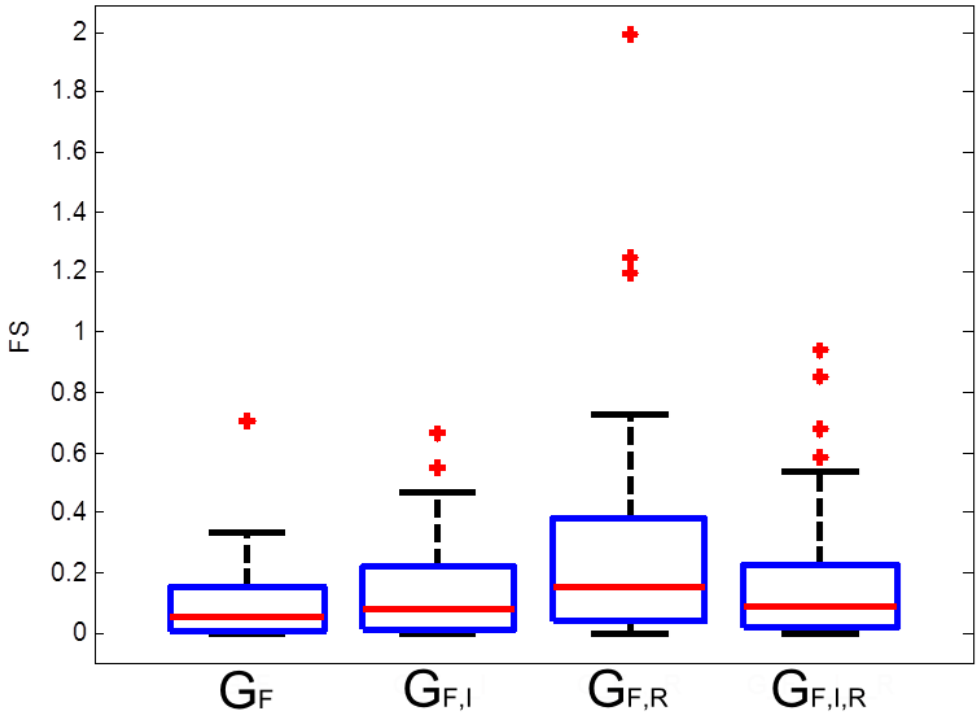


Figure S9. Functional signal to noise ratio (SNR). Box plot showing functional SNR, measured using the Fisher Score (FS), by groups. The presented distribution considers group FS for each one of the 8 NF training runs by subject. To compare differences among the groups repeated measures ANOVA were used. Results show that study groups do not present significant differences in FS, $F(3,16) = 0.781$, $p = 0.522$ ($p > 0.05$).

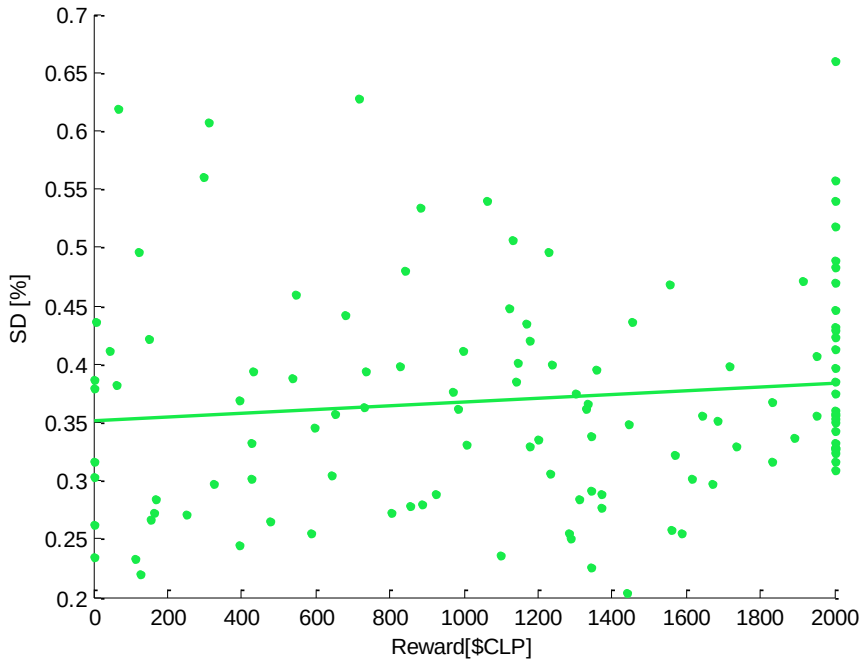


Figure S10. **Variability vs Reward (Block level).** With the purpose of exploring the influence of reward in intra-subject variability, linear correlation between the data of monetary reward delivered during a block within a training run and BOLD signal standard deviation (SD) in the same block was calculated. Only data from $G_{F,R}$ was used for this analysis. Non-significant linear correlation was found ($y=1{,}59*10^{-5}x+0{,}352$; $r_s=0{,}1537$; $p=0{,}094$).

Figure S11. Variability vs Reward (Run level). Linear correlation between the data of monetary reward delivered during a training run and BOLD signal standard deviation (SD) in the same run. Only data from $G_{F,R}$ was used for this analysis. Non significant linear correlation was found ($y=6*10^{-6}x+0,0323$; $r_s=0,418$; $p=0,23$).

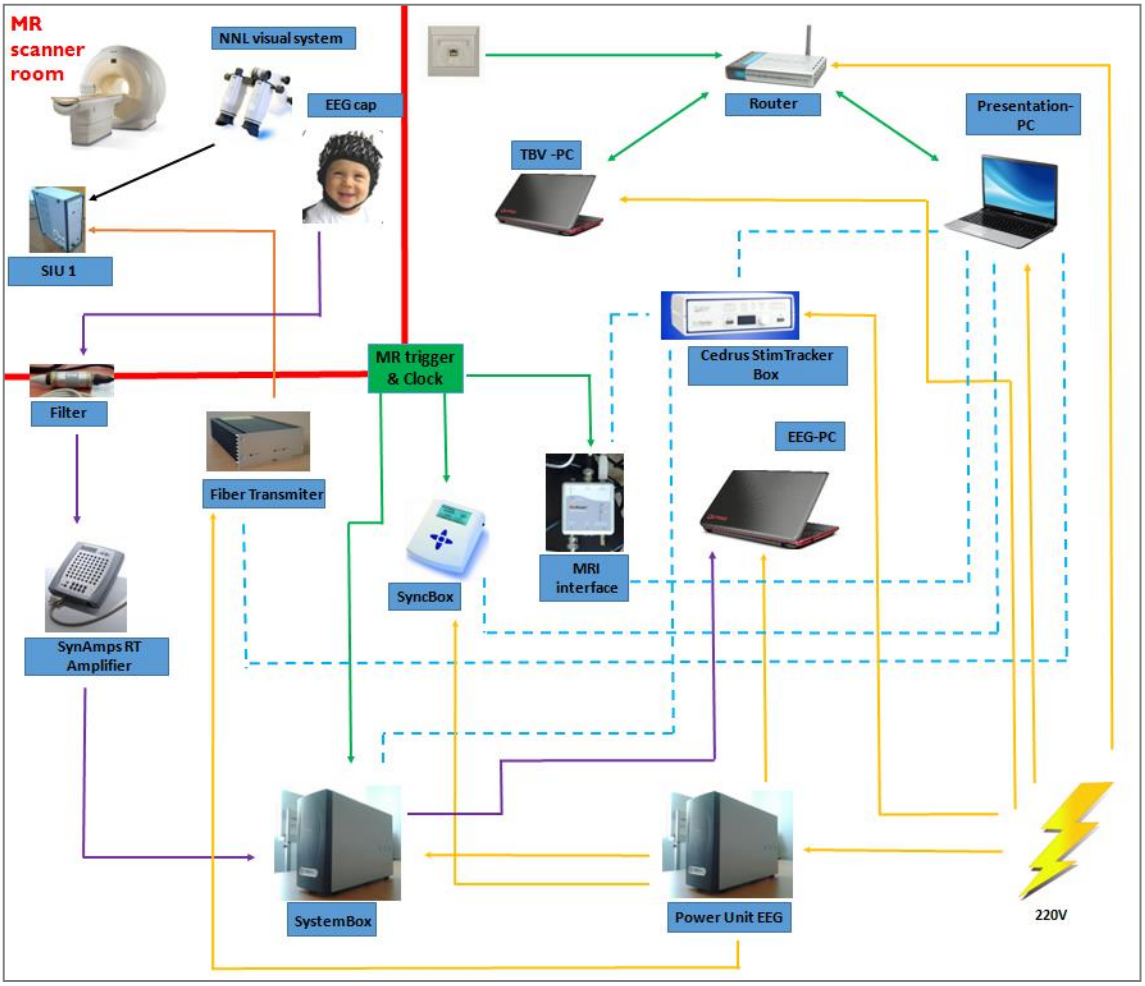


Figure S12. Setup used in experiments real-time fMRI/EEG experiments. Additionally connections for simultaneous EEG acquisition (violet lines) and power lines to (yellow lines) are presented. The current study included the acquisition of simultaneous EEG data during all the experiment (all days and runs). MR trigger and clock wires for synchronization and network connections are shown (green lines). Router connected to MR scanner network allow transference of fMRI volumes through Direct Reconstructor Interface (DRIN, Philips Heathcare, Best, The Netherlands). Connections among the devices that participate in the presentation of stimulus system and its synchronization are shown (light blue segmented line). Equipment is briefly described in the following table.

Table S7. Available equipment for real-time fMRI/EEG acquisition in PUC Biomedical Imaging Center BCI lab. Stimulus presentation system and EEG system brands are NordicNeuroLab AS (Norway) and Compumedics Neuroscan (Australia), respectively.

Equipment	Description	
TBV-PC	Laptop PC to analyse fMRI data using TBV software, calculate feedback and send it to Presentation-PC.	
EEG-PC	Laptop PC to acquire EEG data using Curry 7 software.	
Presentation-PC	Laptop PC to run Presentation experiments, read and show feedback information in NNL stimulus presentation system.	
1.Devices		
1.1 Stimulus presentation		
Headphones	Headphones with noise suppression, allowing communication with the experimenter and auditory stimulus presentation.	
Ear Plugs	Earphone for communication with the experimenter and auditory stimulus presentation.	
Response Grip	Four buttons available for experimental interaction and response. Optic fiber transmission.	
EyeTracker	Camera to record eye movement. Set in Visual System (Googles)	
Visual System (Googles)	Binocular screen for visual stimulus presentation.	

LCD	LCD screen 32” for stimulus presentation.	
1.2 EEG		
EEG Cap	EEG electrode cap with 64 channels.	
2. Power supply units		
2.1 Stimulus presentation		
SIU 1	Shielded Interface Unit: power supply ResponseGrip, Googles, Headphones/EarPlug and EyeTacker. Reception of optic fiber with stimulus information (audio/video) to presentation in VisualSystem (googles) or headphones/earplug. Also, SIU 1 transforms eye-tracker video to optic fiber signals.	
SIU 2	Shielded Interface Unit: LCD screen power supply and intermediary (optic fiber to shielded wire) for video presentation.	
2.1 EEG		
Power Unit EEG	Power supply to EEG system devices.	

3.Consoles and Connectors		
3.1 Stimulus presentation		
Communication Console	Audio stimulus input, communication and volume control (15V).	
Sync Box	Console that receives scanner trigger pulse and ResponseGrip output and sent it to other devices (9V).	
Fiber Transmitter	Electrical signal of audio and video stimulus transformed to optic fiber signals (15V).	
ResponseGrip Box	Receive (and shows) response given through ResponseGrip. Connected to Sync Box (9V).	
Eye tracker beamer	Eye tracker's fiber optic reception and conversion to BNC.	
Eye tracker BNC-USB (Arrington Research)	BNC to USB transformer (PC connection).	

Optical DVI extender	DVI to optic fiber transformer.	
3.2 EEG		
SynAmps RT Amplifier	Signal reception from electrodes and amplification. Connection to SystemBox.	
System Box	Reception of amplifier signal and additional stimulus information (Cedrus StimTracker) and clock.	
SynAmps2 MRI Interface	Reception of scanner trigger signal and connection to Cedrus StimTracker.	
Cedrus StimTracker	Device that allows recognition of stimulus (Presentation codes) and signals (MR trigger) and integration to EEG data (through System Box).	
Pulse-oximeter	Device to measure pulse of subject in the scanner. Optic fiber wire.	

Pulse-oximeter interface	Pulse-oximeter signal reception and connection to amplifier.	
CAP Adapter	Adapter connected in-between cap and amplifier. For experiments out of scanner.	
Analog Filter	Filter located in-between cap and amplifier, through Faraday cage.