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Long-term Motor Training Induced Changes in Regional Cerebral Blood Flow in Both Task and Resting States

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Abstract

Neuroimaging studies of functional activation often only reflect differentiated involvement of brain regions compared between task performance and control states. Signals common for both states are typically not revealed. Previous motor learning studies have shown that extensive motor skill training can induce profound changes in regional activity in both task and control states. To address the issue of brain activity changes in the resting-state, we explored long-term motor training induced neuronal and physiological changes in normal human subjects using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). Ten healthy subjects performed a finger movement task daily for four weeks, during which three sessions of fMRI images and two sessions of PET images were acquired. Using a classical data analysis strategy, we found that the brain activation increased first and then returned to the pre-training, replicating previous findings. Interestingly, we also observed that motor skill training induced significant increases in regional cerebral blood flow (rCBF) in both task and resting states as the practice progressed. The apparent decrease in activation may actually result from a greater increase in activity in the resting state, rather than a decrease in the task state. By showing that training can affect the resting state, our findings have profound implications for the interpretation of functional activations in neuroimaging studies. Combining changes in resting state with activation data should greatly enhance our understanding of the mechanisms of motor-skill learning.

Keywords

motor plasticity; motor skill learning; functional magnetic resonance imaging (fMRI); positron emission tomography (PET)

INTRODUCTION

Motor skill learning, a primary function of the central nervous system, is a process of increasing the spatial and temporal accuracy of movements with practice (Willingham, 1998; Hazeltine

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and Ivry, 2002). Motor skill usually does not develop uniformly across training sessions and is generally characterized by two distinct learning phases: an initial fast learning and a later slow learning (Doyon et al., 2002). In the early stage of learning, considerable improvement in performance can be achieved in a single training session of a few minutes (Classen et al., 1998; Muellbacher et al., 2002). Explicit knowledge of the movement is generally used to facilitate the control and coordination of certain body actions (explicit learning). The latter stage of learning is slow and may take several sessions (or weeks) of practice (Nudo et al., 1996; Karni et al., 1998). As training progresses, motor performance becomes fluent and less attention is required to perform the movement, reflecting implicit learning. With extensive training, skilled behavior becomes resistant to both interference and the simple passage of time. The motor skill can thus be readily retrieved with reasonable performance despite long periods without practice.

Identifying the neural substrates mediating the incremental acquisition of skilled motor behaviors has been the focus of a large body of animal and human studies in the past decade (Grafton et al. 1992, 2002; Karni et al., 1995; Hazeltine et al. 1997; Hikosaka et al., 2002; Doyon et al., 2003; also see Ungerleider et al., 2002 and Doyon & Benali, 2005 for review). Functional neuroimaging studies revealed that the early stage of learning is characterized by a decrease of activation area in the primary motor (M1) region (Karni et al., 1995; Doyon et al., 2002). The time course of changes during the early stage of motor learning (over a 40 min imaging session) has been investigated by Toni and colleagues (1998), who reported progressively decreased neural activity in the premotor area and increased activity in the supplementary motor area (SMA). An imaging-compatible paradigm for studying the later stage of motor learning was introduced by Karni and coworkers (1995, 1998) and Ungerleider and coworkers (2002). Over the course of 3-5 weeks, subjects practiced a finger sequencing task regularly each day. The movement rate more than doubled over five weeks, growing significantly within the initial two weeks and reaching a plateau by the end of the third week. The regional activity in M1 found to be increased after 3 weeks of practice (Karni et al., 1995; 1998). A similar paradigm was used by Hlustik and colleagues (2004), who used a buttonbox to record performance. The early (within two weeks) increase of regional activity in M1 found by Karni and coworkers was replicated by Hlustik et al. (2004). However, Hlustik et al. (2004) also found that regional activity in M1 and S1 stopped increasing and had a trend of decreasing after two weeks of practice. In particular, the S1 activation volume returned to the pre-training level on Week 3 (Hlustik et al., 2004).

So far, most imaging studies investigating motor learning have relied on measuring the differentiated involvement of brain regions in the task performance and the control state. In many situations, the resting state was treated as a control state, and task induced regional activations were determined by comparing images acquired during activation state with those from the resting state (Duff et al., 2007). However, a stable resting state does not necessarily exist. Spontaneous changes in regional neuronal firing occur even when the organism is otherwise in a state of rest. The resting state spontaneous activation can change local blood flow, cause low frequency (0.1 Hz or lower) blood oxygenation level-dependent (BOLD) signal fluctuations, and affect remotely located neurons through efferent output (Golanov et al., 1994). A functional MRI technique has been developed and applied for imaging the resting state to detect task-independent inter-regional connectivity. This fMRI technique is often termed as resting state fMRI and has been successfully applied to study motor (Biswal et al., 1995; Xiong et al., 1999; Cordes et al., 2001; Ma et al., 2007; Duff et al., 2008), visual (Lowe et al., 1998), auditory (Biswal et al., 1996), and language (Hampson et al., 2002) systems for normal subjects and patient populations (Kiviniemi et al., 2003; Quigley et al., 2003; Matsumoto et al., 2004). Resting state functional connectivity networks are often detected by using cross correlation analysis (Fiston et al., 1995; Xiong et al., 1999) and independent component analysis (ICA; Kiviniemi et al., 2003; van de Ven et al., 2004; Yang and Rajapakse,

2004; Beckmann et al., 2005; Ma et al., 2007). Resting state fMRI connectivity offers the possibility of more complete detection of specific neural systems (Xiong et al., 1999).

While numerous data analysis strategies have been developed by different laboratories for analyzing functional neuroimaging data, the foundation of most is still the subtraction (Fox et al., 1984; Horwitz, 1994) (e.g., the t-test) and covariance analyses (e.g., cross-correlation and independent component analysis). A common feature for both analysis techniques is that adding a constant to or subtracting a constant from the data will not alter the outcome. Thus, the commonly used subtraction and covariance analysis strategies are incapable of detecting signals common to both the task and the control states. To detect the background activity, a multivariate, model-free analysis technique has been reported by Kincses and colleagues (2008). The technique is capable of investigating brain changes associated with motor skill learning at the resting state.

Investigation of the resting state is technically challenging, but is important for understanding the mechanisms of the action of motor learning (see Raichle & Gusnard, 2005 for review). Animal studies have shown that extensive motor skill training can induce angiogenesis (Black et al., 1990; Kleim et al., 2002b; Swain et al., 2003), synaptogenesis (Klintsova and Greenough, 1999; Kleim et al., 2002a, 2004) and chronic changes in metabolic rate (Poremba et al., 1997, 1998; Conejo et al., 2004; Sakata et al., 2005). Extensive training in motor and other skills also has been shown to have great impacts in human brain anatomy, as revealed by a comparison of musicians' to non-musicians' brains (Schlaug et al., 1995a; 1995b; Jancke et al., 1997; Gaser et al., 2003 also see Altenmuller, 2003 for review). All these changes may affect both the task and the control states of the human brain and may not be revealed using the classical imaging and analysis strategies. To address this issue, we explored long-term motor training induced neuronal and physiological changes in normal human subjects using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). We hypothesized that long-term neuronal and physiological changes that affect both the task performance state and the control state (in this study, the resting state) may be a critical component of the mechanism of motor skill training and should not be omitted from analysis.

MATERIALS AND METHODS

Subjects

Ten healthy normal volunteers (5 male and 5 female, ranging from 18 to 45 years old) participated in our experiment. None of them was a professional typist or musician by self-report. All subjects were right-handed, free from known neurological and psychiatric disorders, by self-report. Other motor skills have not been assessed. Informed consent was obtained from each subject before beginning the study, according to the protocol approved by the local research ethics committee. During scanning, the subjects lay supine with their heads supported by a foam-padded, hemicylindrical head holder. Each subject's head was immobilized within a tightly fitting, thermally molded, plastic facial mask extending from hairline to chin. All subjects participated in three fMRI sessions and two PET sessions. The interval between two consecutive fMRI sessions was two weeks. The interval between the two PET sessions was four weeks.

Task Paradigm

A finger sequencing task, in which the fingers of the subject's non-dominant hand (left hand) were opposed to the thumb in specific sequences (Karni et al., 1995), was used in our block-design functional activation study. The non-dominant hand was chosen to ensure that subjects were involved in learning the task and did not acquire skill too quickly (i.e., after one session) (Hund-Georgiadis and von Cramon, 1999). During the task, subjects performed one of two

specific sequences (Sequence A and B), which were mirror images of one another, without visual feedback. The order of finger movement in Sequence A was 5, 2, 4, 3, 5 (fingers are numbered as: index finger, 2; middle finger, 3; ring finger, 4; and little finger, 5). In Sequence B, the order of finger movement was 5, 3, 4, 2, 5. Subjects were paired and each was randomly assigned to either Sequence A or B as the training sequence, and the other sequence was used as the control sequence. Five subjects (Group A) were randomly assigned Sequence A as the training sequence, and the other five subjects (Group B) were assigned Sequence B. The control sequence was only performed during scanning and used to enable testing of whether the changes in the brain were dependent on factors other than practice. All subjects practiced the training sequence for four weeks with one practice session each day for a total of 15 minutes, including weekends and the days of the scanning sessions. Subjects were instructed that during practice they were to perform the sequence as accurately and quickly as possible. Each practice session was recorded by a video camera. Video recordings were reviewed in slow motion by an observer, who scored the number of correct sequences completed (movement rate) and the number of errors (out-of-sequence movements) per session. In addition to the practice sessions, there were three fMRI sessions performed during fMRI scanning and two PET sessions during PET scanning. The first fMRI and PET scanning session was performed just prior to the commencement of practice (Week 0). Immediately after the first fMRI scanning, the first PET scanning took place. After two weeks of practice, there was a second fMRI scanning session (Week 2). Finally, the third fMRI scanning session and the second PET scanning session occurred immediately after 4 weeks of practice (Week 4). Some PET scans were performed before the MRI due to schedule issue. But the order is not completely randomized. For the rest condition, subjects were instructed to remain motionless (not to perform any behavioral task), with their eyes closed. For the finger movement condition, subjects were required to perform the tasks with a speed of approximately two movements per second, cued by the acoustic noise of MRI scanner. A vocal instruction was used to cue the subject for starting or stopping the motor task performance.

MRI and PET Data Acquisition

The fMRI images were acquired on an Elscint Prestige 2-T whole-body MRI scanner (Elscint Prestige, Haifa, Israel). For the resting state fMRI, seven contiguous slices were acquired in a transverse plane using a T2*-weighted gradient-echo echo-planar-imaging (EPI) sequence with slice thickness of 5 mm (repetition time (TR) = 700 ms, echo time (TE) = 45 ms, flip angle (a) = 70° , interslice gap = 1 mm, receive bandwidth = 12.21Hz). The in-plane resolution for the images was 3.28×3.28 mm². A total of 400 seven-slice MRI images were acquired in less than five minutes. The matrix size of each slice was 72 × 72 voxels. A block-design fMRI (two off-on cycles for an acquisition time of 8 minutes) was performed for the task activation studies, using the following parameters: 16 contiguous slices, TR = 2 s, TE = 45 ms, a flip angle of 90°, slice thickness = 5 mm, interslice gap = 1 mm, and in-plane resolution $3.28 \times$ 3.28 mm². The total number of volumes for each acquisition was 240. Two fMRI scans were performed for the task activation studies for each session: one for the training sequence and one for the control sequence. The order of the two task scans was randomized across subjects. Two resting state scans were performed prior to and after the task performance scans. Data acquired at the first resting state scan and during performing the training sequence were analyzed and reported here. Data acquired at the second resting state scan and during performing the control sequence will be analyzed and reported elsewhere.

At the end of the EPI data collection, a spin echo, T1-weighted anatomical image (TR = 33 ms, TE = 12 ms, flip angle (a) = 60° , slice thickness = 5 mm, interslice gap = 1 mm, matrix size = 256×256 voxels) in the same slice positions was acquired to facilitate the precise determination of the structures corresponding to the functional activation foci. Off-line

reconstruction algorithms were used to reconstruct the echo-planar images. The first ten images of each run were discarded to allow MRI signal to reach a steady state.

For the PET imaging, water labeled with oxygen -15 ([¹⁵O]H₂O, half-life 122 second) was used as a blood-flow tracer (50 mCi for each intravenous bolus injection). PET data acquisition began at the time of arrival of the tracer bolus in the brain (15–20 seconds after tracer injection) and continued for 40 seconds. For the task condition, task performance started immediately after tracer injection and ended after the completion of data acquisition (approximately 60 seconds). Two [¹⁵O]H₂O PET images (slice thickness = 2.43 mm, in-plane pixel size 2.06 × 2.06 mm²) were acquired for each state (resting state, training task, and control task) in each PET session using an ECAT HR+ PET scanner (CTI, Knoxville, TN, United States). Each volume of PET images included sixty-three contiguous slices in a transaxial field of view of 30 cm. The matrix size of each slice was 128×128 voxels.

Preprocessing

SPM2 (The Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, http://www.fil.ion.ucl.ac.uk) and in-house MATLAB (The MathWorks, Inc., Natick, MA) programs were used in data preprocessing. Motion correction (using least squares algorithm as implemented in SPM2) was applied to all the fMRI images. All the functional MRI images were spatially normalized using a 12 parameter linear transformation to match the EPI template provided by SPM2 to reduce bias due to misplacement of head in different scanning sessions. A linear drift correction (Tanabe et al., 2002) was applied to all functional images. To increase the signal-noise-ratio, all data were spatially smoothed by an isotropic Gaussian filter with a full-width at half-maximum (FWHM) of 4.8 mm. The T1 images were normalized to the T1 template provided by SPM2. All the preprocessing programs were run on a platform of MATLAB 7.0. In addition, the PET images were value normalized to an average voxel value over the brain region of 1000 to normalize for variability of [¹⁵O]H₂O dosage and patient weight. All PET and fMRI images were normalized to a standard voxel size of $2 \times 2 \times 2$ mm³.

Data Processing

Statistical parametric images (SPIs) of task performance data were created by using a cross correlation analysis (Friston et al., 1995; Xiong et al., 1996), in which hemodynamical responses were detected by evaluating the correlation between the time course of each voxel and a reference function (a boxcar function convolved with a hemodynamical response function of BOLD). A cluster of voxels in an SPI image was considered to be part of an active area if it simultaneously satisfied the following conditions: (I) the intensity of each element voxel was over a specified threshold (2.5) (P<0.02, uncorrected); (II) the number of voxels was over 10.

VOI analysis—Seven volumes of interest (VOIs) were selected subject by subject for investigating motor learning effects. Seven brain areas, which are believed to involve in motor skill learning (Hikosaka et al., 2002; Doyon et al., 2002, 2003; Willingham, 1998; Fukai et al., 1999; Doya, 2000; Frank et al., 2001), were selected in our analyses. These included the right and left primary motor area (M1), supplementary motor area (SMA), the right and left dorsal premotor cortex (PMd), as well as the right and left cerebellum (CB) (Hikosaka et al., 2002; Doyon et al., 2002, 2003). The locations of the VOIs were mainly determined by functional activations between the motor task and the resting state. The center of a VOI was defined as the center-of-mass of the activation. The size of each VOI was standardized and was $5 \times 5 \times 5$ voxels (1000 mm³).

When the selected areas had no activations or when an activation spanned two regions (e.g. M1 and primary somatosensory cortex (S1)), we used the following anatomical landmarks to

delimit these areas: The hand M1 area was between the superior and middle frontal gyri, anteriorly bounded by the posterior edge of the precentral gyrus and posteriorly bounded by the anterior bank of central sulcus (Yousry et al., 1997). The PMd area extended from the midline between the central and precentral sulcus, anteriorly to the junction of the superior frontal sulcus and precentral sulcus (Sun et al., 2005). The area of SMA included cortex on the medial wall, dorsal to the cingulate gyrus, and between the central sulcus and the vertical plane through the anterior commissure (AC) (Picard & Strick, 2003). The dentate neuclus (left hand side) was midline and paravermian central to the cerebellum white matter (Saini et al., 2004).

The training induced changes were assessed by evaluating cross-session changes of the VOI values. A two-factor ANOVA, with conditions (resting vs. task) and sessions as factors and with the VOI values across different subjects as repeated measurements, was then performed for each VOI.

Power Spectral Analysis—To perform power spectral analysis on fMRI data, time-courses of the left and right M1 VOIs were first filtered using a high-pass filter (cutoff frequency: 0.01 Hz) to remove the zero frequency and extra-low frequency components (Duff et al., 2008). To test whether any significant differences existed in the power spectra, the spectral peaks at 0.08 Hz (corresponding to the BOLD fluctuation frequency induced by spontaneous firing of neurons (Golanov et al., 1994; Cordes et al., 2001)) and at 0.3 Hz (corresponding to respiratory cycle) were integrated. The integral value represents the total energy in the frequency band and is likely related to the number of neuronal activity at the resting state. A two-factor ANOVA, with VOIs and sessions as factors and with the integral values across different subjects as repeated measurements, was then performed on the power spectra to detect the training induced spectral changes.

RESULTS

Learning curves for the trained sequence are shown in Figure 1. The mean growth curve was modeled by an exponential growth function (with a time constant $\tau = 4.6$ days). Initially, the error rates are low (about 7 per 15-min session or 1.5%) and decrease to near zero within the first week. The video analysis suggests that the improvement in movement rate is apparently due to a decrease in inter-movement delay, with a minor contribution coming from increased finger velocity. A Student t-test revealed that the movement rate at Week 2 is significantly greater than pre-practice (p < 0.05). The difference between Week 4 and Week 2 did not reach statistical significance. A one-way ANOVA analysis based on the movement rate revealed significant difference between the first two weeks and the second two weeks ($F_{(1,276)} = 133.96$, p < 0.001), suggesting that the subjects' skill was mainly improved during the first two weeks.

An fMRI activation map was generated using the traditional differentiated data analysis strategy by contrasting the task performance with the control state (Figure 2). Significant activations were observed in the right M1, SMA, the dorsal premotor cortex, as well as cerebellum. The activation volumes in the right M1 and SMA increased on Week 2 and returned to the pre-training level on Week 4. These observations were confirmed by the quantitative measurements of the activated volumes for M1 and SMA (Figure 3). A simple ANOVA revealed that the activation volumes of M1 and SMA were significantly larger after two weeks of training (p<0.05), when behavioral measures indicated a high rate of skill change. During the second half of training, when behavioral measures indicated a plateau in skill level, the activation volumes of M1 and SMA returned to the pre-training level.

PET functional activation maps on pre-practice and Week 4 are shown in Figure 4. The activation patterns were similar to those detected using fMRI. It also showed no significant difference of the activated volumes for M1 and SMA between pre-practice and Week 4.

Changes in the PET rCBF were evaluated for both the task performance and resting state (Figure 5). A two-factor ANOVA, with conditions (task vs. rest) and sessions as main factors, was performed for each VOI. Our test revealed significant session and condition effects (p<0.05) in the right M1 area (contralateral to the left hand movement). The interaction is insignificant (p>0.05). The rCBF in the right M1 significantly increased in both task performance and resting state (p<0.05). The increase in the resting state appeared to be greater than that in the task state, but was not statistically significant (p>0.05). By contrast, the rCBF in the left M1 area did not significantly change in either task or resting state. The rCBF in SMA appeared to increase in both task and resting state; but the changes were not significant based on our ANOVA test (p>0.05). Changes in other brain areas were also insignificant. A similar voxel-based analysis used to generate functional activation maps has also been performed to generate an SPI between the resting state data acquired at different sessions. The SPI showed no significant differences.

Power spectral analyses were performed for the right and left M1 areas. An ANOVA test revealed a significant increase (p<0.05) in power spectrum in the 0.08 Hz frequency band, corresponding to the spontaneous firing frequency, in the right M1 area after two week and four week training periods (Figure 6A). By contrast, no significant changes (p>0.05) in the power spectrum in the left M1 area were observed (Figure 6B). No significant changes (p>0.05) in the power spectrum in the 0.3 Hz frequency band were observed.

DISCUSSION

A common problem with the literature of motor skill learning is that almost all regional activity studies have relied solely on measuring the differentiated involvement of brain regions in the task performance and the control states. Training induced changes common for both states have not been investigated. To address whether training affects both states, we have investigated long-term motor training induced neuronal and physiological changes in normal human subjects using fMRI and PET. Motor training induced changes in the task performance state and the control state (resting state) were both examined.

Our learning data (Figure 1) replicated the slow-learning effect reported by Karni et al. (1995;1998). Using the classical differentiated data analysis strategy, our fMRI measurements indicated that the activation volumes in the right M1 and SMA increased on Week 2 and returned to the pre-training level on Week 4. The observation that the activity volume of M1 and SMA on Week 4 returned to the pre-training level was further confirmed by the PET data. The changes in regional activity we observed in the first two weeks are consistent with previous findings (Karni et al., 1995;1998;Hlustik et al., 2004). Previous observations on later stage of changes, e.g. after two weeks of practice, did not converge. Karni et al. (1995) reported that regional activity in M1 and S1 stopped increasing and showed a trend towards decreasing after subjects practiced sequential movement of the three middle fingers for 2 weeks. On Week 3, the S1 volume returned to the pre-training level although the M1 volume did not (Hlustik et al. 2004). Compared to Hlustik et al. (2004), there was longer follow-up in the present study (4 weeks). The changes in regional activity we observed in the second two weeks following the trend presented in Hlustik et al. (2004).

While our functional activation data showed that the activation volumes in the right M1 and SMA increase first and then return to the pre-training level on Week 4, our rCBF values in both task performance and resting states increased on Weak 4 when compared to pre-training. The apparent decrease in activation may actually result from a greater increase in activity in the resting state, rather than a decrease in the task state. Our findings may bear profound effects on interpretation of neuroimaging results of functional activation. The classical neuroimaging

data analysis strategy detects the differential involvements of the brain in the control state and during task performance. When the control state does not change, the functional activation data should be adequate to reflect neurophysiological states of the brain. When the control state does change, both the task performance and the control states contain profound information (Raichle & Gusnard, 2005). The neglect of the changes in the control state may only tell a partial story of brain neurophysiological changes induced by learning. Combining changes in control state with activation data should greatly enhance our understanding of the mechanisms of motor-skill learning.

The resting state is a dynamic state and can be influenced by many factors (Sidtis et al., 2004; also see Raichle & Gusnard, 2005 for review). Results reported by Sidtis and colleagues showed that the resting rCBF was significantly affected by the task it was associated with during an experimental imaging session. This influence varied across different tasks and brain regions. More specifically, rCBF was more highly correlated with blood flow during the performance of a paired task than with that during resting states paired with other tasks. Because of great influence of the resting rCBF by the task performed or task to be performed, it is important to dissociate learning effects from the task effects when investigating the resting state rCBF changes induced by motor training. Our motor skill learning is a longitudinal study. The same motor tasks were used across different imaging sessions. Effects of task on the resting rCBF should be similar across imaging sessions. In addition, the speeds of the finger movements were controlled to be the same for all imaging sessions. Effects of task performance speed on the resting rCBF are then constant across imaging sessions. The resting images analyzed here were always acquired before task performance. Effects of task order on the resting rCBF are then expected to be the same across imaging sessions. In short, we do not eliminate the effects of task on the resting state rCBF, but try to control the effects to be the same across different imaging sessions. The only difference between imaging sessions is the practice history of the motor sequence movement task. The resting rCBF changes reported in this manuscript, therefore, should represent the learning effects rather then the task effects.

There are a number of potential explanations for the motor training dependent increases in rCBF. Three potential candidates underlying the increases in the resting rCBF are briefly discussed here.

Synapse formation

The most probable neuronal sequelae of chronic, focal neuronal excitation are synapse formation and expansion of the cortical representation of the exercised region. During development, synapse formation and pruning can be monitored using metabolic rate (Chugani et al., 1987, 1988; 2001), suggesting that synaptogenesis may result in the increase of neuronal activity, which causes greater energy consumption and increased regional cerebral metabolic rate of oxygen metabolism (rCMRO₂). It is generally believed that rCBF and CMRO₂ are coupled at the resting state, thus it can be deduced that synaptogenesis can induce the increase of rCBF. Learning-dependent changes in synapse number within the mammalian brain have been well documented (see Klintsova & Greenough, 1999 for review). With a daily training of reaching condition for 15 minutes over a period of ten days, the number of synapses per neuron within layer V of the caudal forelimb area of adult rats can be increased by as much as 50% (Kleim et al. 2002a; 2004). The synaptogenesis could result in an increase in local neuronal activity and energy consumption, consequently, an increase in rCBF at both task and the resting state.

Angiogenesis

Unlike synaptogenesis, which is learning-dependent, angiogenesis has been reported to be exercise-dependent (Black et al., 1990; Kleim et al., 2002b; Swain et al., 2003). Kleim et al

(2002b) reported that exercise induces angiogenesis but does not alter movement representations within rat motor cortex. The chronic changes in blood flow and blood volume as a result of exercise have been assessed by Swain et al. (2003) using MRI with T2*-weighted and flow-alternating inversion recovery (FAIR) pulse sequences. Their results suggested that prolonged exercise increases the size of a capillary reserve. Thus, motor performance and learning have the potential to increase the capillary perfusion, which may, in turn, cause the increase of rCBF in task and resting state.

Enzyme

Cytochrome oxidase is a rate-limiting enzyme in oxidative phosphorylation, the major energysynthesizing pathway used by the central nervous system (Gonzalez-Lima & Cada, 1994; Sakata et al., 2005). Because cytochrome oxidase is intimately linked to neuronal activity, cytochrome oxidase histochemistry has traditionally been used to assess the metabolic history of a brain area (Wong-Riley et al., 1984; Borowsky et al., 1989). Learning studies in rodents have shown a regional up-regulation of cytochrome oxidase activity (Sakata et al., 2005). Auditory Pavlovian conditioning results in training-dependent increases in cytochrome oxidase in auditory brain regions (Poremba et al., 1997, 1998). Similarly, spatial reference memory results in enhanced cytochrome oxidase activity in cortical, limbic and vestibular regions (Villarreal et al., 2002). In spatial working memory in the water maze, rats show an increase in cytochrome oxidase in the mammillary bodies after 30 days of training (Conejo et al., 2004). This training-dependent up-regulation of cytochrome oxidase suggests increased capacity of the metabolic rate of neural tissues after long-term training. This up-regulation could induce the increase of CMRO₂, which may, in turn, cause the increase of rCBF.

We have observed a significant training induced increase in power spectrum in the 0.08 Hz frequency band, which corresponds to the BOLD fluctuation frequency induced by spontaneous firing of neurons, in the right M1 area after two week and four week training periods. This increase in the power spectrum likely reflects an increase of oxyhemoglobin concentration, which, in turn, may reflect an increase in spontaneous firing in the right M1 area. If the energy consumption is constant or increases as training progressed, the only reason for an increase of oxyhemoglobin concentration is consistent with an increase of rCBF. Thus the increased power spectrum is consistent with the observed increased rCBF. The potential explanations for the increased rCBF should also be applicable to the increased power spectrum. In summary, there are several plausible explanations for our observations of increased rCBF in both task and resting states. Understanding the mechanisms underlying the rCBF increase in both task and resting states can greatly enhance our knowledge of long-term motor skill learning.

CONCLUSION

Using the classical differentiated data analysis strategy, we found that, for the task performance state, regional activity volumes increased first and then returned to the pre-training level as practice progressed. After separating the performance state and the control state (resting state), we observed that motor skill training induced significantly increases in rCBF in both task and resting states. The discrepancy came from the fact that the classical differentiated data analysis strategy has neglected the changes common to both task performance and control states. Our results suggest that the apparent decrease in activation may actually result from a greater increase in activity in the resting state, rather than a decrease in the task state. Our findings may have profound implications for the interpretation of neuroimaging studies of functional activation, as it shows that changes in the control state may underlie activation effects. The changes in control state, we believe, may reflect more fundamental changes in the brain (such as changes in metabolic and hemodynamic capacity, angiogenesis, or synaptogenesis).

Combining changes in control state with activation data should greatly enhance our understanding of the mechanisms of motor-skill learning.

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Fig. 1.

Learning curves for the trained sequence. Each curve depicts the performance (movement speed: sequences per-minute) of a single subject or the statistical performance (mean and standard derivation as denoted by the error bar) as a function of time.

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Fig. 2.

Averaged (across subjects) fMRI activation maps (threshold: Z=2.5). Data were acquired on (A) pre-practice, (B) Week 2, and (C) Week 4. The slices are 44, 38, 32, 26, 20, -36 mm from the AC-PC line, respectively. The letter L indicates the left side of the brain.

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Fig. 3.

Changes in activation volumes as training progressed. The activation volumes in M1 and SMA first increased and then returned to the pre-training state. The error bars represent one SEM (standard error of measurement).

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Fig. 4.

Averaged (across subjects) PET activation maps of motor skill training (threshold: Z=2.5). Data were acquired on (A) pre-training and (B) Week4. The slices are 44, 38, 32, 26, 20, -36 mm from the AC-PC line, respectively. The letter L indicates the left side of the brain.

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Fig. 5.

Mean voxel values of seven VOIs detected by PET. Regional blood flows in both task and resting state were measured at pre- and post-training. Motor skill training induced significantly increases in regional blood flow in both task and resting state (p <0.05). rM1: the right primary motor area, IM1: the left primary motor area, rPMd: the right dorsal premotor cortex, IPMd: the left dorsal premotor cortex, rCB: the right cerebellum, ICB: the left cerebellum, and SMA: supplementary motor area. The error bars represent one SEM.

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Fig. 6.

Power spectral analysis of resting state fMRI data. (A) The right M1 area; (B) the left M1 area. The right M1 area shows a significant increase in spectral power in the 0.08 Hz frequency band (p = 0.05). The left M1 area shows no significant increase in spectral power. The 0.08 Hz frequency band corresponds to the BOLD fluctuation frequency induced by spontaneous firing of neurons. The curves show differences between spectra obtained on Week 4 and pre-practice (Week 0) and on Week 2 and pre-practice (Week 0).