Rapid Modulation of GABA Concentration in Human Sensorimotor Cortex During Motor Learning

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Floyer-Lea, Anna, Marzena Wylezinska, Tamas Kincses, and Paul M. Matthews. Rapid modulation of GABA concentration in human sensorimotor cortex during motor learning. J Neurophysiol 95: 1639–1644, 2006. First published October 12, 2005; doi:10.1152/jn.00346.2005. Movement representations within the human primary motor and somatosensory cortices can be altered by motor learning. Decreases in local GABA concentration and its release may facilitate this plasticity. Here we use in vivo magnetic resonance spectroscopy (MRS) to noninvasively measure serial changes in GABA concentration in humans in a brain region including the primary sensorimotor cortex contralateral to the hand used for an isometric motor sequence learning task. Thirty minutes of motor sequence learning reduced the mean GABA concentration within a 2 × 2 × 2-cm³ voxel by almost 20%. This reduction was specific to motor learning: 30 min of similar, movements with an unlearnable, nonrepetitive sequence were not associated with changes in GABA concentration. No significant changes in GABA concentration were found in the primary sensorimotor cortex ipsilateral to the hand used for learning. These changes suggest remarkably rapid, regionally specific short-term presynaptic modulation of GABAergic input that should facilitate motor learning. Although apparently confined to the contralateral hemisphere, the magnitude of changes seen within a large spectroscopic voxel suggests that these changes occur over a wide local neocortical field.

INTRODUCTION

Movement representations within the human primary motor and somatosensory cortices are plastic and can be altered by motor learning (Floyer-Lea and Matthews 2004; Karni et al. 1995; Nudo et al. 1996) or by chronic changes in afferent input (Weiss et al. 2000). This is associated with local changes in the balance of neuronal excitation or inhibition. Repetitive practice of a specific movement or of a movement sequence is associated with an increase in the excitability of the sensorimotor cortex, which can be measured using transcranial magnetic stimulation (TMS) over periods as short as 10 min (Classen et al. 1998). The increase in excitability is greater during motor learning than with similar simple movements alone (Pascual-Leone et al. 1995). Several studies have provided evidence for local cortical functional reorganization within a similar short motor skill training period (Butefisch et al. 2000; Floyer-Lea and Matthews 2004; Muellerbacher et al. 2001; Sanes et al. 1992).

Modulation of inhibitory activity may play a critical role in motor learning. Long-term cortical plasticity with axonal sprouting and development of new synaptic connections (Darian-Smith and Gilbert 1994) is associated with changes in the number or type of postsynaptic GABA receptors (Skangiel-Kraska et al. 1994). "Unmasking" of existing horizontal connections within the cortex allows rapid changes in sensory or motor representations (Huntley 1997). Unmasking of these connections can be produced experimentally by reducing local cortical inhibition through blockade of GABA receptors in animal models (Jacobs and Donoghue 1991). Reduction in GABA inhibition facilitates long-term potentiation (LTP)-like activity in motor cortex (Castro-Alamanos and Connors 1996; Castro-Alamanos et al. 1995). Pharmacological evidence suggesting that plasticity of human sensorimotor cortex is modulated by changes in local GABA concentration comes from observations such as the suppression of use-dependent functional adaptations of motor and somatosensory cortex by the GABA_A receptor agonist lorazepam (Butefisch et al. 2000; Pleger et al. 2003).

Previous work has established the potential for rapid changes in brain GABA concentrations that appear to be functionally relevant. In a pioneering study, Petroff and Rothman (1999) showed acute increases in cortical GABA with the anticonvulsant inhibitor of GABA breakdown by GABA transaminase, vigabatrin, with a mean rate of increase approaching 20% of the estimated rate of tricarboxylic acid cycle turnover. Similar increases later were seen in both GABA and its metabolites homocarnosine and pyrrolidinone after treatment with topiramate (Petroff et al. 1999). Decreases in GABA concentration were found over the course of 30–40 min in the primary sensorimotor cortex after ischemic forearm nerve block, a context expected to be associated with sensory cortical plasticity in response to the altered afferent input (Levy et al. 2002).

Here we test directly the hypothesis that decreases in local GABA concentration accompany human neocortical functional reorganization with motor learning. We report measurements of GABA levels in the left primary sensorimotor cortex using magnetic resonance spectroscopy (MRS) during 30 min of training in a motor learning task demanding accurate tracking of a short (8 cycle), repeated sinusoidal pattern by varying isometric pressure generation with the (dominant) right hand (Floyer-Lea and Matthews 2004). We also tested for changes during performance of a similar task without a specific learning component and during rest. We show that motor task learning is associated with rapid, reversible decreases in GABA con-
centration in the region of the sensorimotor cortex contralateral to the hand moved.

**METHODS**

**Subjects**

Thirty-six healthy right-hand dominant subjects participated in this study (mean age, 25 yr; range, 20–31 yr). All gave informed consent according to a protocol approved by the local research ethics committee.

**Motor learning task**

The motor learning task has been described in detail previously (Floyer-Lea and Matthews 2004). In brief, two vertical bars were shown on a screen: the right (red) bar indicated the target force, and the left (blue) bar gave a continuous measure of the subject’s response. Subjects were required to track the target force by maintaining the two bars at equal height at all times. Root mean square (RMS) tracking error was measured throughout the experiment. Thirteen subjects tracked a repeating 8-s sequence of force changes. Ten other subjects tracked a nonrepeating, pseudorandom sequence of force changes. A further seven subjects performed no task, but lay relaxed and in the scanner throughout the data collection period. All subjects performed the task (or remained at rest) for 30 min while GABA spectra were acquired continuously.

**MRS**

GABA-edited spectra (3T Varian Innova MRI) using a MEGA-PRESS sequence for simultaneous three-dimensional (3D) voxel localization, water suppression, and editing (Mescher et al. 1998) were acquired from a 2 × 2 × 2-cm³ voxel centered on the hand region of the left primary motor cortex (Yousry et al. 1997), identified on sagittal and axial T1-weighted axial scout scans. A selective double-banded 180° pulse was created from 20-ms Gaussian pulses. The frequency of the first band of this pulse was set to 4.7 ppm to suppress water. The second band was alternated between 1.9 ppm, the resonance frequency of C3 protons (strongly coupled to the observed C4 GABA protons and 3.0 ppm; condition A), and 7.5 ppm, which is symmetrically disposed about the water resonance to equalize off-resonance effects (condition B). The resonance at 1.9 ppm was inverted 180° during condition A but not during condition B. In condition A, the GABA C4 (triplet) resonance (at 3.0 ppm) therefore was fully refocused, whereas in condition B, this peak was not refocused, but phase modulated so that the outer triplet signals were inverted at echo time TE = 68 ms. The difference spectra from conditions A and B (at TE = 68 ms) revealed the edited GABA spectrum without the larger overlapping creatine resonance. One hundred ninety-two acquisition GABA spectra were acquired at rest at the start and end of the experiment, and 10 64-acquisition GABA spectra were acquired serially (and subsequently averaged in consecutive 192-acquisition blocks for purposes of analysis) throughout the task period.

To test for the regional specificity of GABA changes with motor learning, six subjects were studied while performing the learning task with alternating GABA acquisitions from 8 ml volume of interest (VOI) placed over the contra- (VOI 1) and ipsilateral (VOI 2) sensorimotor cortices. Before the experiment began, separate shim settings were optimized for each of the two voxels, and values were changed dynamically as acquisitions from the two voxels alternated between the baseline and the last acquisition block.

**Analysis**

The spectra were analyzed using the linear combination model (LCM) (Provencher 1993), adapted to incorporate the analysis with GABA editing as has been described previously (Wylezinska et al. 2003). Nonspecific effects limiting signal stability (e.g., head movement) can be associated with resonance frequency shifts. The N-acetyl aspartic acid (NAA) resonance frequency was measured in each of the serial spectra, and spectra in which the resonance shifted by >10 Hz (a threshold defined on the basis of preliminary metabolite phantom studies) were not used for measurements. GABA levels were determined from area under the model-fitted GABA resonance peak at 3.0 ppm (Fig. 1). To correct for the expected contribution from mobile brain macromolecules (MM; which include cytosolic proteins) (Behar et al. 1994) to this resonance, a parameterized MM spectrum was included in the basis set of modeled spectra. The parameterized components of the MM spectrum were derived from metabolite-nulled spectra [acquired using condition A of the MEGA-PRESS editing sequence (in which the Cr resonance normally is present) with inversion recovery using a preinversion pulse and recovery time (TI) adjusted to minimize the creatine peak (TI = 0.720s)], which were measured independently in six subjects.

The bandwidth range of the frequency-selective 180 pulses used in MEGA-PRESS allows similar information to be acquired concerning NAA, creatine, and the overlapping glutamate (Glu) and glutamine (Gln) resonance (Glx) at 3.7 ppm, in addition to information on GABA (Fig. 1). The Glx resonance mostly reflects Glu, with only a relatively small contribution from Gln (<15%). concentrations of

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**FIG. 1.** Tracking errors recorded in the scanner during the sequence and random tracking tasks. Performance improved significantly on the sequence tracking task (a, P < 0.01), indicating that learning was taking place. Subjects did not show improvement during the random tracking task (c). Error bars represent SE.
GABA, NAA, and Glx were expressed with respect to Cr (assuming a 6 mM concentration) measured from the a single standard 64-acquisition PRESS spectrum acquired at the start of the experiment.

Statistics

Statistical analyses were performed using SPSS for Windows (version 12). Observations on changes in relative GABA concentration were tested with a general linear model, with repeated measures analysis applied with a significance threshold of \( P < 0.05 \). To test for changes in relative glutamate concentration during the learning task, a paired \( t \)-test was used to compare baseline and 30-min learning time-point values.

RESULTS

Behavioral measures showing motor learning

We first measured GABA levels in the left primary sensorimotor cortex using MRS throughout 30 min of performance of a motor learning task demanding accurate tracking of a short (8 cycle), repeated sinusoidal pattern by varying isometric pressure generation with the (dominant) right hand, and matched tracking of a continuously pseudorandomly varying sinusoidal pattern or rest. There was a significant time-dependent effect on performance during the learning task \( (F = 13.046, P = 0.002) \) and a strong interaction between time and task (repeated sinusoid vs. pseudorandomly varying sinusoid; \( F = 7.844, P = 0.011 \)). As expected, task performance for subjects repeating a fixed sinusoidal pattern \( (n = 13) \) improved over the 30-min training period: the tracking error decreased between the first and last 10-min task periods by \( >20\% \) (paired \( t \)-test; \( t = 9.6; P < 0.001 \)). In contrast, subjects \( (n = 10) \) tracking the nonrepititively varying tracking target did not show a significant reduction of tracking error over a 30-min period (Fig. 1).

GABA concentration decreases in the contralateral primary sensorimotor cortex with motor learning

Spectra averaging signal over 3.2 min were generated from data acquired from a voxel localized to the hand area of the primary sensorimotor contralateral to the hand performing the learning before, during, and after the task period (Fig. 2). The baseline, resting GABA concentration estimate \( (1.49 \pm 0.28 \text{ mM}) \) was similar to those found in other MRS studies (McLean et al. 2002). Significant time-dependent effects were found for GABA concentration overall \( (F = 13.046, P = 0.002) \), and there was a strong interaction between time and task (repeated sinusoid vs. pseudorandomly varying sinusoid; \( F = 7.844, P = 0.011 \)). The GABA concentration measured by MRS decreased \( (18\%) \) during sequence learning \( (1.21 \pm 0.32 \text{ mM}; \text{ANOVA, } F = 10.8; P < 0.01; \text{Fig. 3}) \). However, the GABA concentration did not change with similar force generation tracking with unlearnable, nonrepetitive movements \( (F = 0.90; \text{not significant}) \) or with simply rest \( (F = 1.12; \text{not significant}) \) over the same period of time (Fig. 3). Additional data acquired 20 min after cessation of any short-term motor learning showed a partial recovery of the GABA concentration \( (1.33 \pm 0.21 \text{ mM}) \) by \( ~40\% \) of the maximum decrease.

To test the possibility that these effects may have been a consequence of changes in the Cr resonance with learning (as GABA concentration was estimated relative to Cr), relative changes in the GABA/NAA ratio were assessed for the two tasks. There was a significant time-dependent change in GABA/NAA for the repeated sinusoid learning task \( (F = 10.3, P = 0.007) \). There was no significant time-dependent change with the pseudorandomly varying sinusoid task \( (F = 0.012, P = 0.912) \). There was a significant interaction between the time dependence of GABA/NAA changes and task \( (F = 5.18, P = 0.033) \).

Significant changes in the relative concentration of glutamate, the metabolic precursor to GABA, were not found \( (P = 0.9) \).

Lack of nonspecific changes in NAA with motor learning

To test the possibility that the decrease in the observed GABA concentration was caused by nonspecific changes in the sensitivity or noise of the acquired spectra, MRS resonance intensities of NAA were evaluated during the motor sequence learning period. In contrast to the decrease observed in the GABA signal during motor learning, the NAA signal intensity (which is greater and therefore potentially allows more sensitive assessment of relative changes) did not change significantly \( (F = 0.23; \text{not significant}; \text{Fig. 4}) \).

Specificity of GABA concentration changes with motor learning to the contralateral sensorimotor cortex

A separate experiment was performed to test for the specificity of cortical GABA concentration changes from measurements in spectroscopic voxels of identical sizes placed over the left (contralateral) and right (ipsilateral) primary sensorimotor cortices. Interleaved measurements of relative GABA concentrations were conducted at baseline, before start of the learning paradigm, and after 25–35 min of training. There was a significant difference in the changes associated with learning for the two hemispheres \( (F = 7.24, P = 0.04; n = 6) \). Relative GABA concentration in the left primary sensorimotor cortex
voxel decreases by a mean 27% ($P < 0.05$), whereas GABA in the homologous right hemisphere did not show significant change.

**DISCUSSION**

GABA is synthesized and stored in neurons within the gray matter (Sheikh et al. 1999). The observation that the GABA concentration in the sensorimotor cortex decreased while performing the repetitive tracking but not while tracking an unlearnable, nonrepetitive varying force sequence shows that short-term cortical GABA modulation was specific for learning and not a consequence simply of performance of tracking itself. The rapid partial recovery of GABA concentration after completion of the repetitive tracking task suggests that the GABA modulation may be associated primarily with encoding of the task (which during the period of task performance), rather than its longer-term consolidation (which occurs after training) (Shadmehr and Holcomb 1997). The changes were not found in the ipsilateral motor cortex voxel, suggesting at least some regional specificity to the effects. However, a striking feature is that substantial decreases (almost 20%) were observed using a relatively large voxel, implying that they involve a cortical region at least of similar extent to the tracking-associated activation changes observed by functional MRI (fMRI) in the primary sensorimotor cortex (Floyer-Lea and Matthews 2004; Karni et al. 1995).

The demonstration of a stable relative NAA concentration confirmed that changes were specific for GABA, ruling out trivial measurement confounds as a cause of apparent concentration decrease, such as head movement leading to a decrease in coupling between the head and radiofrequency coil. A decrease in GABA concentration could result from a decrease in GABA production or through an increase in GABA reuptake and catabolism. GABA is produced in the presynaptic terminals of GABAergic neurons from glutamic acid (Glu) by the action of glutamic acid decarboxylase (GAD). We did not find evidence for decreases in Glu concentrations during learning; a previous study showing reductions in cortical GABA concentrations in primary somatosensory cortex during ischemic nerve block also did not report corresponding reductions in Glu (Levy et al. 2002). Together, these findings suggest that any reduction in GABA production likely is related to a down-regulation of GAD rather than at an earlier stage in the GABA synthesis. GAD activity and GABA concentrations are modulated during a variety of physiological conditions (Garraghty et al. 1991; Grattan et al. 1996). Previous studies in both animals (Manor et al. 1996) and humans (Shen et al. 1999) have shown that there is active turnover of GABA. Estimates based on these studies suggest that the rate of GABA synthesis is sufficiently rapid for its inhibition alone to account for the rate of decrease in total GABA observed here.

A theoretical alternative to concentration changes for explaining a decrease in GABA signal independent of any change in glutamate or NAA is that selective relaxation time changes might make the GABA less visible to MRS (e.g., by shortening the spin-spin relaxation time and broadening of the resonance) during the learning period. The GABA signal includes contributions from different pools of GABA. GABA is stored in vesicles in the presynaptic terminal and present as free GABA in the synaptic cleft, as well as in the presynaptic neurons and glial cells after reuptake. Pools of GABA in which GABA is relatively immobilized, e.g., by association with macromolecules, could be less visible using our MRS sequence. Thus it also is possible that a mechanism such as a reduction in GABA...
release, causing a relative reduction of the proportion of GABA in the synaptic cleft and an increase in (MM rich) vesicular GABA, could therefore contribute to the decrease in the observed GABA signal. However, the magnitude of the changes makes this unlikely; such large relaxation time changes with short-term changes in functional state would be unprecedented as far as we are aware. We have direct evidence that nonspecific relaxation effects (e.g., from changes in local magnetic susceptibility), affecting all metabolites, also did not occur, because changes in line-width (reflecting spin-spin relaxation time changes) were not observed for either GABA or NAA (M. Wylezinska and P. M. Matthews, unpublished observations). The BOLD contrast effect associated with motor learning, which arises from a decrease in local relative deoxyhemoglobin concentration (increasing \( T_2^* \)), would be expected to be associated with a relative increase in neurotransmitter resonance intensity if any change was observed, because the magnitude and extent of an fMRI BOLD effect in the sensorimotor cortex decreases over time with this paradigm (Floyer-Lea and Matthews 2004).

Cortical GABA concentration was reduced during tracking of a learnable, repeated sequence, but no significant changes were seen during force tracking identical except for a nonrepetitive variation. The experimental design allowed subjects to be aware of the distinction between the tracking sequences, which led to different cognitive contexts for the two types of tracking contrasted. A limitation of the study is that the cognitive context for the tasks could be controlled only to a limited extent: the two tasks were not fully matched for difficulty or attentional demands. Nonetheless, the most striking distinction between behavior with the two sequences was that there was significant learning (improvement in tracking) with the repeated sequence. Neurons within the basal forebrain are particularly active during learning (Wilson and Rolls 1990), and lesions of the basal forebrain have been shown to abolish the cortical plasticity associated with motor skill learning (Conner et al. 2003). The basal forebrain sends cholinergic and GABAergic projections to wide regions of the cortex including the primary sensorimotor cortex (Semb a 2000). The GABAergic basal forebrain neurons preferentially synapse with cortical GABAergic interneurons (Freund and Meskenaite 1992), suggesting that activation of the basal forebrain during directed learning could reduce GABA levels in the cortex through inhibition of the cortical GABAergic interneurons. A mechanism involving such a relatively diffuse projection system could explain the apparently widespread nature (inferred from the magnitude of changes seen in the large voxel used) of the GABA concentration decreases seen.

Decreases in cortical GABA concentration would be expected to lower relative inhibition in the sensorimotor cortex during motor sequence learning. A recent rodent study using pharmacological modulation of cortical GABA showed a strong negative correlation between the forepaw sensory stimulation-evoked BOLD fMRI response and GABA concentration measured by MRS, consistent with a reduction of inhibition with a decrease in GABA concentration (Chen et al. 2005). LTP-type changes in synaptic strength have been shown to occur in the rodent primary motor cortex only when GABAergic inhibition is lifted (Castro-Alamancos et al. 1995). “Unmasking” latent horizontal connections by disinhibition allows new muscle synergies to be generated rapidly (Schneider et al. 2002). Short-term modulation of cortical inhibition may therefore be an important facilitatory mechanism for the neocortical LTP-like activity critical to motor cortex plasticity with motor skill learning, extending the roles suggested previously in establishing new hippocampal circuits or columnar architecture in the developing visual cortex (Engel et al. 2001; Hensch and Stryker 2004).

Our studies have focused on a single neurotransmitter system and do not address wider issues of changes that may occur with use or adaptive plasticity. Here we are proposing GABA as one element (although potentially an important, facilitatory one) contributing to local cortical changes during fast motor learning. Future work needs to better characterize primary effector neurotransmitter changes, to relate the GABA concentration changes observed here to mechanisms of consolidation and long-term, slow learning, to modulation by other neurotransmitter systems, and to any shifts in use-dependent patterns of energy metabolism (e.g., with altered neuronal–glial interactions).

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