Several lines of evidence suggest that schizophrenia (SCZ) is associated with disrupted plasticity in the cortex. However, there is little direct neurophysiological evidence of aberrant long-term potentiation (LTP)—like plasticity in SCZ and little human evidence to establish a link between LTP to learning and memory. LTP was evaluated using a neurophysiological paradigm referred to as paired associative stimulation (PAS). PAS involves pairing of median nerve electric stimulation with transcranial magnetic stimulation (TMS) over the contralateral motor cortex (for abductor pollicis brevis muscle activation) delivered at 25-ms interstimulus interval. This pairing was delivered at a frequency of 0.1 Hz for 30 min. LTP was reflected by the change in motor evoked potentials (MEPs) before and after PAS. In addition, motor skill learning was assessed using the rotary pursuit task. Compared with healthy subjects, patients with SCZ demonstrated significant MEP facilitation deficits following PAS and impaired rotary-pursuit motor learning. Across all subjects there was a significant association between LTP and motor skill learning. These data provide evidence for disrupted LTP in SCZ, whereas the association between LTP with motor skill learning suggests that the deficits in learning and memory in SCZ may be mediated through disordered LTP.

Keywords: cortex, learning, LTP, paired associative stimulation, rotary pursuit, schizophrenia, transcranial magnetic stimulation

Introduction

Schizophrenia (SCZ) is well known for its broadly based neurocognitive deficits, of which impairment of memory and intellectual abilities are salient findings (Heinrichs and Zakzanis 1998). Neural plasticity has long been conceptualized as a cellular substrate of learning and memory. As theorized by Hebb (1949), neural plasticity is represented by changes in synaptic strength in response to coincident activation of coactive cells, which manifest as long-term potentiation (LTP) or long-term depression. Plasticity is called Hebbian if modifications of synaptic efficacy occur in response to temporal correlations between pre- and postsynaptic activity. A subtype of LTP, induced by activating presynaptic inputs derived from separate origins (rather than from the same presynaptic source) that converge upon common postsynaptic targets is known as an associative LTP (Levy and Steward 1983). This form of LTP provides a framework of how temporally related converging inputs (e.g., thalamocortical, corticocortical, etc.) may elicit enduring synaptic changes in the neuronal circuits, representative of the initial stimulus.

LTP depends, in part, on activation of a double-gated N-methyl-D-aspartate (NMDA) receptor that serves as a "molec-ular" coincidence detector. These calcium-permeable glutamergic receptors are able to provide a long-term augmentation of postsynaptic signal once activated by an input sufficient to depolarize postsynaptic membrane and relieve tonic Mg$^{2+}$ inhibition (Bliss and Collingridge 1993; Rison and Stanton 1995). Of interest, despite the putative association between LTP to learning and memory, the only direct evidence to support the role of hippocampal LTP in learning has only recently been reported in the rat (Whitlock et al. 2006).

Disturbances in neural plasticity have long been hypothesized to underlie major neuropsychiatric disorders such as SCZ (Haracz 1985). Abnormalities of neural plasticity in patients with SCZ are suggested by several lines of evidence. These include a link between the pathophysiology of SCZ and dysfunction of NMDA receptors (Olney and Farber 1995; Akbarian et al. 1996). In addition, genetic and postmortem studies implicated abnormalities in dysbindin, neuregulin, and reelin, proteins involved in synaptic plasticity, as possible contributors to SCZ pathology (Fatemi et al. 2000; Straub et al. 2002; Wess et al. 2002; Stefansson et al. 2003). As well, several neurophysiological experiments in patients with SCZ have provided evidence suggesting that neural plasticity is disrupted in this disorder. Specifically, abnormal responses in SCZ brains were demonstrated by the lack of cortical depression elicited by low frequency repetitive transcranial magnetic stimulation (Fitzgerald et al. 2004) as well as by diminished postexercise facilitation in primary motor cortex of schizophrenic patients (Reid et al. 2002). Nevertheless, direct evidence for aberrant LTP in SCZ is lacking.

A plethora of cognitive deficits have been reported in SCZ (for review see Heinrichs and Zakzanis [1998]) including impaired motor skill learning as demonstrated using rotary pursuit task (Schwartz et al. 1996). In this task, a subject must trace a rotating target, with acquisition of learning skills estimated by the ability of the subject to stay “on target” for longer periods of time with practice. The degree of impairment in motor skill acquisition was found to be associated with general measures of cognitive dysfunction (e.g., conceptual abilities, assessed on the Dementia Rating Scale) but not with motor symptoms nor with the patient’s dose of medications (Schwartz et al. 1996). However, despite significant advances in our understanding of anatomic substrates of motor skill learning (Grafton et al. 1992), the neurophysiological mechanisms that may underlie such changes are poorly understood.

Transcranial magnetic stimulation (TMS) has been extensively used to study plasticity in humans. Significant and persistent elevations of excitability within the human motor cortex have been obtained by stimulation of peripheral nerve...
Electromyography Recording
Subjects were seated in a comfortable chair. Each experimental session lasted approximately 3 h. Surface electromyography (EMG) was recorded from the right abductor pollicis brevis (APB) muscle with disposable disc electrodes placed in a tendon-belly arrangement over the bulk of the APB muscle and the first metacarpal–phalangeal joint. The subject maintained relaxation throughout the experiment, and the EMG was monitored on a computer screen and via speakers at high gain. The signal was amplified (Intronix Technologies Corporation Model 2024F, Bolton, Ontario, Canada), filtered (band pass 2 Hz to 2.5 kHz), digitized at 5 kHz (Micro I-101, Cambridge Electronics Design, Cambridge, UK), and stored in a laboratory computer for off-line analysis.

Transcranial Magnetic Stimulation
TMS of the left motor cortex was performed with a 7-cm figure-of-eight coil and a Magstim 200 stimulator (The Magstim Company, Whitland, UK). The coil was placed at the optimal position for eliciting motor evoked potentials (MEPs) from the right APB muscle. The optimal position was marked on the scalp with a felt pen to ensure identical placement of the coil throughout the experiment. The handle of the coil pointed backward and was perpendicular to the presumed direction of the central sulcus, about 45° to the midsagittal line. The direction of the induced current was from posterior to anterior and was optimal to activate the motor cortex transynaptically.

Median Nerve Stimulation
The median nerve in its distal distribution supplies sensation to the thumb, index, long, and one-half of the ring finger and provides motor innervation of the thenar and 2 lumbrical muscles. Electric stimulation of the right median nerve was performed with a standard stimulation block using constant current square wave pulses with cathode positioned proximally. The pulse width was 20 μs, and the stimulus intensity was delivered at an intensity of 300% of the sensory threshold. The sensory threshold was defined as the lowest stimulus intensity evoking sensory response in distal median nerve distribution.

Experimental Design
Our experimental design consisted of measuring both cortical excitability and cortical inhibition prior to and after (i.e., 0, 15, 30, and 60 min) PAS stimulation (see below). Cortical excitability was determined using the MEP size, which was defined as the intensity of TMS stimulus sufficient to produce a mean MEP amplitude of 1 mV peak-to-peak response at baseline (stimulus intensity of 1 mV or SI1mV). To determine SI1mV, the average MEP of 20 stimuli delivered at a stimulation rate at 0.1 Hz with the subject completely at rest was calculated. Cortical inhibition was assessed using the CSP, in which TMS of the contralateral motor cortex during voluntary muscle activity produces a MEP followed by a period of cessation of EMG activity (Cantello et al. 1992). In this experiment, measurement of the CSP duration was obtained in moderately tonically active APB achieved through isometric contraction at 20% of maximum contraction. This was monitored through visual feedback provided through an oscilloscope placed in front of the subjects. The CSP was generated by stimulating the motor cortex for 10 trials with intensities of 140% of rest motor threshold (RMT) (i.e., CSP40), defined as the lowest intensity that produced a MEP of >50 μV in 5 out of 10 trials in relaxed APB (Rothwell et al. 1999). The CSP duration was the time from the MEP onset to the return of any voluntary EMG activity. This is referred to as the absolute CSP and ends with a deflection in the EMG waveform (Tergau et al. 1999). CSP was determined using our previously published automated method (Daskalakis et al. 2003).

Paired Associative Stimulation
PAS consisted of electric stimuli delivered to the right median nerve, paired with single pulse TMS over contralateral M1, with median nerve stimulation preceding TMS with interstimulus interval of 25 ms. This interstimulus interval, designed to generate approximately synchronous arrival of both inputs in M1 (Fig. 1), was reported to markedly enhance the TMS-induced MEP following paired stimulation (Stefan et al. 2000). Pairs of TMS and electrical stimuli were delivered at 0.1 Hz during a 30 min period, reaching a total of 180 pairs. TMS was delivered at intensity of SI1mV and electrical median nerve stimulation was delivered at 300% of the sensory threshold. Muscle relaxation was ensured throughout the experiment by auditory feedback using a loudspeaker.

Attention
As it has been previously demonstrated that the effects of attention play an important role in LTP (Stefan et al. 2004), attention was evaluated to...
ensure that this was not a confounding factor that could potentially account for group differences as attention deficits have been reliably demonstrated in patients with SCZ (Heinrichs and Zakzanis 1998). To quantify attention, subjects were asked to look at the hand being stimulated and count the total number of stimuli delivered over the course of 30 min and report this at the end of PAS. In this regard, although all subjects were aware that the duration of paired stimuli being delivered occurred 15 minutes before and after 4 minutes of baseline, they were unaware of the frequency at which paired stimuli were being delivered or the total number of stimuli being delivered.

**Rotary Pursuit**

The rotary pursuit apparatus (Lafayette Instrument 30014A Photoelectric Rotary Pursuit) presented a ½ inch-square target that rotated clockwise in a circular path. Participants were required to hold a stylus upon a flat surface under which the target rotated and follow the target as it moved about its path. A photoelectric device measured the time (in seconds) that the stylus was held correctly over the target for each trial. The apparatus was designed such that the target’s speed, in rotations per minute, could be adjusted for each 20-s trial and conducted according to previously published methods (Schwartz et al. 1996). Each participant had to perform an initial set of trials (up to a maximum of 5), in order to determine how many rotations per minute yielded an initial performance of about 5 s. Skill-learning sessions, which consisted of 3 blocks, was then performed, using this “optimal” rotation rate. Each block of skill learning consisted of eight 20-s trials with a 20-s intertrial interval. The 3 blocks were separated by intervals of about 10 min.

**Statistical Analysis**

Groups were compared using repeated-measures analysis of variance (ANOVA). Group membership (i.e., patients and subjects) was entered as a between group independent variable. The post-PAS interval (e.g., 0, 15, 30, 60 min) was entered as the within group independent variable in both experiments. In the evaluation of the PAS-induced MEP facilitation, the change in MEP size, expressed as a ratio of the MEP amplitude of the APB muscle evoked by the SI1mV pulse at baseline was 0.97 ± 0.16 mV for patients with SCZ and 1.04 ± 0.15 mV for healthy subjects. The RMT was 43.7 ± 8.9% of stimulator output for patients with SCZ and 44.2 ± 14.8% of stimulator output for healthy subjects. The sensory threshold was 1.53 ± 0.39 mV for patients with SCZ and 1.72 ± 0.3 mV for healthy subjects. There was no significant difference between SCZ patients and healthy subjects for these measures.

**PAS-Induced MEP Facilitation**

For PAS-induced MEP facilitation, there was a significant main effect of group (SCZ and healthy subjects) (F = 7.64, degrees of freedom [df] = 1, 28, P = 0.01) (effect size: Cohen’s d = 1.01) (Cohen 1988) and a significant group by time interaction (F = 5.22, df = 2, 28, P = 0.03). There was no MEP facilitation for the SCZ group at any post-PAS interval, whereas the healthy subjects showed MEP facilitation, especially at 30 and 60 min post-PAS (Fig. 2).

**Attention**

Patients with SCZ estimated that 168.3 ± 21.6 (out of 180) stimuli were delivered, whereas healthy subjects estimated that 181.3 ± 6.4 stimuli were delivered. This difference was statistically significant (t = 2.23, df = 1, 28, P = 0.04). There was, however, no significant relationship between counting accuracy and PAS-induced MEP facilitation across all subjects (r = 0.161, P = 0.394), in patients with SCZ (r = 0.029, P = 0.919), or in healthy subjects (r = 0.175, P = 0.534). Moreover, when this attention measure was used as a covariate in the repeated-measure ANOVA for PAS facilitation, there remained a significant main effect of group (SCZ and healthy subjects) (F = 4.79, df = 1, 27, P = 0.04).

**PAS-Induced lengthening of CSP**

The CSP40 at baseline was 169.8 ± 39.7 ms for patients with SCZ and 148.9 ± 42.0 ms for healthy subjects, and these differences were not significant and consistent with those previously reported in medicated patients with SCZ (Daskalakis et al., Figure 2).
On measures of PAS-induced CSP facilitation, there was a trend toward a significant main effect of group (SCZ and healthy subjects) \((F = 3.08, \text{df} = 1, 28, \text{P} = 0.09)\) (effect size: Cohen’s \(d = 0.66\)) (Cohen 1988) but no significant group by time interaction. Healthy subjects showed an increase in CSP following PAS, but there was no change in SCZ patients (Fig. 3). Finally, there was no relationship between the change in MEP facilitation following PAS and change in CSP following PAS across all subjects, in patients with SCZ, or in healthy subjects.

**Rotary Pursuit**

The dependent variable of interest (i.e., motor skill acquisition) was represented as time on target per trial. Patients with SCZ and healthy subjects were tested at mean rotations per minute of 23.00 ± 10.66 and 27.33 ± 7.99, respectively and did not differ significantly \((t = 1.28, \text{df} = 28, \text{P} = 0.22)\). There was also no significant difference in baseline performance \((t = 0.975, \text{df} = 28, \text{P} = 0.34)\) between groups. There was a significant main effect of group (SCZ and healthy subjects) \((F = 33.49, \text{df} = 1, 28, \text{P} < 0.0001)\) and a significant group by time interaction \((F = 52.64, \text{df} = 1, 28, \text{P} < 0.0001)\) (Fig. 4) suggesting that patients with SCZ, in contrast to healthy subjects failed to demonstrate any motor skill learning through repeated performance despite no significant differences at baseline. Finally, we examined the relationship between the PAS-induced change in MEP size and the motor skill learning on the rotary pursuit. We found a significant correlation between the induction of PAS-induced MEP facilitation and the extent of motor skill acquisition (time on target at the final block minus time on target at baseline) across all 30 subjects \((r = 0.48, \text{df} = 30, \text{P} = 0.007)\) (Fig. 5).

**Relationship between Medication and the Extent of PAS-Induced MEP Facilitation**

There was no significant relationship between the dose of antipsychotic medication when the aforementioned medications were converted to CPZ equivalents (conversion obtained according from previously published reports) (1997; Woods 2003) and the extent of PAS-induced MEP facilitation \((r = 0.19, \text{P} = 0.49)\) (Fig. 6).

**Discussion**

Our results demonstrate that following PAS, MEP facilitation occurred in healthy subjects but was absent in patients with SCZ. Although patients with SCZ did not attend to the stimulation as well as healthy subjects, there was no relationship between our measures of attention and the extent of MEP facilitation, suggesting that attentional factors were not solely responsible for these group differences. There was also a significant trend toward greater potentiation of CSP following
neuronal assemblies into fine spatial and temporal domains, specific to the incoming stimulus (Buzsaki and Chrobak 1995; Llinas et al. 2005). Binding of the multisensory inputs into a coherent cognitive experience is reliant on this inhibitory “clustering” as diminution of GABAergic inhibition has been shown to alter perceptual selectivity (Kyriazi et al. 1996; Wang et al. 2000) and distort synchronized brain activity (Fingelkurts et al. 2004; Arai and Natsume 2006). Decreased spatial and temporal segregation in response to incoming stimuli may impair delivery of “temporally relevant” inputs to motor pyramidal neurons during PAS. This would ultimately result in altered neural plasticity, particularly relevant in the case of associative LTP plasticity (i.e., reliant on polysynaptic processing).

Our findings of impaired motor skills learning in SCZ patients are consistent with previous observations (Schwartz et al. 1996). In keeping with this study, we found no baseline differences in performance between groups. In addition, in the study by Schwartz et al. (1996), there was no correlation between motor symptoms and impaired learning, further suggesting a disturbance of an actual “learning” rather than of “motor execution” in patients with SCZ.

Positron emission tomography (PET) studies have helped to unravel the functional neuroanatomy of the motor learning and have established that the motor execution of rotary pursuit task was associated with activation (as estimated by relative cerebral blood flow) of diffusely distributed neuronal networks, including cortical, striatonigral, and cerebellar structures. It was found that motor learning, unlike motor execution, was reliant on longitudinal increases of neuronal activity in contralateral primary and supplementary motor cortices and in contralateral pulvinar thalamus (Grafton et al. 1992). These changes in neuronal activity were thought to be a reflection of an augmentation in the magnitude of neural discharges that lead to skill acquisition. Moreover, electrophysiological studies of motor learning in animals have implicated neural plasticity as a neurophysiological mechanism of the acquisition of new motor skills (Asanuma and Keller 1991). In light of these reports, our observation of a positive correlation between the LTP-like plasticity and motor skill learning is particularly interesting, as it provides direct neurophysiological support for an association between learning and LTP, though a closer review of the data suggests that this relationship may be mediated by the fact that patients exhibited poor LTP-like plasticity and poor motor learning and healthy subjects tended to exhibit greater LTP-like plasticity and better motor learning. Consequently, it is important that future studies attempt to establish causative relationship between enhanced LTP and motor learning.

One of the major limitations of this study is the fact that all patients were treated with antipsychotic medications. The effects of antipsychotic medications on PAS measures of LTP-like plasticity have not been evaluated in patients with SCZ, though it remains possible that medications may, in part, account for these group differences. The role of neuroleptics and dopaminergic signaling in induction of synaptic plasticity remains a matter of debate. A number of in vivo and in vitro studies demonstrated attenuation of LTP following treatment with clozapine, risperidone, and trifluoperazine (Finn et al. 1980; Kubota et al. 2001; Gemperle and Olpe 2004), whereas others found that haloperidol (Centonze et al. 2004) and clozapine (Kubota et al. 1996; Gemperle et al. 2003) facilitated...
the induction of LTP. Moreover, recent work in PAS-induced MEP facilitation in Parkinson’s patients found that this MEP facilitation was restored with levodopa treatment (Margonte et al. 2006), whereas the dopamine antagonist haloperidol suppressed LTP-like plasticity induced by PAS in motor cortex of healthy controls (Ziemann et al. 2006). It should be noted, however, that the neurophysiological effects of dopamine antagonists/agonists in healthy controls and patients with nigrostriatal dopaminergic deficits may differ from that of SCZ patients. Enhanced dopaminergic neurotransmission has been postulated to be the key mechanism underlying the pathophysiology of SCZ (Seeman et al. 1976), and increased mesolimbic and nigrostriatal dopaminergic signaling in neuroleptic-free schizophrenic patients has also been demonstrated (Abi-Dargham et al. 1998). Therefore, any antidopaminergic effect of antipsychotic medications in patients with SCZ is likely to restore dopaminergic function contrary to inducing a hypodopaminergic state as would be present in Parkinson’s patients or healthy subjects who receive a single dose of haloperidol. Also, recent non-PAS studies evaluating LTP-like plasticity in medicated and medication free patients with SCZ reported that deficits in plasticity in SCZ were not associated with medications (Daskalakis et al. 2004; Fitzgerald et al. 2004) as both medicated and unmedicated groups demonstrated deficits that were equivalent in magnitude. Finally, the fact that there was no significant relationship between medication dose and the extent of PAS-induced LTP-like plasticity in our study provides additional, albeit limited, evidence that antipsychotic medications may not modulate LTP-like plasticity in patients with SCZ. In fact, although not significant, the association between medication dose and the extent of PAS-induced LTP-like plasticity was positive (Fig. 6), contrary to what would be anticipated if medications did indeed disrupt LTP. Nevertheless, studies confirming our preliminary findings in medication-free patients with SCZ are required.

Another limitation of our study is that no measures of attention were utilized to independently evaluate attention both within clinical groups as well as in healthy subjects as a potential confound in PAS. That is, the act of counting stimuli, albeit practical for the purposes of the study to ensure adequate attention to PAS may represent a somewhat substandard evaluation of sustained attention used in SCZ, in contrast to the continuous performance task that has been repeatedly used to demonstrated deficits in sustained attention in patients with SCZ (Heinrichs and Zakzanis 1998). We suggest that these points (i.e., using an unmedicated sample of patients with SCZ and using independent measures of sustained attention) be considered for future studies.

In summary, our data suggest that patients with SCZ demonstrate associative LTP-like plasticity deficits that may be accounted for by NMDA receptor abnormalities or other cellular mechanisms (e.g., GABA) associated with SCZ. Moreover, our finding of an association between the induction of LTP-like plasticity and motor skill learning provides some evidence to suggest that a disruption of neural plasticity in SCZ may underlie deficits in learning and memory in this disorder.

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Notes

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Address Correspondence to Dr Zafiris J. Daskalakis, Schizophrenia Program, Centre for Addiction and Mental Health, 7th Floor, Clarke Division 250 College Street, Toronto, Ontario, M5T1R8, Canada. Email: Jeff_Daskalakis@c camh.net.

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