Cholinergic Influences on Use-Dependent Plasticity

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INTRODUCTION

Reorganizational changes in the CNS are thought to support learning and memory processes (Bontempi et al. 1999; Klein et al. 1998; Nudo et al. 1996; Plautz et al. 2000; Rioult-Pedotti et al. 1998). In the motor domain, practicing voluntary movements results in use-dependent plasticity (Nudo et al. 1996) that encodes the kinematic details of the practiced movements (Classen et al. 1998) and may contribute to recovery of function following cortical lesions such as stroke (Nudo and Friell 1999; Nudo and Milliken 1996). Previous studies in animal models and in humans proposed the involvement of N-methyl-D-aspartate (NMDA) receptor activation and long-term potentiation (LTP)—like processes in this form of plasticity (Butefisch et al. 2000; Cammarota et al. 2000; Hess et al. 1996).

Central cholinergic neurotransmission through muscarinic receptor activation contributes to learning and memory formation and influences LTP (Dykes 1997; Everitt and Robbins 1997; Maalouf et al. 1998; Sarter and Bruno 1997; Segal and Auerbach 1997; van der Zee and Luiten 1999). Therefore it is possible that cholinergic influences are important for expression of reorganizational changes in the human motor system. To address this issue, we evaluated the effect of scopolamine, a muscarinic receptor blocker (Clissold and Heel 1985; Ridout et al. 1998) on both human corticomotor excitability and use-dependent plasticity.

METHODS

Subjects

Nine healthy volunteers gave written informed consent and participated in this double-blind, placebo-controlled and randomized study to evaluate the effects of scopolamine on corticomotor excitability and use-dependent plasticity. The protocol was approved by the National Institute of Neurological Disorders and Stroke Institutional Review Board.

Study protocol

Transdermal patches containing either scopolamine (Transderm Scoplo, belladonna alkaloid with anti-muscarinic properties) (Clissold and Heel 1985; Whitman and Edean 1990) (1.5 mg) or placebo were placed behind the ear of each subject on different sessions separated by at least 72 h. Corticomotor excitability was measured before and approximately 4 h following each patch to assess drug effects. At this time, plasma concentrations reach >50 pg/ml, a threshold value required for appropriate CSF levels and therefore therapeutic effects such as prevention of motion sickness (Nachum et al. 2001). Use-dependent plasticity was measured once in each session under the effects of scopolamine and placebo. A brief standard questionnaire assessing drowsiness, presence of jitters, dryness of the mouth and/or eye, dizziness, restlessness, and confusion was given to participants at the end of the experiment to evaluate drug-related side effects.

Measures of corticomotor excitability

We measured resting and active motor thresholds (MT rest and MT active), intracortical inhibition (ICI) and intracortical facilitation (ICF) (Chen et al. 1998; Kujirai et al. 1993), recruitment curves (RC) of motor evoked potentials (MEP) to transcranial magnetic stimulation (TMS) (Chen et al. 1998; Ridding and Rothwell 1997), and F- and M-waves (Panayiotopoulos and Chroni 1996). Experiments were carried out with the subjects sitting in a comfortable chair with elbows slightly flexed. Ag/AgCl surface electrodes were placed over the belly of the right first dorsal interosseus (FDI) muscle (active electrode) and on the skin overlying the second metacarpophalangeal joint (reference electrode). Electromyographic signal (EMG) was amplified and filtered (band-pass 50 Hz to 1 kHz) using a Counterpoint Electromyograph (Dantec Electronics, Skovlund, Denmark), digitized (sampling rate 2 kHz), and fed into a laboratory computer for off-line analysis. TMS was delivered from Magstim 200 stimulators via a Bistim module (Magstim, Whitland, Dyfed, UK) to the optimal scalp position for stimulation of the right FDI using a figure-eight shaped coil (Cohen et al. 1990; Kaneko et al. 1996; Rothwell 1997).

MT rest, a measure of neuronal excitability (Mavroudakis et al. 1994), was defined as the lowest intensity of TMS able to elicit MEPs of 50 μV in at least 5 of 10 consecutive trials (Rossini et al. 1994). MT active was determined during 5% background facilitation of the target muscle (Cohen et al. 1998; Kujirai et al. 1993; Ziemann 1999).
and was defined as the minimum intensity of stimulation necessary to induce MEP of at least 100 μV (to be discernible from background EMG activity) in 5 of 10 trials. For recruitment curves, stimulus intensity was increased in 5% steps between 30 and 100% of the maximum stimulator output (Ridding and Rothwell 1997); and five MEPs were recorded at each stimulus intensity. ICI and ICF were studied using a conditioning stimulus of 90% MTactive and a test stimulus intensity set to evoke reproducible MEP of about 1 mV (Ziemann 1999). Ten test stimuli alone and 10 paired pulses per interval were applied pseudorandomly at four different interstimulus intervals (2, 3, 10, and 15 ms). The average of the 10 MEPs was assigned to represent each specific interval and test alone condition. F- and M-waves were recorded after supramaximal electrical stimulation of the right ulnar nerve at the wrist (cathode proximal). After determination of the maximum M-wave, 20 F-waves were recorded and averaged.

**Statistical analysis**

Drug effects on MTrest, MTactive, M and F wave amplitudes, and mean ICI and ICF at each interstimulus interval were compared using a Wilcoxon-Whitney rank test. Results were considered significant at the level of P < 0.05. The RCs were compared using an ANOVA model with repeated measures (main effects intervention and stimulus intensity).

**Use-dependent plasticity**

The experimental setting was the same as that previously reported (Butefisch et al. 2000; Classen et al. 1998). Subjects sat in a dental chair with their right forearm supported in a semipronated position in a molded arm cast. Four fingers were immobilized in slight extension while the thumb was kept completely unrestrained. EMG activity was recorded from surface electrodes placed over the belly of extensor pollicis brevis and flexor pollicis brevis muscles, amplified, band-pass filtered between 10 and 3,000 Hz, and fed into a laboratory computer for off-line analysis. Thumb movements were recorded with a three-dimensional accelerometer mounted on the proximal phalanx of the thumb (Kistler Instrument, Amherst, NY). The direction of TMS-evoked and of voluntary thumb movements was calculated from the first-peak acceleration vector. Acceleration signals were recorded in the vertical (extension and flexion) and horizontal (adduction and abduction) axes and digitized at 3,000 Hz. Data were analyzed using a data collection-analysis program written in LabView (National Instruments, Austin, TX). TMS was delivered from a custom-built magnetoelectric stimulator (Cadwell Laboratories, Kennewick, WA) through a figure-of-eight magnetic coil held on the scalp overlying the left motor cortex, at the optimal scalp position for eliciting mild and isolated right thumb movements (Classen et al. 1998). Movement threshold (MoT) was defined as the minimum stimulation intensity able to elicit consistent thumb movements. MTrest was also determined as part of this experiment. Coil position stability was ensured using a combination of a tridimensional laser coordinate system, aluminum frame constraining the subject’s head, and soft-tip marks on the scalp. Subjects included in this experiment fulfilled the following inclusion criteria: 1) consistent (reproducible) direction of TMS-evoked thumb movements in the baseline condition and 2) posttraining TMS-evoked movement directions matched the training direction. Before training, 60 TMS stimuli were delivered at 0.1 Hz to the

![ FIG. 1. Directional change of 1st peak acceleration vector of movements evoked by transcranial magnetic stimulation (TMS) before and after training. At baseline, TMS evoked predominantly extension and abduction thumb movements. Training movements were performed in a direction approximately opposite to baseline (arrow, a combination of adduction and flexion). Posttraining, the direction of TMS-evoked thumb movements changed from the baseline direction to the trained direction. The mean training direction (arrow) is at the center of the training target zone (TTZ). TMS-induced movement directions after training largely fell within the TTZ, close to a 180° change from baseline direction.](http://jn.physiology.org/)

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optimal scalp position to elicit thumb movements. In these trials, the baseline direction was defined as the mean angle of TMS-evoked movements falling in the predominant direction (Fig. 1). Occasionally, a small percentage of TMS-evoked movements (<5%) fell in other directions and were not computed to determine baseline direction (Butefsch et al. 2000).

Subjects’ relaxation was closely monitored by EMG and by auditory feedback. Trials with background EMG activity were discarded from analysis. After identifying the baseline TMS-evoked movement direction, subjects started the training period performing voluntary brisk thumb movements in a direction opposite to baseline for 30 min at 1 Hz (Butefsch et al. 2000; Classen et al. 1998). Following each voluntary movement, the thumb returned to the start position by relaxation, as confirmed by EMG. The direction and the magnitude of each voluntary training movement were monitored on-line, and subjects were encouraged to perform accurately and consistently. To monitor the consistency of training kinematics across conditions, we measured the angular difference between training and baseline directions, the dispersion of training movement directions, and the magnitude of the first peak acceleration of these movements. After completion of the training period, TMS-evoked movement directions were determined again (TMS delivered at 0.1 Hz for 10 min for a total of 60 trials).

To describe the training effects on TMS-evoked movement directions, we defined a training target zone (TTZ) as a window of ±20° centered on the training direction. Our endpoint measure was the increase in the proportion of TMS-evoked movements that fell within the TTZ after training (Butefsch et al. 2000). By design, the training was in the direction opposite to the baseline direction. Therefore the proportion of TMS-evoked movements within the TTZ before training was very small.

**Statistical analysis**

Increases in the proportion of TMS-evoked movements in the TTZ under scopolamine and placebo, MEP amplitudes, angular difference between training and baseline directions, dispersion of training movement directions, and the magnitude of the first peak acceleration of these movements were analyzed using Wilcoxon signed ranks test. All data are expressed as means ± SE. Results are considered significant if \( P < 0.05 \) after correction for multiple comparisons.

### TABLE 1. Effects of scopolamine and placebo on cortical excitability

<table>
<thead>
<tr>
<th></th>
<th>Scopolamine</th>
<th>Placebo</th>
<th>Significance (( P ))</th>
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<tbody>
<tr>
<td><strong>MT&lt;sub&gt;rest&lt;/sub&gt;</strong></td>
<td></td>
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<tr>
<td>Before</td>
<td>38.9 ± 1.94</td>
<td>40.1 ± 1.14</td>
<td>0.67</td>
</tr>
<tr>
<td>After</td>
<td>38.1 ± 1.72</td>
<td>39.9 ± 1.14</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Significance (( P ))</strong></td>
<td>0.31</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td><strong>MT&lt;sub&gt;active&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>29.4 ± 1.46</td>
<td>30.1 ± 0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>After</td>
<td>28.7 ± 1.33</td>
<td>30.7 ± 0.57</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>Significance (( P ))</strong></td>
<td>0.33</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td><strong>M-wave</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Before</td>
<td>15.54 ± 2.20</td>
<td>20.00 ± 3.09</td>
<td>0.27</td>
</tr>
<tr>
<td>After</td>
<td>14.80 ± 2.18</td>
<td>20.14 ± 2.70</td>
<td>0.10</td>
</tr>
<tr>
<td><strong>Significance (( P ))</strong></td>
<td>0.40</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td><strong>F-wave</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.13 ± 0.04</td>
<td>0.19 ± 0.04</td>
<td>0.31</td>
</tr>
<tr>
<td>After</td>
<td>0.13 ± 0.03</td>
<td>0.15 ± 0.04</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>Significance (( P ))</strong></td>
<td>0.95</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. Motor threshold (MT) is expressed as percentage of maximal stimulator output. M- and F-waves are expressed in mV.

FIG. 2. **A:** mean recruitment curves from right 1st dorsal interosseus (FDI) before and after scopolamine (\( n = 9 \))/placebo (\( n = 6 \)) administration. Stimulation intensity was 30–100% of maximum stimulator output. **B:** drug/placebo effects on mean intracortical inhibition and intracortical facilitation obtained using the paired-pulse paradigm. Conditioned motor evoked potential (MEP) amplitudes are expressed relative to the mean test MEP amplitude.
RESULTS

Effects of scopolamine on corticomotor excitability

The results from this experiment showed that under our experimental conditions neither scopolamine nor placebo alone, in the absence of training, elicited significant changes in MT rest, MT active, and M and F wave amplitudes (Table 1). Similarly, recruitment curves, intracortical inhibition, and intracortical facilitation did not differ after administration of scopolamine or placebo (Fig. 2, A and B).

Effects of scopolamine on use-dependent plasticity

The proportion of TMS-evoked movements within TTZ after training increased by 0.37 ± 0.04 relative to baseline under placebo ($P < 0.05$) and by 0.13 ± 0.06 under scopolamine ($P < 0.05$). The magnitude of training-induced changes under scopolamine was significantly smaller than those observed under placebo ($P < 0.05$; Fig. 3).

The $MT_{\text{antagonist}}$, $MT_{\text{agonist}}$, $\text{MovT}$, $\text{MEP}_{\text{agonist}}$, and $\text{MEP}_{\text{agonist}}$ amplitudes at baseline did not differ across conditions pointing to the similar excitability of the motor system in the two sessions (Table 2). Similarly, parameters reflecting consistency of training kinematics such as magnitude of the first peak acceleration of training movements, dispersion of training movement directions, and angular difference between mean baseline and training angle did not differ across conditions (Table 3).

DISCUSSION

The main finding of the present study is that scopolamine substantially decreased the magnitude of use-dependent plasticity in the absence of global measurable changes in motor excitability.

Use-dependent plasticity has been identified in association with learning motor skills such as playing the piano (Pascual-Leone et al. 1995), performing complex motor sequences (Karni et al. 1995; Nudo et al. 1992), and repetitive voluntary movements such as those used in this study (Classen et al. 1998). Reorganizational changes associated with motor training appear to be a factor contributing to recovery of function after cortical lesions such as stroke (Nudo and Friel 1999).

While the mechanisms underlying these plastic changes are incompletely understood, they may be influenced by NMDA receptor activation levels and GABAergic disinhibition (Aro-

![FIG. 3. A: schematic diagram of directional change of 1st peak acceleration vectors in a representative subject under placebo and scopolamine. Note that training (arrow direction) resulted in substantial directional changes in the placebo condition but not in the scopolamine condition. B: training induced changes in TMS-evoked movement direction ($n = 9$). The bar graph shows the group data. Motor training induced an increase in the proportion of more than 35% in the TMS-evoked movements falling in the TTZ in the placebo condition (white bar) while only about 10% in the scopolamine condition (black bar, mean ± SE).](http://jn.physiology.org/)

### TABLE 2. Measures of motor excitability in the placebo and scopolamine conditions at baseline

<table>
<thead>
<tr>
<th>Condition</th>
<th>$MT_{\text{antagonist}}$</th>
<th>$MT_{\text{agonist}}$</th>
<th>$\text{MovT}$</th>
<th>$\text{MovT}$</th>
<th>$\text{MEP}_{\text{agonist}}$ mV</th>
<th>$\text{MEP}_{\text{agonist}}$ mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>49.4 ± 1.2</td>
<td>49.2 ± 1.3</td>
<td>59.6 ± 1.9</td>
<td>119.9 ± 2.2</td>
<td>0.89 ± 0.03</td>
<td>1.36 ± 0.04</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>48.2 ± 2.2</td>
<td>48.9 ± 2.2</td>
<td>58.7 ± 2.1</td>
<td>119.5 ± 2.7</td>
<td>0.71 ± 0.03</td>
<td>1.45 ± 0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE. Motor threshold (MT) and movement thresholds (MovT) in muscles mediating movements in the training direction (agonist) and in the baseline direction (antagonist) are expressed as percentage of maximal stimulator output. MovT is additionally expressed as percentage of $MT_{\text{agonist}}$. MEP, motor evoked potentials.
Values are means ± SE. Peak acceleration is expressed in m/s². Angular dispersion is expressed as length of unit vector. Angular difference is expressed in degrees.

REFERENCES


The present results demonstrate that scopolamine, a muscarinic receptor antagonist, substantially decreased the magnitude of use-dependent plasticity compared with placebo. Under our experimental conditions, scopolamine did not affect motor training kinematics or concentration (angular difference between training and baseline directions, dispersion of training movement directions, and magnitude of the 1st peak acceleration of voluntary training movements) nor the subjects’ ability to relax as measured with the questionnaire and monitoring of baseline EMG activity. Since scopolamine patch placement results in maximal plasma levels at about 24 h (Nachum et al. 2001) and in our study the patch was kept in position for a maximum of 7 h, the relative paucity of side effects is not unexpected. Similarly, scopolamine did not modify resting or active motor thresholds, recruitment curves, intracortical inhibition, or intracortical facilitation compared with placebo, suggesting a relative stability of excitability levels in corticocortical neuronal connections across conditions as tested with TMS.

In contrast to our results using scopolamine patches, intravenous bolus administration of scopolamine has been reported to elicit a decrease in resting motor thresholds (Di Lazzaro et al. 2000). The reason for this difference may be the rapid climb and overall higher plasma levels reached when the drug is administered intravenously compared with the slower buildup produced by the patch (Ebert et al. 2001). This interpretation is consistent with the finding that subjects receiving intravenous scopolamine exhibited sedation (Vitiello et al. 1997) and deteriorated performance (Preda et al. 1993), while subjects in our study neither experienced sedation nor had signs of deteriorated performance as measured by motor training kinematics. The present findings indicate a facilitatory role of cholinergic function on use-dependent plasticity.