Online Maintenance of Sensory and Motor Representations: Effects on Corticospinal Excitability

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INTRODUCTION

We can cross time intervals interposed between stimuli and responses either by remembering events or by anticipating them (Rainer et al. 1999). Both strategies require the maintenance of task-relevant information in the brain, although the type of information maintained online might be different. When identification of a sensory stimulus and action selection are separated in time, a sensory representation of the stimulus needs to be held online (working memory; Goldman-Rakic 1987; Miyake and Shah 1999). Conversely, when action selection and movement execution are separated in time, subjects can prepare a specific response in advance (motor preparation; Tanji and Evarts 1976). Recent studies suggest that short-term storage of sensory information and movement preparation can be seen as conceptually and neuronally distinct phenomena (Curtis et al. 2004; Di Pellegrino and Wise 1996; Fuster 2000; Mars et al. 2005; Rowe et al. 2000). However, this issue remains controversial because other authors have argued that the same physiological mechanisms might support both motor preparation and working memory (Constantinidis et al. 2001). Furthermore, this debate is complicated by the lability of sensory- and motor-related properties of neuronal populations (Chen and Wise 1996, 1997).

Here, we contribute to this debate by assessing the neurophysiological consequences on the motor system of holding sensory or motor material online. Given the crucial role of the premotor cortex in response preparation (Passingham 1993; Toni et al. 1999, 2002a; Wise and Mauritz 1985) and given that the premotor cortex has direct access to the primary motor cortex and spinal cord (Dum and Strick 2005; He et al. 1993; Matelli et al. 1986), we test the hypothesis that the central and precentral cortex contribute to the online maintenance of movement representations, but not of sensory items. Differently from previous studies on the effects of motor preparation on the corticospinal tract (Chen et al. 1998; Hasbroucq et al. 1997, 1999; McMillan et al. 2006), we directly compared corticospinal excitability evoked while holding sensory or motor material online under identical behavioral conditions. To this end, we use a novel paradigm in which task contingencies, rather than explicit verbal instructions, induce participants to use either sensory or motor codes to cross temporal delays interposed between instructions and responses (Toni et al. 2002b). This approach is important for isolating the informational codes used to cross a delay period, rather than the control of short-term storage driven by verbal instructions. Furthermore, we manipulated the length of the maintenance delays, to evoke robust preparatory activity during the delay interval (Schoffelen et al. 2005; Toni et al. 2002b). These features are important for capturing the temporal dynamics of delay-related activity supporting online maintenance and for assessing neural phenomena comparable to those observed in animal studies (Funahashi et al. 1993; Quintana and Fuster 1999).

Participants solved a delayed-nonnatch-to-sample (DNMS) task between two “sample” visual patterns and a “test” pattern, centrally presented. The sample and test patterns were separated by a time delay. The task involved a comparison of their shape (Fig. 1). On some trials, the pattern configuration allowed the participants to prepare the correct response immediately after the presentation of the sample. In other words, we influenced the type of information carried over the delay period by manipulating the relevance of the shape of the test cue for...
correct performance. These settings optimized the ability of our DNMS task to induce participants to cross temporal delays interposed between instructions and responses by using either sensory or motor codes. Using single-pulse transcranial magnetic stimulation (TMS) over the “hot spot” of the prime mover [first dorsal interosseus (FDI)], we measured corticospinal excitability evoked during the delay period of the DNMS task. Importantly, by recording EMG from both the prime mover and from muscles not involved in providing the response, we could distinguish between generalized and response-specific effects.

**METHODS**

**Participants**

Nine right-handed volunteers [six males, 23–24 yr old; handedness score: 80 ± 16% (mean ± SD); Oldfield 1971] participated in the TMS experiment after giving written informed consent. Nine additional volunteers (five male) participated in a control experiment designed to assess possible changes in background EMG activity between experimental conditions. All participants had normal or corrected to normal visual acuity and no history of neurological illness. Participants gave informed consent according to institutional guidelines of the local ethics committee (CMO region Arnhem-Nijmegen, Netherlands).

**Behavioral procedures**

There were three behavioral training sessions followed by a test session. Single-pulse TMS was used only during the test session. The same stimuli, responses, and stimulus–response associations were used throughout the experiment.

Participants were first trained to perform a visuomotor associative task relating four shapes to two different movements, using either their right or left hand (Fig. 1A). Participants were instructed to respond by pushing a button that was held between the extended index finger and thumb. As a consequence, the first dorsal interosseus (FDI) muscle needed to be activated to press the button. In the beginning of a trial, one of the four visual stimuli was presented for 300 ms. Then a variable, randomized delay (0.1–1.0 s in steps of 0.1 s) was followed by a tone (trigger cue, presented for 300 ms). Participants were asked to respond as quickly as possible after the auditory trigger cue. On each trial, immediately after the response, a visual feedback stimulus (a green tick or red cross) was presented for 200 ms. This feedback stimulus informed the participants whether the response was correct. Participants were trained until no errors were made in 80 successive trials.

In the second training session, participants were trained in a delayed-nonmatch-to-sample (DNMS) task. Two of the four visual stimuli (Fig. 1, “Sample”) were briefly presented (Sample, 300 ms). A variable delay (Delay, 1.5–9.0 s, 2.5-s steps) was followed by the brief presentation of one of the four stimuli (Test, 300 ms). Participants were instructed to respond with the hand specified by the nonmatching shape between the set of 3 shapes presented in that trial (Sample and Test). Sample and Test shapes were paired as to evoke movement preparatory activity (B, PREPARATION; in these trials the pattern configuration allowed the participants to prepare the correct response immediately after the presentation of the Sample); or maintenance of sensory items (C, MEMORY: in these trials the pattern configuration required the participants to compare the shape of the Test and Sample stimuli). Transcranial magnetic stimulation (TMS)–free trials were also presented in both PREPARATION and MEMORY conditions (D), which allowed us to measure behavioral performance unaffected by the TMS pulse and to diminish any possible anticipatory effects of the 2nd TMS pulse to the visual test stimulus.
shapes that could have instructed 1) the same movement or 2) different movements. When the two sample shapes instructed the same movement (Fig. 1B), then the test shape invariably matched one of the two instruction stimuli. It follows that the correct response was completely specified by the instruction shapes. In these trials (preparation trials), the participants could have selected the response after the presentation of the sample stimuli and hold the movement ready during the delay.

When the two sample shapes instructed different movements, then the test shape could have matched (70%) or not one of the two sample stimuli. It follows that the correct response was specified by the comparison between sample and test shapes. In these trials (memory trials, Fig. 1C), the participants needed to wait until the presentation of the test shape to be able to compare the sensory characteristics of test and sample stimuli and select the appropriate response. In those trials in which the test shape did not match any of the two sample stimuli (30%), the participants were required to press the finger specified by the test shape (catch trials). Their presence allowed us to probe whether the participants were solving the memory trials by applying an alternative strategy to the one detailed above; that is, during memory trials, the participants could have simply opted to perform the movement that was not instructed by the test cue. This alternative strategy did not require the participants to hold the sample stimuli online, but it relied on the trigger cue being invariably matched to one of the two sample stimuli. Therefore if participants used this alternative strategy, they would have been unable to correctly perform the catch trials, achieving only chance-level performance.

In the third training session, the participants were trained in the DNMS task as used in the test session (100 trials). The task was the same as that used in the second training session, apart from the absence of feedback on performance and RT cutoff. Furthermore, the interstimulus interval (ISI) was variable (from 1.5 to 9.0 s in steps of 2,500 ms) and randomized throughout the experiment.

Finally, during the test phase, participants sat in a chair in front of a 19-in. computer screen (viewing distance: 130 cm). The experimental timing (delivery of visual stimuli and TMS, recording of motor responses) was controlled by Presentation 9.13 (Neurobehavioral Systems, Albany, NY). Both hands lay in pronated position on a pillow that was placed on the subject’s lap and both elbows were flexed to about 90°, allowing maximal relaxation of the forearm muscles. Two response buttons (one for each hand) were taped to the pillow. TMS was applied during performance of the DNMS task. In each trial two single pulses were administered. The first pulse, given 1,000 to 900 ms before presentation of the sample stimuli, served as baseline. The second pulse could be either a test pulse or a catch pulse (Fig. 1). Test pulses were applied during the variable ISI (2,000 ms after presentation of the sample stimuli and 500 ms before presentation of the test stimulus), whereas the catch pulses were applied 2,000 to 2,500 ms after presentation of the test stimulus. These TMS catch pulses were included to diminish any possible anticipatory effects of the second TMS pulse to the visual test stimulus (these were called TMS-free trials; see Fig. 1D). Because the distribution of pulses is equal during both conditions, our design controls for the potential confounding effects of the sound produced by the application of the TMS pulse. Furthermore, unknown to the participants, there were no trials of the catch condition during the experimental session. Catch trials were removed to keep the length of the experimental session to a minimum.

Transcranial magnetic stimulation

TMS was delivered using a figure-of-eight shaped coil (diameter 70 mm) connected to a Magstim 200 stimulator (Magstim, Whitland, UK). The coil was held tangentially on the left hemiscalp with its handle pointing backward at an angle of about 45° from the midsagittal axis.

In the beginning of the experimental session, surface markings were drawn onto a tightly fitting Lycra swimming cap worn by the participants. These markings served as reference points for positioning the coil. The point at which stimuli of a given intensity consistently yield the largest motor-evoked potentials (MEPs) in the FDI muscle of the right hand was marked as the FDI motor hot spot. After the motor hotspot was determined, TMS was applied at decreasing intensities to determine the subject’s resting motor threshold (Rossini et al. 1988). Resting motor threshold (rMT) was defined as the intensity needed to evoke an MEP in relaxed muscle of $\geq 50 \mu V$ peak-to-peak amplitude in five of ten consecutive trials [rMT across the participants was 32 ± 2% (mean ± SD) of maximum stimulator output]. Then, stimulation intensity was increased again to determine stimulator output that yields peak-to-peak MEP amplitudes of about 1 mV. This was the stimulation intensity used during the test phase (38 ± 3% of maximum stimulator output).

Electromyography

EMG was recorded using 23 × 35-mm self-adhesive AgCl surface electrodes (Kendall-LTP, Chicopee, MA). Electrodes were placed in differential pairs over the FDI and the abductor digitii minimi (ADM) muscles of the right hand, using a belly-tendon montage. The EMG signals were recorded using an Oxford Synergy electromyograph and were amplified (amplifier range: 100 mV; 10 channels) and digitally filtered (20 Hz to 3 kHz) using Medelec Synergy Software (version 11.1, Oxford Instruments). Acquisition duration was set to 50 ms. During the experiments, EMG activity was monitored by the experimenter with visual and auditory feedback.

Behavioral analysis

We considered the mean response times of correct trials (RTs) and error rates (ERs) measured during those trials of the test session without TMS pulse during the instructed delay. These behavioral measures were analyzed with an $2 \times 2 \times 4$ ANOVA [with factors TASK (two levels: preparation, memory); HAND (two levels: left, right); and DELAY (four levels: 1.5, 4.0, 6.5, 9.0 s)]. Participants were considered as a random factor. The alpha-level was set at $P = 0.05$, multivariate approach, Pillai’s Trace corrected.

Electrophysiological analysis

Mean peak-to-peak MEP amplitudes were calculated offline. Recordings with peak-to-peak EMG activity of $>50 \mu V$ before MEP onset (mean 3.15% of total trials per participant, SD = 3.32%) were excluded from analysis. For each participant, mean peak-to-peak MEP amplitudes evoked during performance of the DNMS task were normalized to the mean peak-to-peak MEP amplitudes evoked during baseline (before presentation of the sample stimuli). These normalized MEPs were analyzed with a $2 \times 2 \times 2$ ANOVA [with factors TASK (two levels: preparation, memory); HAND (two levels: left, right); MUSCLE (two levels: FDI, ADM)]. The alpha-level was set at $P = 0.05$, multivariate approach, Pillai’s Trace corrected.

Background EMG activity

We performed an additional control experiment (nine participants) to verify that the preparation and memory trials evoked comparable levels of background EMG activity. The participants performed the same training as during the main experiment and 300 experimental test trials (150 trials in the memory and 150 trials in the preparation condition, randomly intermixed), using the same parameters as those in the main experiment. EMG activity was recorded during the entire experimental session, although TMS was not applied. The root mean square of the EMG signal recorded in the 1,000 ms before the test cue...
was compared between the memory and preparation trials by means of a paired t-test.

RESULTS

Behavioral performance

During TMS-free trials, participants were faster and made fewer errors during the preparation trials than during the memory trials [Fig. 2; main effect of TASK: RT: $F(1,8) = 136.4, P < 0.01$; ER: $F(1,8) = 17.0, P < 0.01$]. Crucially, a longer delay between Sample and Test stimuli resulted in longer RT during memory, but not during preparation [TASK $\times$ DELAY interaction - RT: $F(3,6) = 6.5, P < 0.03$]. There were no significant effects of hand, indicating that participants responded equally fast with both effectors under all task circumstances.

The modulation of RT by delay was further investigated within each condition with a post hoc analysis, using a linear regression on the RT of each participant separately for the memory and the preparation trials. The participants’ standardized beta weights were then tested at the second level using a two-tailed paired-samples t-test. This post hoc analysis revealed increases in RT with increasing delay length in memory ($P = 0.006$), but no significant modulation with increasing delay length in the preparation condition ($P = 0.14$). Taken together, these results indicate that the mental representations used to bridge the temporal gap between sample and test during the memory trials were more labile than those used during the preparation trials, suggesting that different mental representations were used to cross the instructed delay in the different conditions.

Electrophysiological data

MEP amplitudes were affected by our experimental manipulations. There was a significant main effect of HAND [$F(1,8) = 10.228, P = 0.013$] and MUSCLE [$F(1,8) = 10.000, P = 0.013$]. The effects of HAND were modulated by the task [TASK $\times$ HAND interaction: $F(1,8) = 20.813, P = 0.002$] and differed for the different muscles [MUSCLE $\times$ HAND interaction: $F(1,8) = 9.102, P = 0.017$]. Crucially, we found a TASK $\times$ HAND $\times$ MUSCLE interaction [$F(1,8) = 5.951, P = 0.041$; see Fig. 3], indicating that excitability of the prime mover (FDI) and the control muscle (ADM) were differentially modulated in the two hands as a function of task.

To test the specificity of these MEP changes, we compared the MEPs to the baseline MEP using two-tailed paired-samples t-test. We hypothesized that the MEP recorded over the prime mover would be increased during the preparation delay period, whereas the MEPs recorded over the nonprimary movers would be reduced (Tomberg 1995). Indeed, during right-hand responses, the MEPs recorded over the right FDI during preparation of a right-hand response were increased compared with baseline during the preparation delay [$t(8) = 5.0314, P =$...
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We probed the level of corticospinal excitability evoked by holding online either sensory material (memory trials) or motor responses (preparation trials), well before participants were required to execute a finger movement that involved FDI contraction. When participants could prepare the movement in advance (preparation trials), the excitability of the motor cortex contralateral to the moving hand increased, whereas the excitability of the ipsilateral motor cortex decreased. The increase in excitability was confined to the prime mover (FDI), whereas the decrease in excitability extended to cortical territories controlling muscles unrelated to the response (ADM). Crucially, these changes in excitability were evoked only during preparation trials and not during memory trials. We infer that short-term storage of sensory information and preparation of motor responses have different access to the output stage of the motor system.

Behavioral effects

Participants solved the DNMS task well above chance, indicating that they were effectively engaged in the task. During memory trials, performance became slower as a function of delay length (Fig. 2). Conversely, during preparation trials there was no modulation of RT by delay length. This finding of differential effects of delay length on RT in the preparation and memory trials is in line with previous results (Toni et al. 2002b).

A difference between the present results and those obtained by Toni et al. (2002b) can be observed in the response profile of the memory trials. In this study RT increase linearly as a function of delay length (Fig. 2), whereas in Toni et al. (2002b) there was a U-shaped relationship between RT and delay length. This difference is likely related to the use of a shorter minimum delay between Sample and Test presentation (1.0 s in Toni et al. 2002b; 1.5 s in the present study) because the shortest delay used in Toni et al. (2002b) was likely to introduce interference between storage of the Sample stimuli and encoding of the Test stimulus. In this study we increased the duration of the shortest delay to avoid this possible confound.

The differential effect of delay length on preparation and memory trials (Fig. 2) cannot be explained by a speed-accuracy trade-off because there was no significant task × delay interaction on ER. Overall, the behavioral data indicate that the type of information retained during the memory trials was more vulnerable to the length of the maintenance delay, and thus of a different kind than that used during the preparation trials.

Electrophysiological effects

In this study we found both increases of corticospinal excitability over the cortical representation of the prime mover and generalized decreases of excitability over the muscles of the hand that should not be moved when the response is required (Fig. 3). There was a strong trend (P = 0.057) of decreased excitability in the muscle contralateral to the prime mover. This trend is consistent with the significant effect seen in another unrelated muscle of the nonresponding hand (ADM) and with the general observation that it is harder to isolate a decrease in excitability in muscles contralateral to the prime mover than in muscles unrelated to task performance (Leocani et al. 2000).

These results are in line with previous findings in both human and nonhuman primates (Fetz and Finocchio 1972; Leocani et al. 2000; Rossini et al. 1998; Tanji and Evarts 1976). Here we further qualify the contribution of the human precentral cortex to motor preparatory processes, showing that corticospinal excitability changes when motor representations are held online, but not during the maintenance of sensory items (Fig. 3). This finding fits with a previous study showing that during a choice RT-task, the interference generated by single-pulse TMS follows a precentral rostrocaudal gradient of interference as the time of response execution approaches (Schluter et al. 1998). Furthermore, in a recent MRI study using the same paradigm as that in this report, we showed that short-term storage of sensory information and preparation of motor responses are supported by spatially segregated portions of the precentral cortex, organized along a similar rostrocaudal gradient (Mars et al. 2005). In this perspective, the pattern of differential corticospinal excitability we report here appears to fit with the notion of a rostrocaudal gradient of sensory-motor properties along the precentral gyrus (Picard and Strick 2001; Simon et al. 2002).

It might be argued that the scope of our inferences is limited to the primary motor cortex because we probed corticospinal excitability over the FDI hot spot (Terao et al. 1998). However, the excitability of primary motor cortex is likely to be a nonlinear integration of influences from a spatially distributed cortical circuit, reflecting not only the effects of local circuitry but also distal effects from premotor and posterior parietal cortex. Therefore, finding a change in corticospinal excitability during movement preparation does not imply that primary motor cortex is the site of maintenance of the motor representation. Indeed, several electrophysiological and neuroimaging studies converge in suggesting that preparatory activity supporting movement representations is reliably evoked in precent-
eral and parietal regions, but less consistently in primary motor cortex (De Lange et al. 2005; Johnson et al. 1996; Kalaska and Crandall 1995; Thoenissen et al. 2002; Toni et al. 1999, 2002a; Wise et al. 1997). By the same token, it remains to be seen whether the present changes in corticospinal excitability occur at the cortical level and/or at the subcortical level (Van Elswhijk et al. 2007).

A second finding of this study concerns the temporal dynamics of delay-related activity supporting movement preparation. We show that cortical excitability increases not only immediately before response delivery (Chen et al. 1998), but also up to several seconds before motor execution. This prolonged modulation of corticospinal excitability during preparation trials could be related to the characteristics of our experimental paradigm, that is, full advance knowledge on the response to be executed, combined with limited information on the timing of the response (Fig. 1). More precisely, the timing of the response was constrained by the uniform distribution of delays between Sample and Test stimuli, making the timing of the response unpredictable. Therefore the participants experienced an increased conditional probability of providing a response at a given time, given that no response had been yet required (Schoffelen et al. 2005). Conversely, previous studies either did not probe the time interval preceding the Go-signal (Aoki et al. 2002; Chen et al. 1998) or tested corticospinal excitability during a single (i.e., fully predictable) time period before an imperative cue, often reporting a decrease in corticospinal excitability before the test cue (Hasbroucq et al. 1997, 1999; Touge et al. 1998). Furthermore, it was previously shown that using temporally predictable TMS pulses can also lead to a decrease in corticospinal excitability (Takei et al. 2005). In summary, our results indicate that, given a task in which participants need to be ready to respond at any time, their motor system maintains the motor plan online resulting in an increased corticospinal excitability. It remains to be seen whether the increase in corticospinal excitability obtained in the present study is modulated by the time-varying probability of providing a response, as suggested by the time-varying features of hemodynamic activity along the precentral gyrus (Mars et al. 2005).

Third, we report how interhemispheric inhibition could target not only the prime mover (Di Lazzaro et al. 1999; Ferbert et al. 1992; Gerloff et al. 1998; Mochizuki et al. 2004; Sterns et al. 2002), but also task-unrelated muscles, provided that the contralateral hand has already been selected for providing the response. This effect is reminiscent of previous reports on the effects of motor imagery on corticospinal excitability (Facchini et al. 2002; Fourkas et al. 2006; Kasai et al. 1997; Stinear et al. 2006), i.e., muscle-specific MEP increases during kinesthetic motor imagery and MEP decreases during negative motor imagery (Sohn et al. 2003). It is an open issue whether this effect is exclusively driven by the primary motor cortex or whether other portions of the precentral gyrus contribute to the reduction of excitability observed over the nonresponding hand. Furthermore, our study concentrated on participants with strong dominance of the right hand. Because motor cortex excitability is modulated by the degree of hand dominance (Brouwer et al. 2001), the effects we report might differ in subjects with weaker hand dominance. Furthermore, our results do not directly address the issue of stimulating the motor areas controlling the nondominant hand. It remains an open issue whether these effects generalize to the nondominant hand.

In conclusion, the present results indicate how the human precentral cortex is specifically interested in preparing a motor response, over and above the effects of elapsing time or holding sensory items online. It remains to be seen how different corticocortical circuits converging onto primary motor cortex contribute to the effects observed in this study.

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