

The Role of the Cerebellum in Subsecond Time Perception: Evidence from Repetitive Transcranial Magnetic Stimulation

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Abstract

■ In three experiments, we investigated the role of the cerebellum in sub- and suprasedond time perception by using repetitive transcranial magnetic stimulation (rTMS). In Experiment 1, subjects underwent four 8-min 1-Hz rTMS sessions in a within-subject design. rTMS sites were the medial cerebellum (real and sham rTMS), left lateral cerebellum, and right lateral cerebellum. Following each rTMS session, subjects completed a subsecond temporal bisection task (stimuli in the range 400–800 msec). Compared with sham rTMS, rTMS applied over the right lateral or medial cerebellum induced a leftward shift of the psychophysical function (perceived lengthening of time). In Experiment 2, a separate sample of subjects underwent the identical rTMS procedure and completed a suprasedond bisection task (stimuli in the 1000–2000 msec range). In this experiment, rTMS to the

cerebellar sites did not produce any significant changes compared with sham rTMS. Experiment 3 employed a within-subject design to replicate findings from Experiments 1 and 2. Subjects underwent four rTMS conditions (sub- and suprasedond tasks following medial cerebellar and sham rTMS). rTMS induced a significant leftward shift of psychophysical function in the subsecond bisection, but not in the suprasedond bisection. In this study, we have demonstrated that transient cerebellar stimulation can differently affect the ability to estimate time intervals below and above a duration of 1 sec. The results of this study provide direct evidence for the role of the cerebellum in processing subsecond time intervals. This study further suggests that the perception of sub- and suprasedond intervals is likely to depend upon distinct neural systems. ■

INTRODUCTION

According to a theory of interval timing (the scalar expectancy theory [SET]), processing temporal information involves different processes that are working in concert: an internal clock (short- and long-term) memory and decisional processes (see Malapani, 2002; Wearden, 1999; Gibbon & Church, 1990, for reviews). Recent developments in the neurobiology of interval timing have revealed that critical brain structures engaged in temporal processing include the prefrontal and parietal cortices (lateralized to the right), thalamus, basal ganglia, and cerebellum (Ivry & Spencer, 2004b; Matell & Meck, 2004; Gibbon, Malapani, Dale, & Gallistel, 1997). Isolation of each process for localization in the brain is difficult because these processes are working together as a system to process temporal information. Despite difficulties of localizing components of this dynamic system, there is a developing consensus that the internal clock is subserved by the basal ganglia “and/or” cerebellum.

There is considerable evidence that the basal ganglia function as the internal clock for interval timing (Rao, Mayer, & Harrington, 2001). Interval timing deficits around 300 or 600 msec are well described in patients with Parkinson’s disease (PD) (Harrington, Haaland, & Hermanowicz, 1998). Patients with PD were also impaired in the estimation and reproduction of suprasedond intervals (3–27 sec) (Pastor, Artieda, Jahanshahi, & Obeso, 1992). It is possible that the focal brain abnormality underlying timing disorder resides in dopaminergic systems such as the substantia nigra and striatum. Further evidence for this comes from the observation that interval timing dysfunction in patients with PD is reversible with dopaminergic treatments (Malapani et al., 1998). Manipulation of dopamine levels in rats and humans has been shown to alter the rate of perceived time, an effect attributed to the slowing or speeding of the internal clock (see Meck, 1996). A current neurobiological model of interval timing proposes that dopamine-mediated striatal–frontal circuitry is a mechanism for the internal clock (Matell & Meck, 2000, 2004).

Evidence of cerebellar involvement in interval timing is firstly provided by clinical cases. Mangles, Ivry, and Shimizu (1998) showed that patients with cerebellar damage

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were impaired in the perception of both milliseconds (400 msec) and seconds (4 sec) interval ranges, whereas patients with prefrontal damage had an impairment only in the seconds range together with working memory deficits. In children with cerebellar dysgenesis, smaller cerebellar volumes were associated with impaired interval discrimination (400 msec) as well as motor timing dysfunction (Dennis et al., 2004). Consistent with this is the finding in a recent neuroimaging study that implicates the cerebellum specifically in interval timing in the subsecond range (Lewis & Miall, 2003). Here, cerebellar and premotor areas were activated to a greater extent during temporal discrimination of stimuli around 600 msec, compared with that of stimuli around 3 sec. However, the involvement of the cerebellum in interval timing has recently been questioned by Harrington, Lee, Boyd, Rapcsak, and Knight (2004a). They used subsecond time intervals (around 300 or 600 msec) to examine interval timing in a relatively large group of patients with cerebellar stroke. They observed only a trend toward time perception deficits in the patients, which was attributed to a generalized slowing of cognitive processing speed.

The aim of the present study was to investigate the role of the cerebellum in sub- and suprasedond interval timing (400/800 and 1000/2000 msec). To this end, we used repetitive transcranial magnetic stimulation (rTMS) to induce a transient change in cerebellar function in healthy volunteers. TMS is a noninvasive method to change neural excitability (Barker, Jalinous, & Freeston, 1985). rTMS over the motor cortex at low frequency (≤ 1 Hz) decreases neuronal excitability of the stimulated brain area (Chen et al., 1997), whereas rTMS at high-frequency ranges (> 5 Hz) increases neuronal excitability (Pascual-Leone, Valls-Sole, Wassermann, & Hallett, 1994). Since the reported observation that cerebellar TMS influenced motor cortex function (Werhahn, Taylor, Ridding, Meyer, & Rothwell, 1996; Ugawa, Uesaka, Terao, Hanajima, & Kanazawa, 1995), cerebellar function has increasingly been studied using rTMS. One-hertz rTMS applied over the cerebellum has been reported to increase temporal variability in finger tapping (Theoret, Haque, & Pascual-Leone, 2001) and to interfere in visuo-motor movement (Miall & Christensen, 2004) and procedural learning (Torriero, Oliveri, Koch, Caltagirone, & Petrosini, 2004). Finally, Oliveri, Koch, Torriero, and Caltagirone (2005) have recently shown that 1-Hz rTMS applied over the cerebellum produces an increase in the excitability of the motor cortex, suggesting the possibility that there may be some reciprocal relationship between cerebellar activity and other brain regions.

EXPERIMENT 1

The aim of Experiment 1 was to examine whether the cerebellum is involved in subsecond interval timing

(400/800 msec). We used a temporal bisection task that has been used in experimental animals and, more recently, in humans (Allan & Gibbon, 1991; Wearden, 1991). In the task, subjects are initially asked to memorize two standard (referent) durations of tones. They are then asked to indicate whether a probe tone is more “similar” to one of two referent tones, a “short” or a “long” tone. For the analysis, the mean proportion of long responses is plotted against the probe stimulus duration, yielding a *psychophysical function*. This function is used to determine the bisection point where short and long responses are made with equal frequency, and the difference limen to represent the gradient of the function (steeper gradient represents better temporal sensitivity). A leftward shift of the function would occur if subjects perceived tone durations longer than they actually were; hence, a lengthening of subjective time (Wearden, Pilkington, & Carter, 1999). Application of psychopharmacological stimulants (i.e., methamphetamine) in experimental animals produces a leftward shift of the function, whereas anti-psychotic agents such as haloperidol induce a rightward shift (Cevik, 2003; Meck, 1986). The difference limen is the smallest difference that can be reliably discriminated in the set of intervals used in a temporal bisection experiment. It has been associated with temporal sensitivity or precision. In developing children (aged 3, 5, and 8 years), the difference limen becomes smaller with age (Droit-Volet & Wearden, 2001), but in old age, the difference limen tends to be larger especially in an attention-demanding condition (Lustig & Meck, 2001).

Eleven right-handed healthy volunteers underwent four 1-Hz rTMS sessions, in a within-subject design. Stimulation sites were the medial cerebellum (real and sham rTMS), left lateral cerebellum, and right lateral cerebellum, with the order randomized across subjects. Immediately after completion of each rTMS session, subjects completed a block of temporal bisection trials. Before the rTMS experiment started, they completed two consecutive blocks of temporal bisection in order to induce a stable performance level in the subjects before any changes produced by rTMS and to ensure familiarity with the experimental setup.

Based on the finding that 1-Hz rTMS over the cerebellum increases the excitability of the motor cortex (Oliveri et al., 2005), we hypothesized that decreasing cerebellar activity with 1-Hz rTMS, which would lead to disinhibition of the motor cortex (hence excitation), would induce a leftward shift of psychophysical function, similar to the effect of psychopharmacological stimulants (Meck 1986). As functional neuroimaging findings on the lateralization of cerebellar activation during interval timing have been inconsistent (see General Discussion for further details), we investigated both cerebellar hemispheres as well as the medial cerebellum.

Methods

Subjects

Eleven healthy male volunteers (mean age 30 years, $SD = 5.1$, range 21–41) participated in Experiment 1. They were academic staff or postgraduate students from the University of Sheffield. All subjects were right-handed, as assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). After complete description of the study to the subjects, written informed consent was obtained. The study was approved by the local Research Ethics Committee.

Temporal Bisection Task and Experimental Procedure

Within a repeated measures design, all subjects completed a total of six blocks of temporal bisection; they completed two blocks of temporal bisection before the rTMS procedure started and a block of temporal bisection immediately after each of four rTMS sessions. The stimulus durations used in this experiment are 400, 467, 533, 600, 667, 733, and 800 msec.

After subjects arrived at our TMS laboratory, they received a training session for the temporal bisection task. In this session, subjects heard two standard tones (400 and 800 msec) each being repeated five times. Subsequently, they were trained to press one of two buttons on the response box in response to the standard short and long tones, each of which were presented 10 times. The subject received feedback for incorrect responses. Training was completed if the subject made 100% correct responses for a block of 10 trials.

In each block (two blocks before rTMS and one block following each of four rTMS sessions), they were asked to respond whether a randomly presented tone from the seven test stimuli (standard short and long together with five intermediate stimuli) was more similar to the standard short or long tone by pressing the left button for short or the right for long. Each block consisted of 18 trials for each of seven stimuli and, therefore, a total of 126 trials. The response stimulus interval was varied randomly from 1000 to 1400 msec (1000, 1100, 1200, 1300, and 1400 msec). A Power Macintosh computer controlled experimental events and recorded data using Psycscope (Cohen, MacWhinney, Flatt, & Provost, 1993). Responses were made on the right and left buttons of a Psycscope response box. All tones had a frequency of 700 Hz and were delivered via the computer speaker.

rTMS Protocol

Immediately after completion of each rTMS session, subjects completed a block of temporal bisection lasting approximately 4–5 min, followed by a 10-min rest before

the next rTMS session. Test stimuli were randomly presented within a block.

We applied 1-Hz rTMS for 8 min (480 TMS pulses) to all subjects in randomized order to (1) the medial cerebellum (1 cm below theinion), (2) the left lateral cerebellum (1 cm below theinion and 3 cm left of midline), and (3) the right lateral cerebellum (1 cm below theinion and 3 cm right of midline), and (4) 1 cm below theinion (sham TMS) (Theoret et al., 2001). rTMS was performed with a 70-mm figure-of-eight coil and a Magstim Super Rapid magnetic stimulator (Magstim Company, Whitland, UK). rTMS was applied at an intensity of 90% motor threshold. Individual motor threshold was determined by using the thumb movement visualization method after stimulation over the motor cortex (Pridmore et al., 1998). The coil was positioned vertically with the handle pointing upward. For sham rTMS, the coil was held at 90° to the scalp over the medial cerebellum. A physician who has experience with rTMS attended all stimulation sessions. During and following rTMS, none of our subjects reported any adverse effects (e.g., nausea) (Satow et al., 2002). The total testing lasted approximately 2 hr.

Data Analyses

Proportions of right button press (reported “long responses”) for each tone duration were calculated for each individual. These psychophysical functions showed a logistic growth (sigmoidal) pattern that increased gradually at first, more rapidly in the middle, and slowly toward the end. A sigmoidal curve-fitting method was applied to the psychophysical function in each condition for each individual to find the best fit by using the software CurveExpert 1.3 (curveexpert.webhop.biz/). In most subjects, a sigmoidal curve model shown below shows the best fit for individual data, that is, proportions of long response, $P(L)$:

$$P(L) = \frac{ab + cx^d}{b + x^d}$$

Parameters were not weighted, as our null hypothesis was that there was no difference between conditions. Using the function, we then calculated the bisection point and the difference limen based on the psychophysical function for each condition in each individual. The bisection point indicates the stimulus duration at which the subject will provide 50% long responses. The difference limen, which represents the slope of psychophysical function, is calculated as half the difference between the duration providing 75% and 25% long responses. All statistical test results reported in this experiment were two-sided and statistical significance was set at .05.

Results and Discussion

Figure 1 (left) shows subjects' bisection performance on two consecutive blocks before the rTMS procedure. The psychophysical function was shifted to the left in the second block, $t(10) = 2.63, p < .03$, compared with that of the first block. There was also a trend toward steeper gradient of psychophysical function in the second block as indicated by a decrease in difference limen value, $t(10) = 1.84, p = .096$. Figure 1 (right) displays psychophysical functions following rTMS sessions. A leftward shift of psychophysical function after medial or right lateral cerebellar rTMS compared with sham rTMS is evident. Repeated measures analysis of variance showed significant differences in bisection point across rTMS conditions, $F(3,30) = 4.9, p < .01$, whereas there were no significant differences in "difference limen" between rTMS conditions. Post hoc pairwise comparisons showed that the bisection point after either medial ($p = .023$) or right lateral ($p = .013$) cerebellar stimulation was significantly shorter than after the sham rTMS condition. Examination of individual data confirmed that 10 of 11 subjects showed the same directional effect. There was no significant difference in bisection point between left lateral cerebellar (582 msec) and the sham rTMS (590 msec) conditions. As TMS intensity was dependent upon an individual's motor threshold, we examined whether the leftward shift of psychophysical function after rTMS was related to TMS output intensity. There was no significant rTMS output intensity correla-

tion with bisection point in any conditions (medial, $r = -.43, p = .18$; right lateral, $r = -.35, p = .30$; left lateral, $r = -.16, p = .64$; sham, $r = -.11, p = .75$).

In this experiment, we found practice effects across two pre-rTMS blocks of temporal bisection, producing a leftward shift and a trend toward steeper gradient of psychophysical function. Following medial or right lateral cerebellar rTMS, compared with responses after the application of sham rTMS, there was a leftward shift of psychophysical function indicating that subjects made long responses more frequently (hence "lengthening of perceived time"). This perceptual alteration is specific as evidenced by an absence of temporal sensitivity change (i.e., the gradient of psychophysical function). The results provide direct evidence for the involvement of the cerebellum in perceiving subsecond intervals.

EXPERIMENT 2

In the framework of the SET, the internal clock contains a pacemaker generating pulses to an accumulator via a switch that allows pulses to be registered. The pacemaker generates pulses in a multiplicative manner. The pacemaker speed is considered to be constant to produce the scalar property of interval timing, unless its speed is influenced by experimental manipulations (Wearden, 1999). If the pacemaker speed increases, its cumulative effect would be greater on longer intervals (during which a proportionally greater number of pulses

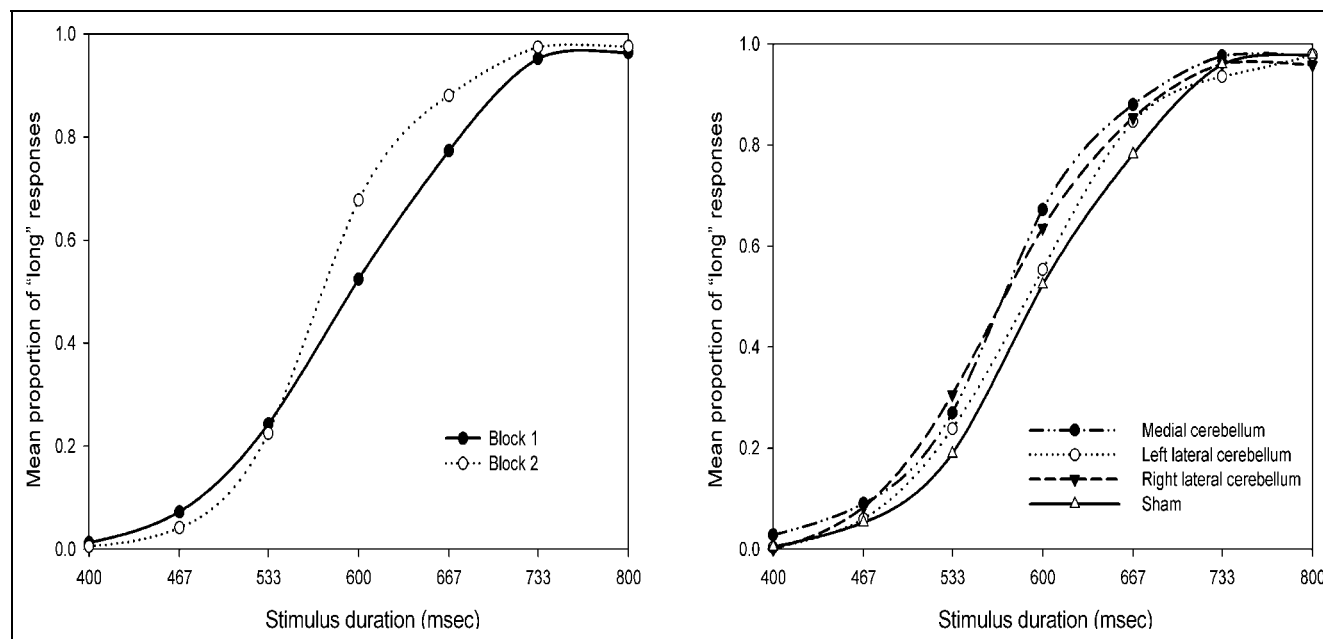


Figure 1. The mean proportion of long responses plotted against stimulus duration for two blocks of practice (left). With practice, the psychophysical function was shifted to the left ($p < .03$), and the gradient of the function became steeper at a trend level ($p = .096$) compared with that of the first block. The right shows psychophysical function after each of four rTMS applications. After rTMS over medial ($p = .023$) or right lateral ($p = .013$) cerebellum, the psychophysical function was shifted to the left without changes in the gradient of the psychophysical function. The bisection point was larger in sham rTMS compared with that of Block 2 ($p < .01$), suggesting that the learning effect did not last long enough to influence the performance in rTMS conditions.

can accrue) than on shorter intervals (during which few pulses accrue). The switch is closed to connect the path between pacemaker and the accumulator with the arrival of a significant, attention-triggering signal (Lejeune, 1998). When the duration of the signal is ended, the switch is opened to disconnect the path between the pacemaker and the accumulator. The latent interval for the closure and opening of the switch (i.e., the latency) is not dependent upon the length of an interval to be timed (Droit-Volet & Wearden, 2002).

Some studies have successfully manipulated variables affecting pacemaker speed, using repetitive sensory stimulation (flashes or clicks) or psychopharmacological stimulants such as dopamine agonists. For example, Droit-Volet and Wearden (2002) used two temporal bisection tasks (200 and 800 or 400 and 1600 msec), both presented with and without visual flicker. They found that subjects judged intervals as longer (i.e., a leftward shift of psychophysical function resulted) when stimuli to be timed were preceded by visual flicker. Furthermore, the flicker had a more marked effect in the condition using longer intervals (400/1600 msec). This greater effect of the flicker on the longer intervals suggested the pacemaker was being speeded up, as the effect of the switch would be independent of intervals employed. In studies with rats, pacemaker speed was increased with amphetamine and decreased with the dopamine D₂ receptor antagonist, haloperidol (Drew, Fairhurst, Malapani, Horvitz, & Balsam, 2003; Maricq & Church, 1983; Maricq, Roberts, & Church, 1981). This phenomenon was more pronounced on interval timing of longer duration stimuli.

Droit-Volet (2003) observed a switch effect in a temporal bisection task (0.5 and 2 or 1 and 4 sec) by introducing a preparatory signal before a stimulus presentation. In the preparatory signal condition, subjects showed a leftward shift of psychophysical function compared with a no preparatory signal condition. This effect was constant across the duration ranges tested. Thus, in order to determine whether a leftward shift of psychophysical function is related to pacemaker or switch effects, more than one range of test durations need to be used. This is because a leftward shift might be either due to the speeding up of pacemaker or due to switch effects (either being closed earlier or being open later, to allow more pulses into the accumulator).

Experiment 1 suggested that the cerebellum might function as either (or is connected to) the pacemaker or the switch within the internal clock system. Experiment 2 was designed to examine whether the rTMS effect observed in Experiment 1 was due to pacemaker or switch effects. In this experiment, we investigated the same rTMS effect to the cerebellum on suprasecond intervals (1000 and 2000 msec). A greater rTMS effect on these suprasecond intervals, compared with those observed in Experiment 1, would suggest a pacemaker effect, whereas the same amount of effect as in Exper-

iment 1 would imply a switch effect. A new sample of 11 healthy volunteers took part. The rTMS procedure was identical to that of Experiment 1. As in Experiment 1, no side effects were noted in any individuals. Statistical test results reported in this experiment were two-sided and statistical significance was set at .05.

Methods

In Experiment 2, a new sample of 11 healthy volunteers (mean age 32 years, $SD = 5.2$, range 25–43) participated. They were all male and right-handed. As were subjects who participated in Experiment 1, they were academic staff or postgraduate students from the University of Sheffield and were thus of homogenous (high) intelligence and educational level. Apart from intervals included in the temporal bisection task (1000, 1170, 1340, 1500, 1660, 1830, and 2000 msec), the identical experimental procedures, rTMS protocol, and data analyses techniques in Experiment 1 were used. Each subject in this experiment received rTMS in the same order as the correspondingly numbered subject in Experiment 1.

Results and Discussion

Figure 2 (left) displays subjects' bisection performance on two consecutive blocks before the rTMS procedure. There were no statistically significant differences in bisection point, $t(10) = 1.8$, $p = .1$, or difference limen between the two blocks, $t(10) = 1.3$, $p = .212$. Figure 2 (right) shows psychophysical function after each rTMS condition. There were no significant differences in bisection point, $F(3,30) = .2$, $p = .88$, or difference limen, $F(3,30) = 1.1$, $p = .35$, across rTMS conditions. There was also no significant correlation between bisection point in each rTMS condition and TMS output intensity (medial, $r = .29$, $p = .38$; right lateral, $r = .35$, $p = .30$; left lateral, $r = .47$, $p = .15$; sham, $r = .31$, $p = .36$).

In Experiment 2, practice effects across two pre-rTMS blocks of temporal bisection were not statistically significant. Nonetheless, the psychophysical function in the second block in Experiment 2 (Figure 2, left) was in the same direction as in Experiment 1 (Figure 1, left). On the other hand, there were no rTMS effects detected in any of stimulation conditions. This experiment indicates that the leftward shift of psychophysical function observed in Experiment 1 cannot be attributed to either pacemaker or switch effect, which suggests specificity of the cerebellum in processing subsecond intervals.

EXPERIMENT 3

The aim of this experiment was to replicate the finding of the leftward shift of psychophysical function in subsecond temporal bisection but not in suprasecond bisection following cerebellar rTMS. We were also

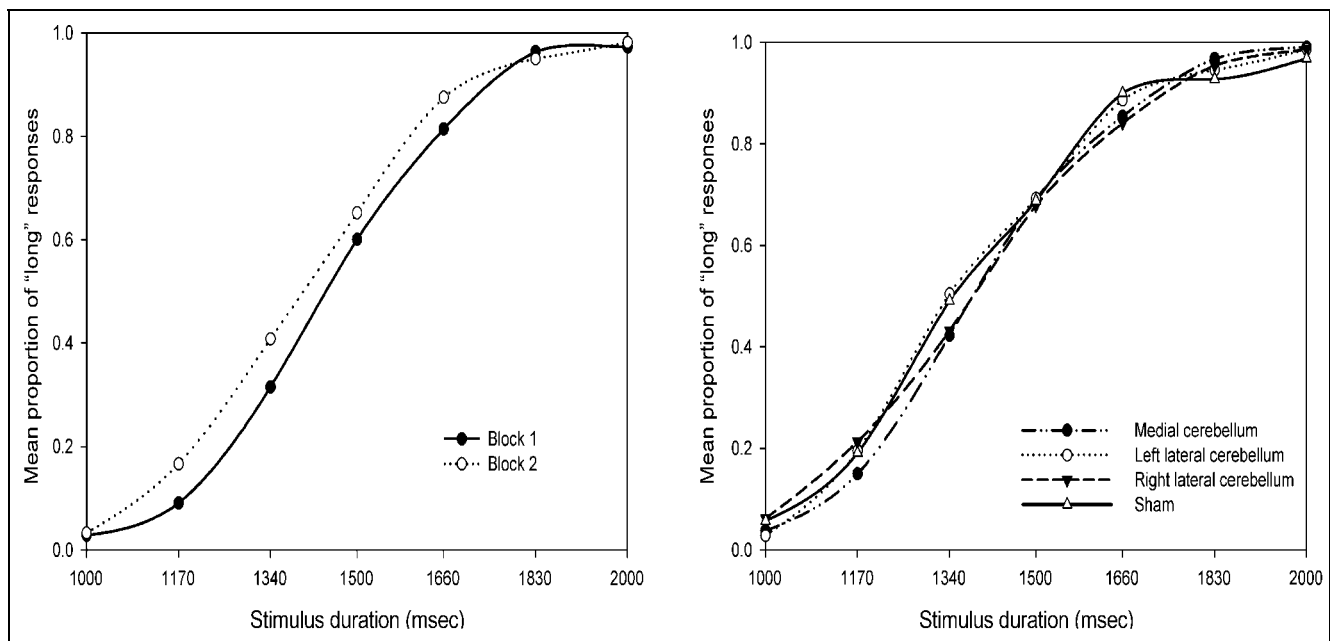


Figure 2. The mean proportion of long responses plotted against stimulus duration for two blocks of practice (left). There were no statistically significant effects of practice. Cerebellar rTMS did not produce any significant effects on the psychophysical function (right). There was no significant difference in bisection point among sham and two practice bisection conditions, but as one can see, the sham function was more similar to the Block 2 function.

interested in investigating whether the significant effect of practice observed in Experiment 1, but not in Experiment 2, could be replicated. In this experiment, the medial cerebellum was chosen as the site of stimulation because the location of the coil was identical with sham rTMS except the angle of the coil applied.

Methods

A new sample of 18 right-handed healthy male volunteers participated (mean age 27 years, $SD = 7.8$, range 20–46).¹ Three subjects were left-handed, and the remainder of subjects were right-handed. The order of stimulation (real or sham) was counterbalanced across subjects within each bisection task. Half of the subjects performed subsecond bisection task first, and the rest of the subjects performed suprasedond bisection first. All subjects completed two consecutive blocks of temporal bisection before the rTMS procedure. Temporal bisection tasks, rTMS protocol, and data analyses techniques were identical to those in previous experiments. One subject reported that he had a mild headache after the experiment, which responded well to over the counter nonprescription analgesic. As a replication experiment, all statistical test results reported in this experiment were one-sided and statistical significance was set at .05.

Results and Discussion

Subjects' subsecond bisection performance on two consecutive blocks before the rTMS procedure revealed

that, with practice, the psychophysical function was shifted to the left in the second block, $t(15) = 1.95$, $p = .035$, compared with that of the first block. However, there were no significant changes in suprasedond bisection performance following practice, $t(15) = 0.9$, $p = .40$. Figure 3 (left) displays psychophysical functions following medial cerebellar and sham rTMS sessions in the subsecond bisection. A leftward shift of psychophysical function after medial cerebellar rTMS compared with sham rTMS was evident, $t(15) = 2.04$, $p = .028$. As can be seen in Figure 3 (right), there were no significant changes in suprasedond bisection performance following rTMS, $t(15) = -0.23$, $p = .82$.

Variations in “goodness of fit” between model and data could be a potential confounding variable on the reported difference between medial cerebellar and sham rTMS conditions, because goodness of fit for individual data might be different across rTMS conditions. To address this issue, we calculated a residual (error term) for each distribution for each individual. The error term was used to examine the covariation effect of goodness of fit (Wichmann and Hill, 2001). When goodness of fit was controlled for, the leftward shift of psychophysical function after medial cerebellar rTMS remained significant, $F(1,13) = 5.1$, $p = .021$.

In this experiment, we replicated the practice effect across two pre-rTMS blocks of subsecond temporal bisection in Experiment 1. This practice effect was not observed in the suprasedond temporal bisection task, consistent with the finding from Experiment 2. Following medial cerebellar rTMS, compared with responses

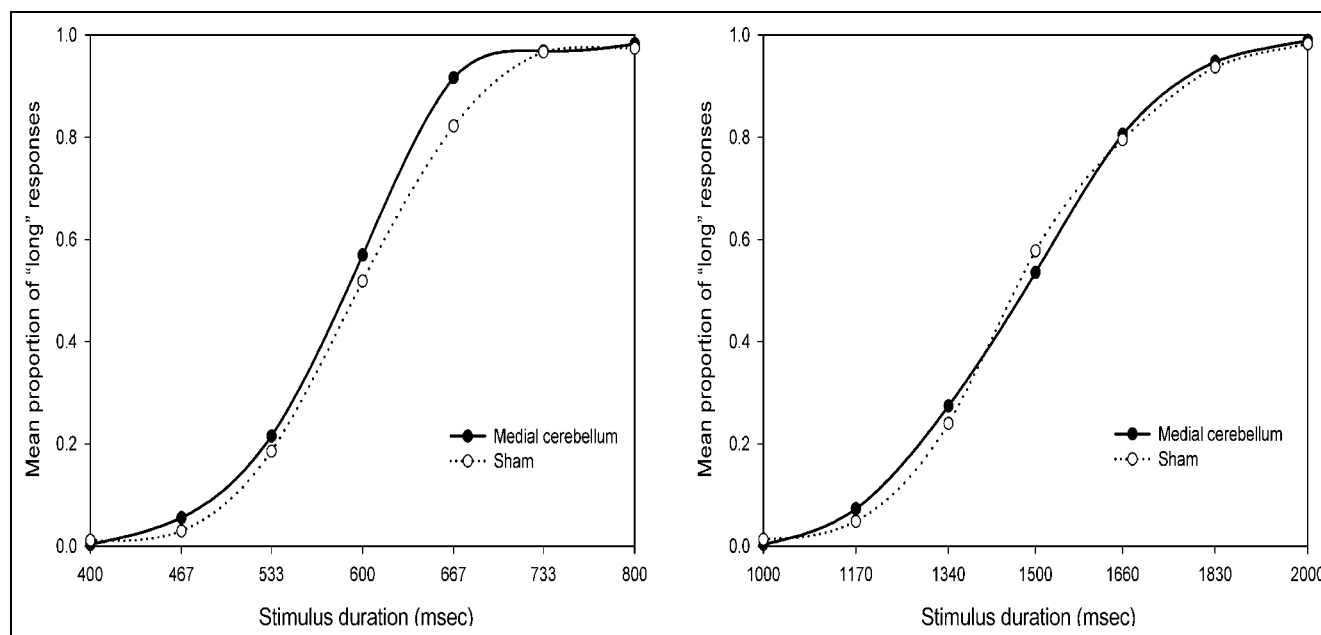


Figure 3. The mean proportion of long responses plotted against stimulus duration for sub- and suprasedond tasks after each of two rTMS applications. After rTMS over the medial cerebellum, the psychophysical function in the subsecond bisection was shifted to the left ($p = .028$, one-tailed) without changes in the gradient of the psychophysical function, compared with that of the sham rTMS condition (left). On the other hand, rTMS applied over the medial cerebellum did not produce any significant effects on the psychophysical function in the suprasedond bisection (right).

after the application of sham rTMS, there was a leftward shift of psychophysical function in subsecond temporal bisection but not in suprasedond bisection. Together, this experiment closely replicated findings from Experiments 1 and 2 (Table 1). We also observed that participation of suprasedond temporal bisection first resulted in a high proportion of long responses even to the

standard short tone in the following subsecond temporal bisection in two subjects. When asked to perform the two tasks in succession, 4 out of 101 healthy subjects showed this interference effect in our previous study (Lee, Dixon, Spence, & Woodruff, 2006); hence, the rate was higher in our current experiment, possibly due to the consequences of long and intense testing procedure

Table 1. Means and Standard Deviations of Temporal Bisection Variables for Each Cerebellar rTMS Condition in Each Experiment

	<i>Medial</i>	<i>Left</i>	<i>Right</i>	<i>Sham</i>
Experiment 1				
400/800 BP	565.2 (25.7)	582.3 (21.9)	565.8 (25.9)	590.5 (23.8)
400/800 DL	79.6 (12.7)	86.5 (8.7)	89.6 (19.6)	79.9 (15.7)
Experiment 2				
1000/2000 BP	1384.7 (131.0)	1361.7 (139.2)	1369.8 (155.0)	1350.5 (159.1)
1000/2000 DL	187.7 (42.9)	204.8 (32.9)	199.6 (30.7)	211.9 (25.9)
Experiment 3				
400/800 BP	576.0 (27.0)	–	–	586.6 (26.1)
400/800 DL	83.9 (5.7)	–	–	85.9 (7.1)
1000/2000 BP	1453.0 (100.7)	–	–	1457.1 (90.7)
1000/2000 DL	214.7 (21.6)	–	–	220.4 (25.6)

BP = bisection point; DL = difference limen.

Values are given in msec.

in our current experiment. In conclusion, this experiment highlighted the robust nature of our findings in Experiments 1 and 2.

GENERAL DISCUSSION

The aim of the present study was to investigate whether subsecond interval timing depends on the cerebellum and to further examine its role in interval timing in the context of SET. In Experiment 1, we tested whether the cerebellum was involved in subsecond interval timing. rTMS applied to the medial or right lateral cerebellum produced a “lengthening of perceived time” (hence, subjects made long responses more frequently), compared with responses after the application of sham rTMS. On the other hand, in suprasedond temporal bisection in Experiment 2, there were no significant effects of cerebellar rTMS. The results of Experiment 1 provide direct evidence for the involvement of the cerebellum in perceiving subsecond intervals. On the other hand, the results of Experiment 2 indicate the absence of cerebellar involvement in the perception of suprasedond intervals. The dissociation between sub- and suprasedond interval timing following cerebellar rTMS was replicated by using a within-subject design in Experiment 3. Taken together, the results of this study are in line with the proposal that different neural systems are involved in sub- and suprasedond timing, with the former subserved by the cerebellum.

Throughout our three experiments, subjects performed two blocks of temporal bisection prior to the rTMS procedure in order to induce a stable performance level in the subjects before any changes produced by rTMS and to ensure familiarity with the experimental setup. We found practice effects across two pre-rTMS blocks of temporal bisection. In subsecond intervals, the psychophysical function was shifted to the left with practice. By contrast, there were no statistically significant effects of practice on suprasedond intervals. Lang and Bastian (2002) have shown that practiced novel movement become more automatic to a degree that it does not interfere performance in a secondary task in healthy volunteers. Patients with cerebellar lesions in their study, however, showed little evidence of learning; hence, performance deteriorated during a dual task condition. Our finding of a practice effect during subsecond interval timing along with the demonstration of the cerebellar involvement of automaticity of practiced movement suggests that the cerebellum may be involved in the perception of, and movement within, relatively short (subsecond) time intervals. Nonetheless, it should be noted that our current study was not designed to address this specific issue of practice on time perception.

The rTMS results of this study are in line with a number of studies in patients with cerebellar lesions that

report impaired subsecond interval timing in these patients (Dennis et al., 2004; Casini & Ivry, 1999; Mangels, Ivry, & Shimizu, 1998; Nichelli, Alway, & Grafman, 1996; Ivry, Keele, & Diener, 1988). In a series of temporal bisection experiments in patients with cerebellar degeneration, Nichelli et al. (1996), using a similar stimulus interval range to ours (100–900 msec), found a leftward shift of psychophysical function. Our study contrasts with the failure to find significant subsecond interval timing dysfunction in patients with cerebellar stroke (Harrington et al., 2004a). However, these patients did exhibit impairment in subsecond interval timing at a trend level of significance. Functional reorganization that occurs following a brain injury such as stroke might have contributed to their findings of a lack of association between cerebellar damage and timing difficulty. Although the role of the cerebellum in interval timing is currently under active debate (Harrington et al., 2004a; Harrington, Lee, Boyd, Rapcsak, & Knight, 2004b; Ivry & Spencer, 2004a), our study findings provide positive evidence for the critical role of the cerebellum in subsecond interval timing.

Interval timing studies in patients with cerebellar lesions have reported that these patients are impaired in both sub- and suprasedond interval timing ability. However, in this study, rTMS applied to the cerebellum modulated subsecond interval timing alone. As to why cerebellar rTMS effect was only on subsecond interval timing, a recent functional magnetic resonance imaging study by Lewis and Miall (2003) may provide an insight. They showed that the cerebellum and motor cortex activations were greater when subjects were engaged in estimating duration of stimuli of 600 msec, compared with when estimating stimulus duration of around 3 sec. Furthermore, electrophysiological studies of mismatch negativity (MMN) showed that intervals up to 800–1000 msec are measured by an automatic and preattentive brain mechanism. MMN evidence for automatic brain processing mechanisms is not apparent when subjects estimate intervals of duration >1 sec (Grimm, Widmann, & Schroger, 2004; Näätänen, Syssoeva, & Takegata, 2004). The dissociation between sub- and suprasedond interval timing following cerebellar rTMS in our study suggests different neural timing systems for sub- and suprasedond intervals. This proposal is supported by the observation that rTMS over the right dorsolateral prefrontal cortex altered the ability to reproduce suprasedond (2 sec), but not subsecond (500 msec) intervals (Jones, Rosenkranz, Rothwell, & Jahanshahi, 2004).

In the framework of the SET, a performance change might have resulted from any processing changes of the internal clock, memory, or decision making. If the cerebellum were responsible for the internal clock, cerebellar rTMS would produce either a greater effect for the longer stimulus intervals (due to a pacemaker effect) or the same shift effect across both interval ranges (due to a switch effect). Hence, our observed

leftward shift of psychophysical function following cerebellar rTMS is unlikely to be attributed to the internal clock system. However, a decay of the memory trace in the long-term (reference) memory could produce a similar effect to that observed in our study. As the interval stored in reference memory decays, future intervals appear to last relatively longer. Another possibility is that cerebellar rTMS might have led to an altered decision-making process. Against this, however, is the finding that a shift effect occurred in the subsecond bisection, despite the same level of task difficulty across both interval ranges (note that the ratio between two referent tone durations was set at 1:2 in both experiments, i.e., 400–800 and 1000–2000 msec).

One might further speculate a physiological mechanism underlying the dissociation between sub- and suprasedond time perception following cerebellar rTMS. Given that multiple cortical–subcortical brain areas are involved in time perception, it is highly likely that each area of the brain acts as an oscillator to interact with each other (Matell & Meck, 2004; Miall, 1989). The interaction between these oscillators may provide the means by which the brain stores and recalls time intervals, for instance, via a phase-synchronization mechanism (Lee, Williams, Breakspear, & Gordon, 2003). Miall (1989) proposed that frequencies for oscillators were between 5 and 15 Hz, and that the activity threshold for these oscillators determined the temporal resolution (the more activation and the better temporal resolution, as synchrony between oscillators determines temporal resolution). It is possible that a specialized neural mechanism exists to measure very brief time intervals (hundreds of milliseconds) for perception and action that might be different from that of relatively long intervals (seconds). For example, synchronized high-frequency (typically 40 Hz, but varying from 20 to 90 Hz) neural oscillations were observed in the motor cortex during the components of movement (i.e., preparation to move, movement, and maintenance of posture; for a review and further references, see Lee et al., 2003). This oscillatory activity in the motor cortex synchronized with that of the cerebellum in zero lag over the 15- to 39-Hz range during a precision grip task in the monkey (Soteropoulos & Baker, 2006). The synchronization of high-frequency oscillatory activity between the cerebellum and motor cortex may provide a physiological correlate of the effect of cerebellar rTMS on subsecond timing.

The connectivity between the cerebellum and cortex is poorly understood (Daskalakis et al., 2004; Voogd, 2003). The effect of our rTMS application over the cerebellum on the bisection task in Experiments 1 and 3 was similar to that previously observed with psychopharmacological stimulants (i.e., a leftward shift of psychophysical function) (Meck, 1986). Two previous studies have applied 1-Hz rTMS over the cerebellum, and both studies observed an increased excitability of

the contralateral primary motor cortex (Oliveri et al., 2005; Gerschlager, Christensen, Bestmann, & Rothwell, 2002). The increase of the motor cortex excitability in these studies might be related to a decrease of cerebellar inhibitory output (i.e., Purkinje cells) to the deep cerebellar nuclei, and hence to excitatory thalamocortical circuits. The laterality effect observed in this study (right more than left) is similar to that of a study examining the effect of 1-Hz cerebellar rTMS on procedural learning (Torriero et al., 2004). In this study, rTMS over the right lateral cerebellum produced a performance decrease regardless of the hand used, whereas rTMS over the left cerebellum affected procedural learning involving the ipsilateral hand only. However, cerebellar interhemispheric difference in interval timing is inconsistent across neuroimaging studies: Bilateral (Pastor, Day, Macaluso, Friston, & Frackowiak, 2004; Belin et al., 2002; Rao et al., 2001; Jueptner et al., 1995), left (Lewis & Miall, 2003; Maquet et al., 1996), or right (Tracy, Faro, Mohamed, Pinsk, & Pinus, 2000) cerebellar activation has been reported. This inconsistency in the laterality of cerebellar activation is in contrast to the strong cortical lateralization to the right during interval timing.

There are some issues to consider in interpreting the results of this study. As the stimulus durations used in each experiment were different (i.e., sub- and suprasedond stimuli), a block of temporal bisection in Experiment 2 would last approximately 2 min longer than that in Experiment 1. It could be argued that rTMS duration might be increased proportional to the increase in task performance time. However, on the basis that rTMS duration is not proportional to rTMS effect duration (Touge, Gerschlager, Brown, & Rothwell, 2001), we decided to deliver the same physical stimulation across both experiments. It seems unlikely that either the clicking sound or the sensation produced by rTMS would explain the observed leftward shift of psychophysical function on subsecond interval estimation, as these effects would be negated by the randomized rTMS design.

In conclusion, our study shows that the perception of subsecond time intervals *does* depend on cerebellar function. This finding is consistent with neuropsychological and neuroimaging studies reporting the specificity of the cerebellum in subsecond interval timing. However, our study does not support the hypothesis that the cerebellum acts as (or is associated with) the internal clock because cerebellar rTMS does not change suprasedond interval timing. The dissociation between sub- and suprasedond interval timing after the transient alteration of cerebellar function observed in the present study together with growing evidence from neurophysiological and rTMS studies suggests that the brain mechanism responsible for timing subsecond intervals is different from that responsible for timing suprasedond intervals. It may be that subsecond interval perception particularly relies on the cerebellum as part of a neural

timing network comprising the cerebellar–basal ganglia–thalamocortical circuit.

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Note

1. Two subjects who performed the suprasecond bisection task first showed more than 25% of long responses to the standard “short (400 msec)” stimulus during the subsecond temporal bisection task. As the stimulus duration at which the subjects will provide 25% long responses was outside of the stimulus range, they were replaced with new subjects.

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