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RESEARCH ARTICLE

The role of the human ventral premotor cortex in counting successive stimuli

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Abstract Adult humans have the ability to count large numbers of successive stimuli exactly. What brain areas underlie this uniquely human process? To identify the candidate brain areas, we first used functional magnetic resonance imaging, and found that the upper part of the left ventral premotor cortex was preferentially activated during counting of successive sensory stimuli presented 10–22 times, while the area was not activated during small number counting up to 4. We then used transcranial magnetic stimulation to assess the necessity of this area, and found that stimulation of this area preferentially disrupted subjects' exact large number enumeration. Stimulation to the area affected neither subjects' number word perception nor their ability to perform a non-numerical sequential letter task. While further investigation is necessary to determine the precise role of the left ventral premotor cortex, the results suggest that the area is indispensably involved for large number counting of successive stimuli, at least for the types of tasks in this study.

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Introduction

Many animals can use small numbers precisely up to four (Gallistel and Gelman 2000; Hauser et al. 2003; Uller et al. 2003). There is also some evidence that human adults may use distinct processes to manipulate small and large numbers (Cipolotti et al. 1991; Trick and Pylyshyn 1994). Neuropsychological and lesion studies, in animals, human infants, and human adults, have suggested that proficiency in the precise use of large numbers is a uniquely human quality, although there may exist similar neuronal substrates for animals, human infants, and human adults in manipulation of small numbers.

Several previous neuroimaging studies have investigated the neuronal basis for small number enumeration in the human brain. Two have specifically focused on the "subitizing" process: enumeration of quantities up to 4 with a rapid, accurate, and confident way, for visual objects in a spatial array (Sathian et al. 1999; Piazza et al. 2002). Activations between subitizing (1–4) and counting (5–8 or 6–9) processes were compared, and the activated areas for subitizing were mainly in the occipitoparietal areas, and the areas for the counting process were more widespread than for the subitizing process. Sathian et al. found posterior parietal activation for counting but not for subitizing. In contrast, Piazza et al. detected posterior parietal activation for both counting and subitizing. Our group performed neuroimaging studies investigating small number enumeration up to 4, not for a spatial array but for successive sensory and motor events (Kansaku et al. 2006). Subjects counted successive auditory, visual, and somatosensory stimuli, and responded with a corresponding number of tapped movements of a finger or a foot. We studied the network of brain areas activated during counting the successive sensory and motor events, and showed the involvements of the lateral premotor cortex, pre-supplementary motor area, posterior temporal cortex, and thalamus. The posterior parietal cortex was activated during sensory counting but not motor counting, and the anterior parietal cortex activated specifically during motor counting.

If presented numbers of the successive stimuli become large, it is likely that adult humans will still be able to easily and precisely enumerate them; but it is not known which areas of the brain are additionally recruited in the process. The present study aimed to demonstrate the brain areas that are additionally involved when a large number of sequential stimuli are delivered and the subjects are required to keep an accurate count of them. We performed three experiments to address the question. First, we delivered exactly the same successive stimuli to subjects in two sessions but asked the subjects to count the stimuli in two different ways (Experiment 1). In the large number task condition, subjects counted sets of 10–22 stimuli, and responded with the total number of stimuli. In the small number task condition, subjects experienced the same sets of 10–22 stimuli, but counted stimuli up to 4 before starting to count again at 1. The differences in activation between these two task conditions could help us to evaluate neural processes that are additionally recruited for large number counting compared to small number counting. The task paradigm was used for the combined experiments using functional magnetic resonance imaging (fMRI) and transcranial magnetic stimulation (TMS). The fMRI was utilized to localize an area (or areas) additionally recruited more for large compared to small number counting, and the TMS was applied to disrupt the function of that area to determine whether it plays an indispensable role in the exact large number counting process (Hallett 2000).

Second, we tried to improve the determination of the specificity of the area for large number counting, and performed two additional TMS experiments. The experiments were designed because it might be reasonable to attribute the area detected by the first experiments to internal verbalization or other linguistic processes. To assess whether the area was related to internal verbalization of number words, we presented number words for subjects to internally verbalize while stimulating the area detected in the first experiment with TMS (Experiment 2). Third, we presented successive stimuli, and asked subject to count them (a Counting task) or recite letters alphabetically in concert with each stimulus (a Letter task) and gave TMS pulses to determine whether the stimulation to the area significantly disrupted counting stimuli but not sequentially reciting letters (Experiment 3). In so doing, the present study demonstrated the possibility of the involvement of the ventral premotor cortex in our large number counting.

Materials and methods

Experimental procedures

Subjects

Volunteer adult healthy subjects (age 20–42 years) participated in the experiments. All subjects were neurologically normal and strongly right-handed according to the Edinburgh Inventory (Oldfield 1971). The study received approval from the Institutional Review Board. All subjects gave written informed consent according to institutional guidelines.

Experiment 1

fMRI experiments

Thirteen subjects, six males (age 20–42 years) and seven females (age 23–40 years), participated in the fMRI experiments. Three different types of modalities were presented in two sets of three sessions: visual stimuli in the form of white squares on a black screen; auditory stimuli in the form of 440 Hz beeps; and somatosensory stimuli in the form of air puffs. Each stimulus lasted 200 ms and was separated from the next by 300 ms. In each trial, stimuli appeared between 10 and 22 times. The stimuli appeared, 8 s later a white circle appeared prompting the subject to respond, and the next set of stimuli appeared 8 s after the response cue. Each fMRI session consisted of 20 trials (Fig. 1a). During the large number phase of the experiment, we asked the subject to count all the stimuli and respond with the number counted. For the small number phase, we asked the subject to count the stimuli up to 4, start again at 1, and respond with the last number counted. For example, if we presented six stimuli, the subject counted up to 4, started again at 1, and responded with

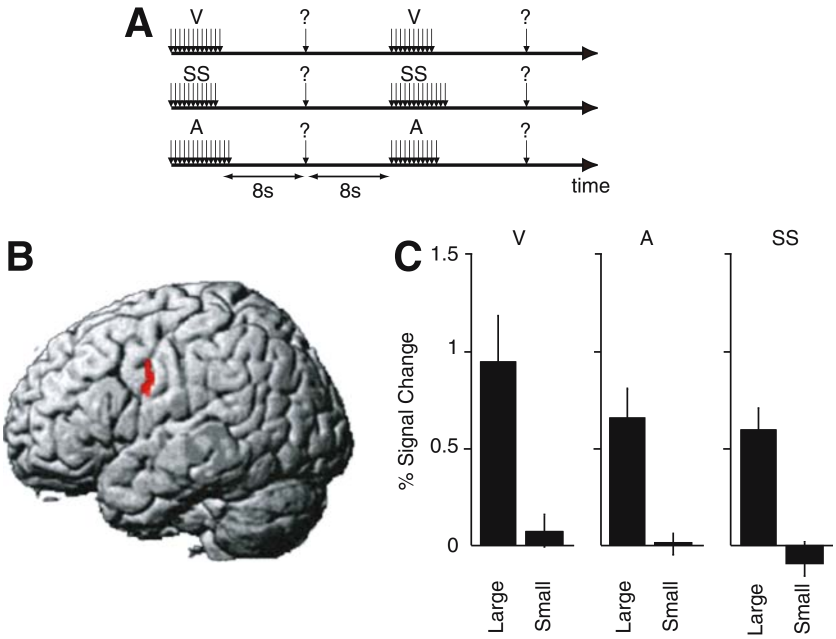

Fig. 1 Brain activations during counting ($n = 13$) in Experiment 1. **a** Timing of task paradigm. Three types of sensory stimuli were presented in separate sessions—*V* visual, *A* auditory, and *SS* somatosensory. The same sensory stimuli were presented between sessions for large and small number counting. **b** Activation maps obtained from group analyses—stronger activation in large number counting than in small number counting. *Red dots* represent pixels for *t*-scores larger than 2.56 ($P < 0.05$, family-wise error correction for multiple comparisons) in the conjunction analysis. The cluster was located in the *upper part* of the left ventral premotor cortex [$x = -48$, $y = -2$, $z = 28$ in Talairach coordinates (adapted from Talairach and Tournoux 1988)]. **c** The percentage of signal change was calculated individually within spherical volumes of interest with a diameter of 10 mm in the area, and the fMRI activations were significantly greater during large number counting than during small number counting ($P < 0.00005$, Wilcoxon signed rank test), and it was also confirmed that the significant difference was detected in each of the three sensory modality conditions ($P < 0.05$)

the correct answer of 2. The critical comparison between large and small number counting is a subtraction between two conditions with the exact same timing and rhythm. Any resulting difference in activation between these conditions thus could not be attributed to timing or rhythm, which is identical between both conditions. Responses were recorded to ensure subject's participation using a hand-response device (Button Response Unit, MRI Devices Corporation, Waukesha, WI, USA), with an assigned number (1, 2, 3...9, 0) to each button. Subjects were required to push 0 first if the number was less than 9. Of 20 trials, mean number of trials with correct answers was 18.7 (SD, 1.3) in large number counting sessions, and 19.2 (SD, 1.2) in small number counting sessions. The order of the experimental conditions was randomized among subjects.

BOLD contrast images were acquired at 3.0 T (Signa, GE Medical Systems, Milwaukee, WI, USA) using gradient-echo echo-planar imaging (TR/TE = 2,500/25 ms, FA = 90°, slice thickness/gap = 5/1 mm, FOV = 22 cm × 22 cm, matrix size = 64 × 64). We improved magnetic field homogeneity by using both linear and second order shims. This method was developed in spectroscopic imaging at 1.5 T (Spielman et al. 1998), and the effectiveness of the method for gradient-echo echo-planar imaging was evaluated at 3.0 T (Kansaku et al. 2000). We used statistical parametric mapping (SPM2, Wellcome Department of Cognitive Neurology, Institute of Neurology, University College London, London, UK) for data analyses. For each individual subject, scans were realigned to the first image and stereotactically normalized to a standard stereotactic space [Montreal Neurological Institute (MNI) brain template]. During the spatial normalization process, scans were resampled into voxels that were 2 mm × 2 mm × 2 mm in the x (right–left), y (rostral–caudal), and z (dorsal–ventral) directions, respectively. All scans were then smoothed with a Gaussian filter (6 mm full-width at half-maximum) to accommodate individual anatomical variability. A hemodynamic response function was used to characterize condition effects. We applied a conjunction analysis (Price and Friston 1997; Friston et al. 1999, 2005) to find brain regions consistently activated in all three sensory conditions. Because the conjunction analysis is based on the minimal statistic, if we apply tasks consisting of different sensory modalities but contain a common cognitive process, this analysis effectively works to remove activations related to primary and lower sensory cortices and shows candidate brain regions that may be involved in the common cognitive process (Bremmer et al. 2001; Kansaku et al. 2004, 2006). We also performed a subtraction analysis of the large number counting task paradigm minus the small number counting task paradigm to isolate regions of the brain specific to large number processing. For group analysis, we performed a multiple regression, second-level conjunction analysis ($P < 0.05$, family-wise error correction for multiple comparisons). In the group analysis we applied inclusive masking at a threshold P value of 0.05 by the results of the large number counting for each sensory modalities: visual, auditory, and somatosensory, in order to avoid confounding effects related to deactivations. It should be noted that our significant conjunction does not mean all the contrasts were individually significant (i.e., a conjunction of significance). It simply means that the contrasts were consistently high and jointly significant. This is equivalent to inferring one or more effects were present (Friston et al. 2005). Based on the thresholding issue of the conjunction analyses, to further evaluate the fMRI activations in the area detected by the analyses, we calculated the percentage of signal change individually within spherical volumes of interest with a diameter of 10 mm, and made sure that the activations were conjointly and significantly greater during large number counting than during small number counting. We also made sure that a significant difference was detected in each sensory modality condition. To report activity, we converted the MNI coordinates into Talairach coordinates (1988) using a linear transformation matrix and listed the Talairach coordinates.

TMS experiments

Eight subjects, five males (age 20–35 years) and three females (age 25–34 years), participated in the TMS experiments. TMS experiments were conducted with the same numbers of stimuli that were used in the fMRI experiments. In each trial, sequential sets of stimuli appeared 10 and 22 times, followed by a response cue. Stimuli for large number and small number counting conditions were identical; only the task differed between conditions. During the large number counting condition, we asked subjects to count all stimuli in a set and respond with the total number following the response cue. For the small number counting condition, subjects counted stimuli in sets of up to 4. For example, if ten stimuli were presented, the subject would count a set of four stimuli, then a set of four stimuli, then a set of two and respond with "two." We performed small and large number counting sessions separately; the order of the experimental sessions was randomized between subjects (Fig. 2a).

Transcranial magnetic stimulation was performed using a Magstim Rapid stimulator (the peak magnetic field strength: approximately 2.2 T; Magstim, Wales, UK) through a figure-of-eight-shaped coil (diameter 70 mm, model 9925-00). The task paradigm involved the presentation of one of four trump card shapes—spade, heart, diamond, or club—for brief presentation of 10 ms (Corthout et al. 1999), followed by a TMS pulse 10 ms later, and a second TMS pulse 50 ms after the first. One of these shapes was presented in each trial. As in the fMRI experiment tasks, the subjects counted simple sequences of sensory stimuli. To alert the subject, we presented an example of the shape to be counted 2 s before the sequential presentation of the visual shapes. TMS pulses were delivered randomly with a minimum inter-pulse interval of 4 s. The first TMS pulses were not given after any of the first four visual stimuli. A question mark followed 500 ms after the last shape presentation, prompting the subject to identify the number of shapes presented (Fig. 2a). Responses were recorded with a number key in a

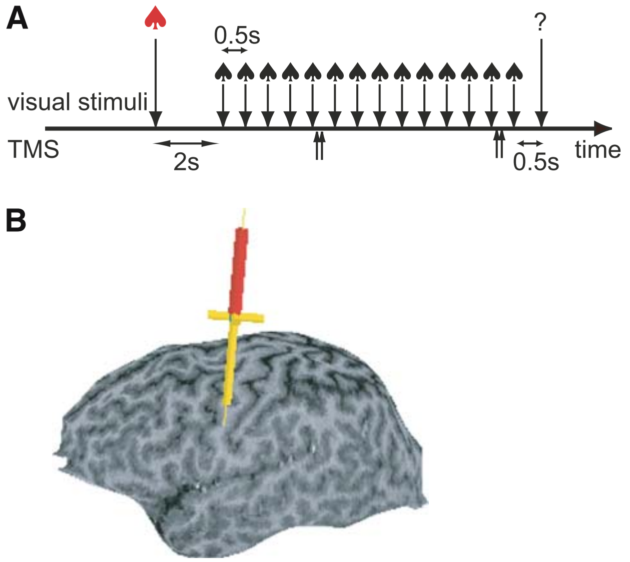

Fig. 2 Experimental procedures for the TMS study of counting for Experiment 2. **a** Timing of task paradigms. Subjects ($n = 8$) were required to count the number of an indicated *tramp shape*, which was repetitively presented on a display. Large number counting sessions (10–22) and small number counting sessions (up to 4) were done separately. *Double arrows* indicate timing of the paired TMS pulses. **b** Peeled brain MRI 3D images from a subject showing site of stimulation. Location of the scalp of the stimulation was determined using Brainsight (Brainsight Frameless ver. 1.5b7, Rogue Research), after co-registering the subject's head with the subject's MRI 3D images

keyboard (RT7D00, Dell Computer, Round Rock, TX, USA). We requested that the subjects push 0 first if the number was less than 9, and push 0–0 if they completely lost the number. Experiments were controlled with Presentation software (Neurobehavioral Systems Inc., San Francisco, CA, USA). A Polaris optical tracking system (Northern Digital, Waterloo, Ontario, Canada) was used to measure the position of the subject's head, and Brainsight software (Brainsight Frameless ver 1.5b7, Rogue Research, Montreal, Quebec, Canada) was used to co-register the subject's head with the subject's MRI 3D images. We identified the stimulus strength from the motor threshold (MT) of right primary motor cortex stimulation (160% of MT). MTs were recorded in the FDI muscle and ranged between 54 and 70% of the stimulator output.

We stimulated over the upper part of the left ventral premotor cortex, its homologue area in the right ventral premotor cortex, and the supplementary motor area proper. Stimulus sites were determined using the subject's individual MRI 3D images (Fig. 2b). In the sham condition, we put the coil perpendicular to the scalp, so the subjects heard the stimulation sounds, but were not stimulated. The order of the TMS task conditions was counterbalanced across subjects. Accuracy of counting during the stimulus and sham conditions was evaluated using analysis of variances (ANOVAs).

Experiment 2

TMS experiments

Eight subjects, five males (age 20–35 years) and three females (age 25–34 years), participated. This was a control experiment to ascertain that the disruption of counting was unrelated to linguistic processing, such as internal verbalization or verbal memory. Number words were presented instead of the visual shapes, followed by TMS pulses 10 ms later and a second TMS pulse 50 ms after the first. A question mark followed 2 s after the TMS pulse, prompting the subject to identify the number, by hitting number keys on a keyboard (Fig. 5a).

Experiment 3

TMS experiments

Nine subjects, five males (age 26–39 years) and four females (age 24–32 years), participated. Every 500 ms, visual stimuli in the form of black squares were presented for brief presentation of 10 ms (Corthout et al. 1999) on a white screen (Fig. 6a). The stimuli were presented 10–22 times in one session. In one task we presented a number (e.g., 3) to start the counting, 2 s before the sequential presentation of the visual shapes (a Counting task). The subjects were required to count when they saw the visual stimuli (e.g., 4–5–6–..., if "3" was presented). A question mark followed 500 ms after the last shape presentation, prompting the subject to identify the last number that the subject counted. Responses were recorded with a number key in a keyboard (SKB-109LU, Sanwa Supply, Japan). We requested that the subjects push 0 first if the number was less than 9, and push 0–0 if they completely lost the number. Again the experiments were controlled with Presentation software (Neurobehavioral Systems Inc.).

In the other experimental task, the Letter task, we similarly presented visual stimuli every 500 ms in the form of black squares for brief presentation of 10 ms (Corthout et al. 1999) on a white screen (Fig. 6a). However, for this experiment we presented a letter (e.g., C) 2 s before the sequential presentation of the visual shapes. The subjects were required to internally say letters in alphabetical order (e.g., D–E–F–..., if "C" was presented). A question mark followed 500 ms after the last shape presentation, prompting the subject to identify the last letter that the subject forwarded. In both tasks we stimulated over the upper part of the left ventral premotor cortex, and its homologue area in the right ventral premotor cortex. In the sham condition, we put the coil perpendicular to the scalp, so the subjects heard the stimulation sounds, but were not stimulated. The order of the TMS task conditions was counterbalanced across subjects. Accuracy of the subjects' performance during the stimulus and sham conditions was evaluated.

Transcranial magnetic stimulation pulses were delivered randomly with a minimum inter-pulse interval of 4 s, by using a Magstim Rapid stimulator (Magstim) through an air-cooled double 70-mm coil system (model 1600-23-04; Magstim, Wales, UK). A Polaris optical tracking system (Northern Digital) was used to measure the position of the subject's head, and Brainsight software (Brainsight Frameless ver 1.5, Rogue Research) was used to co-register the subject's head with the subject's MRI 3D images. The stimulus strength was set at 70% of maximum machine output. The TMS coil was held by a Point Setter System (Mitaka Kohki Co., Tokyo, Japan) during the experiments.

Results

Experiment 1

First, we utilized fMRI, hypothesizing that regions uniquely involved in large number counting are essentially inactive in processing smaller numbers. In order to discover the candidates, we specifically focused on the ability that humans can count successive stimuli delivered by different types of sensory modalities. Our task paradigm used sequential stimuli delivered in different sensory modalities to identify regions involved in large number processing that are activated regardless of sensory modalities. In each trial, stimuli appeared between 10 and 22 times. During the large number phase of the experiment, we asked the subject to count all the stimuli and respond with the number counted. For the small number phase, we asked the subject to count the stimuli up to 4, then start again at 1, and respond with the last number counted. For example, if we presented six stimuli, the subject counted up to 4, started again at 1, and responded with the correct answer of 2 (Fig. 1a). Identical stimuli were used for the large and small number counting conditions; the only difference between the conditions was the task performed.

Brain regions consistently activated regardless of input sensory modalities were evaluated, and in the small number counting condition, such brain regions were mainly observed over the bilateral premotor cortices, pre-supplementary motor areas, posterior superior/middle temporal cortices, and the results agreed with our former studies (Kansaku et al. 2006). The activated area in the lateral premotor cortices could be between the dorsal and ventral premotor cortex, because there are multimodal neurons in both dorsal and ventral premotor cortex in monkeys.

Spatial distribution of activation during large number counting was similar to activation during small number counting, but subtraction analysis showed an additional activation during large number counting in the left premotor cortex, specifically an upper part of the ventral premotor cortex (Table 1; Fig. 1b). The additional activation during large number counting was shown as an extended activity to ventral side from the activation observed during small number counting. The left ventral premotor activation was the only cluster detected in the analysis ($P = 0.05$, family-wise error correction for multiple comparisons), with a Talairach coordinate (Talairach and Tournoux 1988) of ($x = -48$, $y = -2$, $z = 28$). The percentage of signal change was calculated individually within spherical volumes of interest in the area, and the signal increase in the area during large number counting was much higher than that of small number counting, and the difference was confirmed to be significant ($P < 0.00005$, Wilcoxon signed rank test). It was also confirmed that a significant difference was detected in each of the three sensory modality conditions ($P < 0.05$, Wilcoxon signed rank test; Fig. 1c).

Results of the fMRI experiment suggest that the left ventral premotor cortex may have a role in counting large numbers; however, the fMRI does not give firm evidence that the area is necessary for large number counting. In order to determine the functional relevance of the area (Hallett 2000), we used TMS. Just as in the fMRI experiment, the TMS task paradigm involved counting sequential stimuli with 500 ms interstimulus interval. Visual stimuli in one of four trump card shapes—spade, heart, diamond, or club were used in the trials. In each trial, stimuli appeared between 10 and 22 times. During the large number phase of the experiment, we asked the subject to count all the stim-

Table 1 Group analysis of areas of consistent activation in Experiment 1

Brain region (Brodmann's area)	*x*	*y*	*z*	*t*-Statistic	*P*-value
Large number counting					
Medial frontal gyrus (BA 6)	−2	1	63	6.44	0.000
Left premotor cortex (BA 6)	−50	−2	44	5.48	0.000
Right premotor cortex (BA 6)	53	−2	42	5.28	0.000
Left inferior frontal gyrus (BA 6/44)	−61	3	18	3.85	0.000
Left posterior superior/middle temporal cortex (BA 22)	−50	−43	4	3.12	0.000
Right posterior superior/middle temporal cortex (BA 22)	55	−39	0	2.93	0.000
Left posterior cerebellum	−32	−69	−23	2.63	0.000
Small number counting					
Left premotor cortex (BA 6)	−50	−2	46	5.73	0.000
Medial frontal gyrus (BA 6)	0	3	59	4.88	0.000
Right premotor cortex (BA 6)	53	0	44	4.16	0.000
Right posterior superior/middle temporal cortex (BA 22)	55	−39	6	3.71	0.000
Left inferior frontal gyrus (BA 44/6)	−61	7	14	3.70	0.000
Left posterior superior/middle temporal cortex (BA 22)	−48	−46	10	3.00	0.000
Left posterior cerebellum	−34	−69	−23	2.77	0.000
Large minus small					
Left ventral premotor cortex (BA 6)	−48	−2	28	2.68	0.000

uli and respond with the number counted. For the small number phase, we asked the subject to count the stimuli up to 4, then start again at 1, and respond with the last number counted. Identical stimuli were used for the large and small number conditions; the only difference between the conditions was the task performed. In both large and small number counting sessions, three cortical regions (left and right ventral premotor cortices, and supplementary motor area-proper) were stimulated in separate sessions and a control session was added. Figure 3a and b shows the effect of the stimulation on accuracy. In the large number counting session, the ANOVA showed a significant effect; a multiple comparison test (Tukey's honestly significant difference criterion) found significant difference in stimulation of the left ventral premotor cortex (Fig. 3a; $df = 3$, $F = 9.0$, $P = 0.0002$). Such an effect was not observed with left ventral premotor cortex stimulation during small number counting (Fig. 3b; $df = 3$, $F = 1.3$, $P = 0.28$). Nor was it found in the right ventral premotor cortex and supplementary motor area-proper during counting both large and small numbers.

Transcranial magnetic stimulation to the left ventral premotor cortex showed a striking effect when subjects attempted to count large numbers of stimuli. We further analyzed the subjects' performance during left ventral premotor cortex stimulation, and demonstrated the frequency of responses in numerical positions relative to the position of the correct number (Fig. 3c). Numerical positions were widely and approximately normally distributed in the large number counting condition (Fig. 3c, left), whereas they were not in the small number counting condition (Fig. 3c, right). Subjects were told to say that they were "not sure" if they had no idea regarding the number; in the large number counting condition such responses were given in six trials. In some of these trials, the subjects told us that "I forgot where I was." We evaluated the accuracy for the large number counting condition in two bins, 5–13 and 14–22 (Fig. 3d). Mean accuracy was less in the higher bin, but this difference was not significant ($P = 0.15$, Wilcoxon signed rank test).

The TMS effect on the left ventral premotor cortex might depend on the number of TMS pulse-pairs in the task trial. Therefore we divided the data of 160 trials according to the number of TMS pulse-pairs. TMS pulse-pairs were delivered one time ($n = 71$), two times ($n = 82$), and three times ($n = 7$) during the trials, and the accuracy was 79, 56, and 43%, respectively. The trials that gave the TMS pulse-pairs three times during the trial were rare ($n = 7$), thus we further analyzed only the frequency of responses in trials in which TMS pulse-pairs were delivered one or two times. Frequency of responses in numerical positions relative to the position of a correct number was shown in Fig. 4. When the TMS pulse-pairs were given one time throughout the trial (one-time TMS, $n = 71$; upper panel, Fig. 4a), the most frequent error was observed at -1 of the numerical position, and second most frequent error was observed at 1 of the numerical position. When the TMS pulse-pairs was given two times throughout the trial (two-times TMS, $n = 82$; lower panel, Fig. 4b), the numerical positions were more widely distributed than the one-time TMS trials, and the most frequent error was observed at -2 of the numerical position, and second most frequent error was observed at 2 of the numerical position.

Experiment 2

It is possible that the TMS effect is related to linguistic processing for a number word, such as mental verbalization and verbal memory. In order to exclude this possibility, we performed an additional control experiment. We presented number words on a visual display, followed by paired TMS pulses, and asked subjects to mentally verbalize and memorize the number. This stimulation had no significant effects on the subjects' accuracy, including stimulation to the left ventral premotor cortex (Fig. 5b). We also considered the possibility that differences in activation between large and small number counting conditions could relate to the increased phonological complexity of large number words. However, stimulation to the left ventral premotor cortex did not disrupt mental verbalization and verbal memory, irrespective of the phonological complexity of the number words that were employed. It is conceivable that number words are important in human precise counting as was postulated recently (Gordon 2004; Pica et al. 2004), but in our results, effect of TMS to the left upper part of the ventral premotor cortex was not obviously related to mental verbalization and verbal memory of the large number words.

Experiment 3

The possibility still remains that the TMS effect in Experiment 1 is related more generally to working memory, and is not necessarily specific to counting. To test the specificity of this effect to counting, we conducted Experiment 3, in which we compared a sequential Counting task to a sequential Letter task.

In the Counting task, the ANOVA showed that TMS stimulation had a significant effect; a multiple comparison test (Tukey's honestly significant differ-

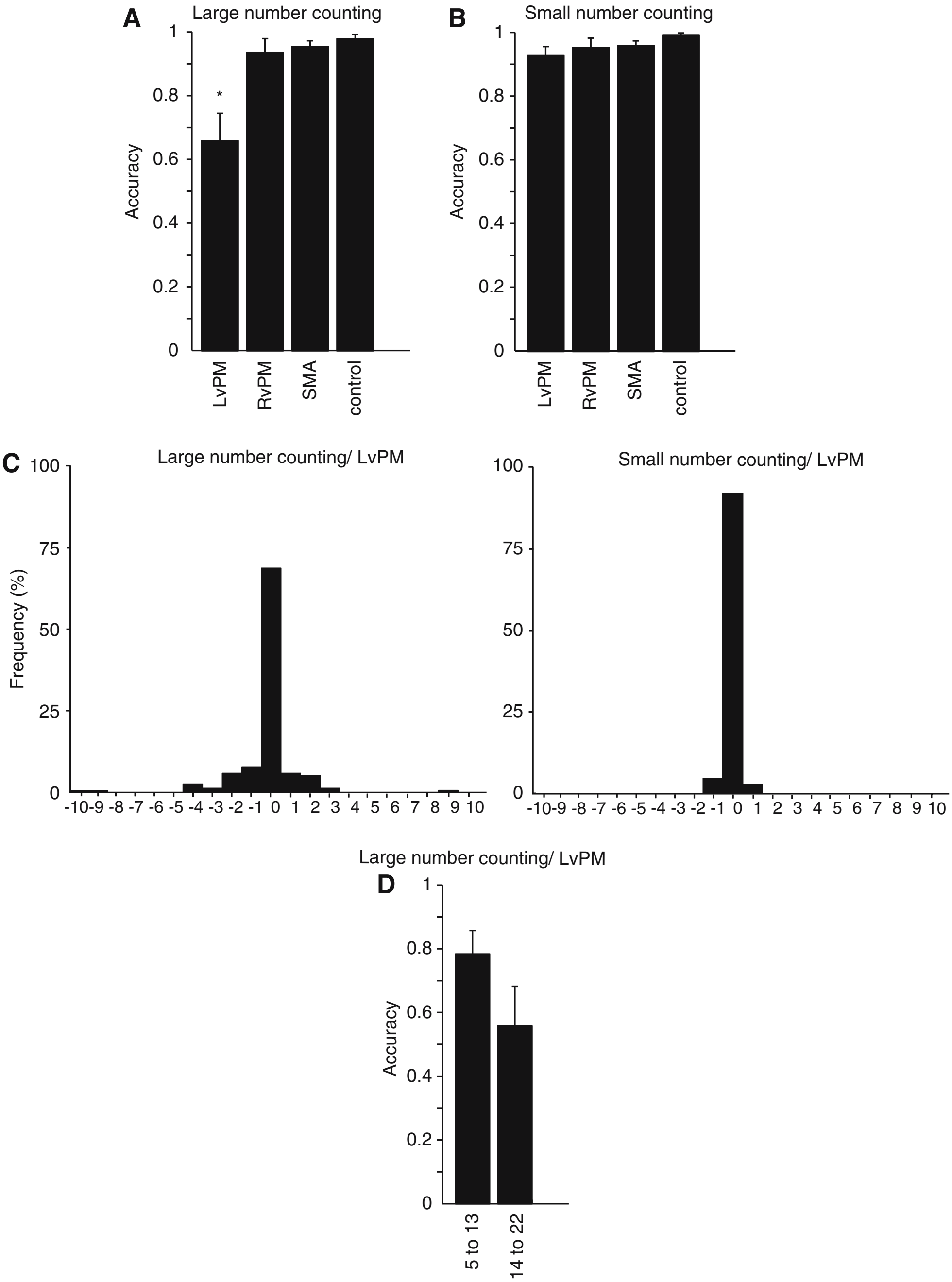

Fig. 3 Disruption of the counting process induced by TMS in Experiment 1. **a** Large number counting was disrupted by TMS stimulation to the left ventral premotor cortex. **b** TMS had no effect on subjects' accuracy during small number counting. **c** Frequency of responses in numerical positions relative to the position of a correct number. Numerical positions were widely and approximately normally distributed in the large number counting condition (*left panel*), whereas they were not in the small number counting condition (*right panel*). **d** Accuracy of counting is shown in two bins, 5–13 and 14–22. Mean accuracy was lower in the larger bin, but the difference was not significant ($P = 0.15$, Wilcoxon signed rank test)

ence criterion) found significant difference in accuracy during stimulation of the left ventral premotor cortex (Fig. 6b; $df = 2$, $F = 3.7$, $P = 0.048$). Such an effect on accuracy was not observed with left ventral premotor cortex stimulation in the Letter task (Fig. 6b; $df = 2$, $F = 1.0$, $P = 0.38$). The effect was not found in the right ventral premotor cortex during either Counting or Letter tasks. There was no difference in accuracy between control conditions of both the Counting task and the Letter task, thus there were no differences in difficulties between the two task conditions ($P = 0.84$).

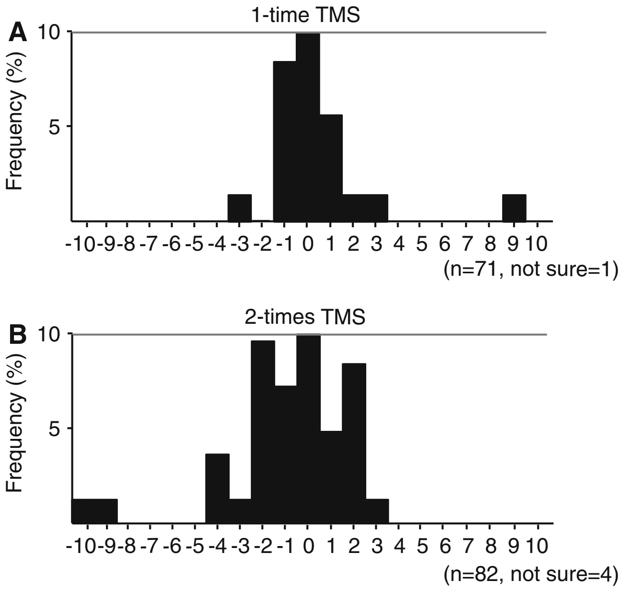

Fig. 4 Effect of the number of TMS pulse-pairs in the left ventral premotor cortex stimulation condition during large number counting (Experiment 1). **a** Frequency of responses in numerical positions relative to the position of a correct number according to the number of TMS pulse-pairs given during each trial. Note that the most common frequencies were observed at −1 and +1 when the TMS pulse-pairs were given one time throughout the trial (one-time TMS, $n = 71$; *upper panel*). **b** When the TMS pulse-pairs were given two times throughout the trial, the most common frequencies were observed at −2 and +2 (two-times TMS, $n = 82$; *lower panel*)

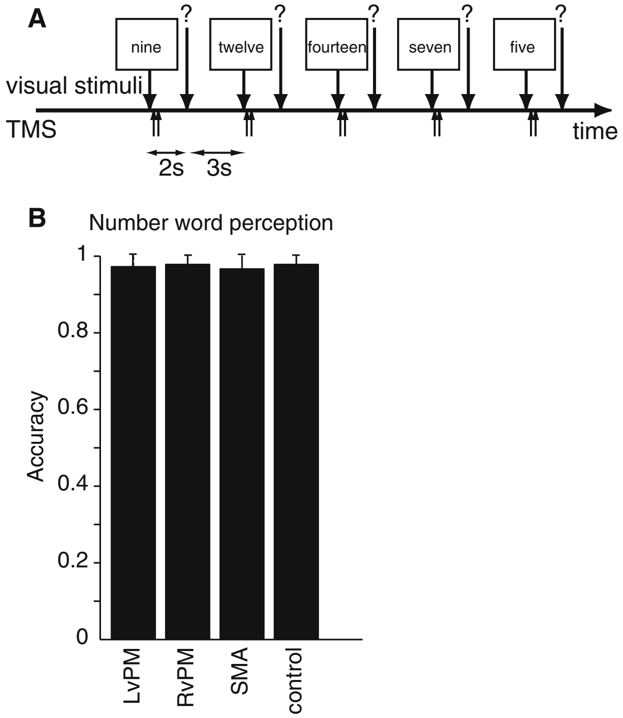

Fig. 5 TMS study of number word perception (Experiment 2). **a** Timing of task paradigms. *Number words* were presented, followed by paired TMS pulses—a TMS pulse 10 ms later and a paired TMS pulse 50 ms after the first. **b** TMS had no significant effect on subjects' accuracy, including stimulation to the left ventral premotor cortex

Discussion

In this study we showed that stimulation to the left ventral premotor cortex disrupted exact counting of large number of successive stimuli. At least for our task conditions, it appeared that the left ventral premotor cortex is included in the entire network as one of the critical areas in the operation of exact counting of large numbers of successive stimuli. Previous studies of numerical processing have focused primarily on parietal–prefrontal networks of activation. Recent monkey studies explored the neuronal representation of quantity in the parietal and prefrontal cortices (Nieder et al. 2002; Sawamura et al. 2002), and these areas are known to be active in human imaging studies using complex arithmetic tasks (Chochon et al. 1999; Simon et al. 2002; Dehaene et al. 2004). However, the processes underlying counting sequential stimuli and the significance of the premotor cortices for numerical competence have not yet been widely discussed. Our former studies investigated neural correlates of counting small numbers of successive stimuli up to 4, and demonstrated the most significant activation in the lateral premotor cortex (Kansaku et al. 2006). The lateral premotor cortex activations for small number counting were bilaterally observed, but in the present study the most significant difference between large and small number counting was detected to be left lateralized in the ventral premotor cortex, which is more inferiorly located than the areas for small number counting. Although it was not investigating areas for counting but for calculation and number comparison, there is a study that demonstrated the effect of number size on neural activation: bigger activation in large numbers, in the left inferior frontal gyrus, left precentral sulcus, and left intraparietal sulcus (Stanescu-Cosson et al. 2000). The area of activation in the left precentral sulcus may have overlapped with the activation in the left ventral premotor cortex found in our study. Large number processing and representation may thus involve the ventral premotor cortex in the human brain.

Transcranial magnetic stimulation to the left ventral premotor cortex showed a striking effect when subjects attempted to count large numbers of stimuli, and the errors of numerical position were widely and approximately normally distributed. Gallistel and Gelman have suggested a particular neuronal mechanism for precise large number counting that maps from magnitudes to

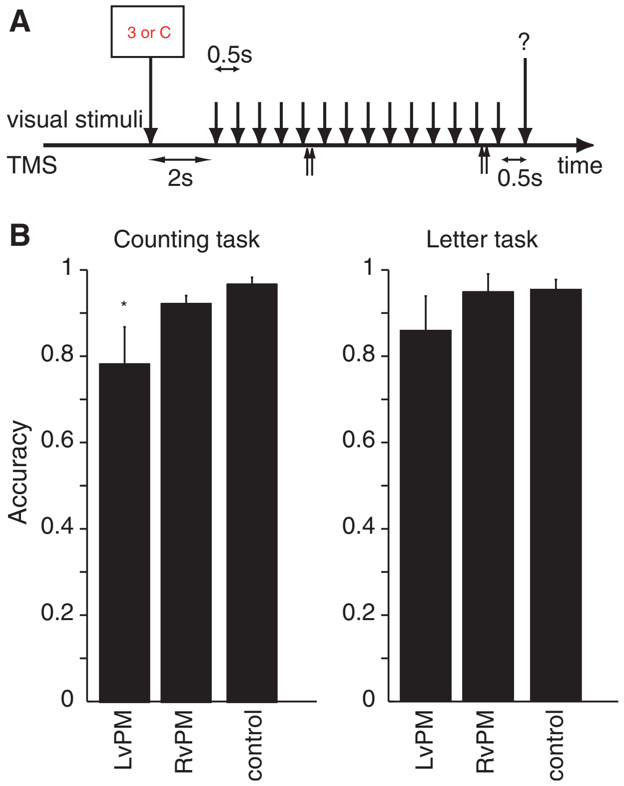

Fig. 6 TMS study using Counting and Letter tasks (Experiment 3). **a** Timing of task paradigms. *Number words* (Counting task) or *letters* (Letter task) were presented, followed by sequential presentation of the *square shape*. Again *double arrows* indicate timing of the paired TMS pulses. **b** Note that the significant effect was only observed in the Counting task

precise numbers, in order to overcome large memory noise caused by large memory magnitude (Gallistel and Gelman 2000). If there is such a mechanism, which can be a bridge between approximate and exact processes in numerical representations, it is also possible that the TMS disrupted the process for mapping from approximate magnitudes to exact numbers.

The results in this study do not show that the role of the left ventral premotor cortex is only involved in large number counting: the function of this area might be shared with other cognitive/motor processing. The results from our TMS experiment of Experiment 1 indicate that stimulation of this area disrupted the ability of participants to count large numbers of successive stimuli exactly. Nieder recently suggested that “true” counting in humans is operated with the support of language (Nieder 2005). It is also proposed that humans recruit networks involved in word-association process for exact arithmetic (Dehaene et al. 1999). A recent study showed that adult humans who do not have fully elaborated number words appear unable to perform precise large number counting (Gordon 2004). Existence of a language-based counting system was also recently proposed (Pica et al. 2004). There may exist a language related process for exact large number enumeration. Therefore, although in a control experiment we showed that the effect of TMS to the left upper part of the ventral premotor cortex was not obviously related to mental verbalization and verbal memory of the large number words, it may be still possible that TMS stimulation to the ventral premotor cortex disrupted a sort of linguistic process, which enables humans’ exact large number counting.

If large number counting is a recursive process that is expressed as $X = X + 1$, the process for counting might involve a kind of syntactic rules in language. Some of the errors during TMS stimuli could be explained by disrupting the process of $X = X + 1$, especially in trials when numerical positions were at -1 in the one-time TMS, and at -2 in the two-times TMS. However, more interestingly, each TMS caused not only under- but also over-estimation. Survival of the approximate process might be a reason that the errors were observed bi-directionally.

Large number counting may place unique demands on other non-numerical parameters, which might account for the essential involvement of this area. Large number counting may place a greater demand on working memory, and the effect during large number counting might be more obvious because more memory was required. It has been postulated that memory noise size becomes larger when the processed number is larger (Gallistel and Gelman 2000). Subjects were, however, able to maintain approximate magnitude representations even when TMS disrupted the premotor cortex in most of the trials, suggesting that the role of the premotor cortex in counting is more than simply a function of working memory load. The approximate mathematical thinking may require working memory, as suggested by Dehaene et al. (1999). However, in 6 trials out of 160 trials, the subjects completely lost the number and a subject told us that “I forgot where I was” at that time. This seems to imply that a sort of memory processes was disturbed by the TMS. Therefore there still remains some possibility that the TMS disrupted not only one process but also the other processes such as working memory, and that the ventral premotor cortex might be involved in both processes of incrementing the number by 1 and temporarily memorizing the current number.

Timing/rhythm might also be suggested as a non-numerical parameter. There is a recent proposal that number and time are similarly processed in the brain because magnitude representation is similar (Walsh 2003). However, because timing and rhythm was not different between large and small number conditions, timing and rhythm of the sensory stimuli could not account for the differences in activation between these conditions. Also, because our TMS experiment (Experiment 3) showed that the left ventral premotor cortex is more involved in the performance of counting than saying a letter in alphabetical order when they felt each of the sensory stimuli, it is not plausible that the differential involvement of the left ventral premotor cortex is related to the production of rhythms from the successive stimuli, e.g., for the demand of making the chunking structure of 1–2–3–4 in the Experiment 1.

Traditionally we have thought that the preparation and organization of movements and actions are the function of the premotor cortex (Wise 1985). For example, the premotor cortex's important role in complex sequential finger movements was reported (Catalan et al. 1998). Recent neuroimaging studies in humans challenged this traditional view, and have found non-motor cognitive roles in the premotor cortex (Schubotz and von Cramon 2003). They proposed that the premotor cortex has a role in predicting sensory sequences and planning sequential actions. In this view, the sequential prediction and planning functions of the premotor cortex could be co-opted and recruited for counting. The premotor cortices' cognitive roles could have evolved as an extension of essential sensorimotor abilities of the premotor cortex, such as performing sequential movements. Using dual tasks of self-paced sequential finger movement and letter counting, one study observed overlapped activations in the lateral premotor cortices (Wu et al. 2004). Further investigations are necessary to clarify the involvement of the premotor cortex in motor and non-motor information processing.

Several studies have proposed that parietal and prefrontal areas play essential roles in numerical processing; our results do not indicate that these areas are differently recruited for large and small number counting, but in no way rule out their essential role in both small and large number counting. By focusing particularly on the differences between large and small number counting, our experiments suggest that the human left ventral premotor cortex plays an essential role for counting large numbers of stimuli. To further investigate the neuronal mechanisms underlying large number counting, a possible approach could be to prepare pseudo-counting task paradigms that lack one of the necessary principles of counting described by Gallistel and Gelman. Such further investigations of the roles of this area could provide clues to understanding how humans evolved their numerical competence.

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