

# Neurobiology of perceptual and motor timing in children with spina bifida in relation to cerebellar volume

Maureen Dennis,<sup>1,5,6</sup> Kim Edelstein,<sup>1</sup> Ross Hetherington,<sup>4,6</sup> Kim Copeland,<sup>10</sup> Jon Frederick,<sup>7</sup> Susan E. Blaser,<sup>2</sup> Larry A. Kramer,<sup>9</sup> James M. Drake,<sup>3,5</sup> Michael Brandt<sup>7</sup> and Jack M. Fletcher<sup>8</sup>

<sup>1</sup>Brain and Behaviour Program, Departments of <sup>2</sup>Radiology and <sup>3</sup>Surgery, <sup>4</sup>Community Health and Knowledge Transfer, Hospital for Sick Children, Departments of <sup>5</sup>Surgery and <sup>6</sup>Psychology, University of Toronto, Toronto, Ontario, Canada, <sup>7</sup>Center for Computational Biomedicine, Departments of <sup>8</sup>Pediatrics and <sup>9</sup>Radiology, University of Texas Health Science Center at Houston and <sup>10</sup>Department of Psychology, University of Houston, Houston, TX, USA

Correspondence to: Maureen Dennis, PhD, Brain and Behaviour Program/Psychology, Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G 1X8, Canada

E-mail: maureen.dennis@sickkids.ca

## Summary

The cerebellum is important for perceptual and motor timing in the mature brain, but the timing function of the cerebellum in the immature brain is less well understood. We investigated timing in children with spina bifida meningocele (SB), a neural tube defect that involves cerebellar dysgenesis, and in age-matched controls. Specifically, we studied perceptual timing (judgements of 400 ms duration) and motor timing (isochronous motor tapping); measured cerebellar volumes; and related perceptual and motor timing to each other and to cerebellar volume measurements. Children with SB had impairments in the perception of duration (around 400 ms) but not frequency (around 3000 Hz), showing that their perceptual timing deficit was not a generalized auditory impairment. Children

with SB had motor timing deficits on unpaced but not paced isochronous tapping, and their unpaced timing performance was associated with clock variance rather than with motor implementation. Perceptual and motor timing were correlated, suggesting that children with SB have impairments in a central timing mechanism. Children with SB, especially those with upper spinal cord lesions, had significant cerebellar volume reductions in grey and white matter, as well as different regional patterns of grey matter, white matter and CSF. Duration perception was correlated with cerebellar volumes, and the number of valid tapping trials was correlated with cerebellar volumes in the SB group, which data demonstrate structure–function relations between timing and cerebellar volumes.

**Keywords:** spina bifida; hydrocephalus; cerebellum; timing; MRI volumetrics

**Abbreviations:** ADHD = attention deficit hyperactivity disorder; SB = spina bifida meningocele

Received September 18, 2003. Revised January 15, 2004. Accepted January 16, 2004. Advance Access publication April 6, 2004

## Introduction

Timing mechanisms are involved in motor control and movement co-ordination (Ivry, 1996; Mangels *et al.*, 1998; Mauk *et al.*, 2000; Ivry and Richardson, 2002). The role of the cerebellum in timing has been well established, from observations that cerebellar lesions impair the rhythmic composition of movement (Holmes, 1922) to current views

about the role of the cerebellum as an adaptive, self-tuning controller important for the processing of time-varying signals (Massaquoi and Topka, 2002).

The cerebellum is activated during time discrimination tasks (Rao *et al.*, 2001). Increased functional cerebellar activation is observed during paced finger tapping, a short-

duration time production task (Rao *et al.*, 1997; Penhune *et al.*, 1998; Jancke *et al.*, 2000). Virtual lesions of the cerebellum by means of repetitive transcranial magnetic stimulation produce increased variability in paced finger tapping (Théoret *et al.*, 2001).

Adults with cerebellar lesions provide evidence for a cerebellar role in both perceptual and motor timing. They show inappropriate timing in rapid limb movements, increased variability during repetitive finger tapping, and impaired performance on perceptual tasks requiring precise timing of short (half-second) intervals (Ivry and Keele, 1989; Nichelli *et al.*, 1996; Ivry *et al.*, 1988; Mangels *et al.*, 1998).

The timing function of the cerebellum in the immature brain is less well understood. Developmental disorders of the cerebellum produce deficits in short-duration timing. Children with ataxia-telangiectasia, an autosomal recessive disorder with diffuse degeneration of the cerebellar cortex, are impaired in judging duration, although not pitch (Mostofsky *et al.*, 2000). Adult survivors of childhood cerebellar tumours show impairments in perception of short duration (around 400 ms), although they can make judgments about frequency and can estimate long time intervals (Hetherington *et al.*, 2000).

How congenital malformations of the cerebellum affect perceptual and motor timing has not been investigated, and spina bifida meningocele (SB) is a condition in which this issue may be explored. SB involves dysraphism of the spinal cord, with a loss of sensory and motor function below the level of the spinal lesion, and profound disturbances of brain development that include abnormal formation and maturation of the cerebellum, midbrain, corpus callosum, and posterior cortex and white matter (Fletcher *et al.*, 1992, 2000). Most children with SB develop hydrocephalus, which involves enlarged cerebral ventricles and produces a range of primary and secondary effects on the brain (del Bigio, 1993; Fletcher *et al.*, 2000).

Cerebellar dysmorphology and hypoplasia are prominent parts of the neuropathology of SB (Barkovich, 1995). The Arnold-Chiari II malformation, the defining pathology in SB, involves a small posterior fossa in which cerebellar development is restricted, the cerebellar hemispheres are reduced, the vermis is pushed upwards, and the cerebellar tonsils and flocculonuclear lobe are herniated downwards through the exits of the fourth ventricle.

The general objective of the present study was to investigate timing in children with SB in relation to the extent of cerebellar volume, which is a measure of developmental cerebellar dysmorphology. Specifically, we aimed to study perception of short durations and production of isochronous motor tapping in children and adolescents with SB and in age-matched controls; to measure cerebellar volumes; and to relate perceptual and motor timing to each other and to cerebellar volume measurements.

## Methods

### Participants

Participants were 140 children and adolescents between 8 and 19 years of age. One group ( $n = 103$ ) had been diagnosed with SB at birth and had been treated with a shunt shortly thereafter. Twenty-eight of those children had no shunt revision, 34 had one revision, 28 had two to four revisions, 12 had five to nine revisions, and one child had 14 shunt revisions. The other group comprised typically developing, age-matched controls ( $n = 37$ ). All participants had estimated intelligence quotient (IQ) scores within two standard deviations of the population mean of 100; that is, they had one or more IQ scores of  $\geq 70$  on the Verbal Reasoning and/or Abstract/Visual Reasoning subtests of the Stanford Binet Test of Intelligence—Revised (Thorndike *et al.*, 1986). In our sample, children with SB had lower average IQs than controls [ $t(1,138) = 8.59$ ,  $P < 0.001$ ; group mean  $\pm$  SEM, control,  $108.4 \pm 1.64$ ; SB,  $88.3 \pm 1.27$ ], although each group IQ was within the normal range (i.e. within  $\pm 1$  SD of the population mean). The sample included 103 Caucasian, 22 Hispanic, six Asian, five African American and four other children. Individuals were excluded from participation if they had neurological disorders unrelated to SB, a severe psychiatric disorder that precluded adequate cooperation (autism, psychosis, oppositional-defiant disorder), uncontrolled seizure disorder, uncorrected sensory disorder, or inability to control the upper limbs. The exclusions were ascertained by Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition questionnaires completed by the parents (SNAP-IV; Swanson, 1992), including a Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition checklist for autism and pervasive developmental disorders, a medical history chart review by a research nurse, and observations of the child's behaviour when evaluated. Participants in each group were recruited from clinics around two sites, The Hospital for Sick Children in Toronto ( $n = 79$ ) and the University of Texas—Houston Medical School in Houston ( $n = 61$ ). Participants and their families gave informed assent/consent in accordance with the guidelines of the research ethics boards at the two sites.

Individuals with SB have lesions at various levels of the spinal cord, which provides a source of principled, within-group variability. The level of spinal lesion is related to a mutation in methylenetetrahydrofolate reductase, the enzyme that regulates folate-dependent remethylation of homocysteine (van der Put *et al.*, 2001). The incidence of the methylenetetrahydrofolate reductase mutation is higher in mothers of children with upper spinal lesions than in typically developing controls and in mothers of children with lower spinal lesions (Volcik *et al.*, 2001). To explore one source of biological variability, participants with SB were divided into upper spinal lesion (T12 and higher;  $n = 25$ ) and lower spinal lesion (L1 and lower;  $n = 78$ ) groups, according to current taxonomies (Park *et al.*, 1992).

### Materials

Auditory stimuli were administered using an IBM-compatible personal computer, and Sony™ Dynamic Stereo Headphones MDR-V100. Tones were generated with a Creative Technology Ltd CT4170 sound card. Stimuli were presented at a level of 70 dBA at the headphone cup. For each trial in the frequency and duration tasks, participants responded by pressing the left or right mouse button. For each trial in the tapping task, they responded by tapping a

3 cm long plastic lever covering a microswitch connected to the computer. Tasks were coded and scored in Delphi.

### **Cerebellar tasks**

#### *Perceptual timing: duration and frequency discrimination*

The duration and frequency perception tasks were presented as similar two-alternative, forced-choice trials. Each trial in the duration perception task consisted of two intervals defined by 50 ms, 1000 Hz boundary tones at the beginning and end of each interval, and separated by an empty interval of 1000 ms. The target duration was 400 ms, and the foil duration was always longer. Order of target and foil durations was randomly determined at each trial. On each trial, participants were required to press the left mouse button if they judged the first trial to be longer and the right button if they judged the second trial to be longer. Working memory demands for the task were minimized with on-screen cues in the form of coloured, numbered boxes mapping the trials to the left–right mouse buttons, and flashes to register participant responses. Feedback about performance was not given. The intertrial interval was 1500 ms. An up–down transformed-response adaptive procedure (Wetherill and Levitt, 1965) was used to track 80% response accuracy. The foil duration was adjusted up or down in 8 ms increments depending on the accuracy of the previous response. The procedure stopped after six reversals of direction, and the last five reversal values were averaged to produce the estimate of the discrimination threshold. The frequency perception task followed a similar procedure. In each frequency trial, the target frequency was 3000 Hz, the foil tone always being higher. Participants heard two 400 ms tones separated by an empty interval of 1000 ms, and judged whether the first or second tone was higher. The foil duration was adjusted in 1 Hz steps depending on the accuracy of the response. Parameters were set based on pilot studies and Monte Carlo simulations to minimize the number of trials to convergence and overall run time while optimizing performance.

The measures of interest were the estimates of the difference from the target duration and frequency that could be discriminated with 80% accuracy.

#### *Motor timing*

Motor timing was measured with an isochronous rhythmic finger-tapping paradigm that required participants to tap along with a series of computer-generated tones, and then to continue tapping without being paced by the computer tone. They listened to a series of isochronous tones and began tapping in synchrony with the computer tone at will, and then continued tapping, attempting to maintain the same intertap interval after the computer tone ceased. Each trial consisted of 10 paced taps, followed by at least 30 unpaced taps. A valid trial was one that had 30 consecutive unpaced taps with an intertap interval not more than 25% above or below the mean paced value for that trial. Hand order was counterbalanced across subjects. The testing session was discontinued after 12 valid trials or after six invalid trials with each hand.

The outcome of interest was the variance of the intertap interval for the unpaced portion of each trial. Variability in isochronous rhythmic tapping can be decomposed into two components: a motor implementation component, involving the initiation and termination of the response, and a central or clock component (Wing and Kristofferson, 1973). Unpaced motor variance was computed using

the lag 1 serial covariance and unpaced clock variance was defined as the difference between total variance and motor variance.

### **Brain imaging procedures**

One hundred and sixteen children (89 with SB, 27 controls) had structural MRI brain scans. Of these, 68 (54 with SB, 16 controls) had scans that were quantitatively segmented; scans that were not segmented either had artefacts or had not been processed at the time of the study. The qualitative features of the scans were coded by two radiologists.

#### *Image acquisition*

Three sets of images were acquired, including a T1-weighted coronal series for assessment of white and grey matter and a T2-weighted coronal series for assessment of CSF. To co-register and position-normalize the scans, external fiducial markers were placed on the nasion and external meatus. An initial series (spin echo T1-weighted sagittal localizer, FOV 24, TR 500, TE14, 256 × 192 matrix, 3 mm skip 0.3, two repetitions) was used for anatomical landmark identification. One whole-brain coronal series consisted of fast spin-echo proton density and heavily T2-weighted images (FOV20, TR 4000, TE1 15, TE2 112, 256 × 192 matrix, with two repetitions). This series was obtained in contiguous 1.5 mm slices across the whole brain. Another whole-brain coronal series consisted of a 3D SPGR (3D-spoiled grass) gradient echo contiguous 1.5 mm coronal series (TR21, TE4, flip angle 35°, 124 locations, 256 × 192 matrix, one repetition).

#### *Image preprocessing*

Prior to tissue segmentation, each slice series was stored in a single-volume file and the pixel greyscale limits were expanded by increasing the gain within the 0–255 (byte data) range. Each sequence volume was then reformatted so that voxel dimensions were isotropic. The T1- and T2-weighted reformatted volumes were aligned with each other through the use of the fiducial markers. Rigid-body translation and rotation routines programmed in IDL software were used for the realignment procedure itself, which was manually and visually checked at each step. Each volume was placed within a 256 cubic voxel bounding box with the fiducial marker cross-point placed at the centre of the volume. The two reformatted and aligned volumes were filtered using a non-linear anisotropic diffusion filter, which increased the overall signal-to-noise ratio of each volume an average of 100% (Gerig *et al.*, 1992). This automated non-linear filter served to sharpen areas of high intensity gradient (boundaries) and to smooth regions of low-intensity gradient within the tissue borders. Finally, a separate interactive C program operating on the T2-weighted, reformatted, aligned and filtered volume was used to separate the cerebellum using a combination of interactive intensity thresholding and manual delineation. The cerebellum was then filled automatically to its borders, thereby defining an image mask, the masking process being performed on a slice-by-slice basis. Cerebellum volume measures were computed automatically following mask generation.

#### *Automatic segmentation*

The method used a fully automated fuzzy cluster analysis (Pao, 1989) that obtained whole brain and regional brain tissue and CSF

volumes (Brandt *et al.*, 1992, 1994, 1996). The T1-weighted scan volume, which provides superior white–grey contrast compared with the T2-weighted scan, was used to obtain white and grey matter tissue volumes. The T2-weighted scan was fuzzy clustered separately from the T1-weighted scan to extract CSF volumes, and this was used to adjust the white and grey matter volume measures obtained from the T1-weighted volume.

### Derived measures

Solution images were derived from the final computed fuzzy cluster membership values for each voxel, which could then be viewed graphically on screen and compared with the actual scan images. Separate tissue volumes (white matter, grey matter, CSF) were obtained for the whole cerebellum, medial cerebellum and lateral cerebellum.

The cerebellums of individuals with SB are highly dysmorphic, which makes it difficult to reliably visualize multiple landmarks and thence to estimate regional cerebellar volumes. We therefore developed an algorithm to estimate volumes that would roughly correspond to medial and lateral cerebellar regions. We identified the midsagittal cerebellum slice from the coronal series and identified the primary fissures to the left and right of the middle cerebellar slice in MRI scans from typically developing children. We found that the vermis represented on average 11% of the total cerebellum, and we used this estimate to define a medial cerebellar volume by identifying the areas 5.5% on either side of the midline, the remainder being defined as the left or right lateral regions (Fig. 1). The medial cerebellar volume is therefore a proxy for the vermis volume, but the procedures developed do allow reliable estimates of the medial cerebellum in individuals with major dysmorphologies of the cerebellum. Because the primary goal was to differentiate medial and lateral regions of the cerebellum to assess relations with the neurocognitive measures, this procedure appeared to be appropriate. Fig. 1 shows a segmented control brain.

## Results

### Perceptual timing

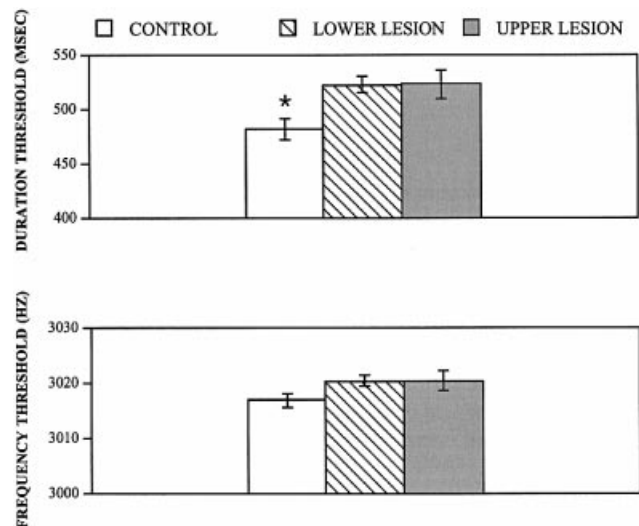
Comparisons of performance on the duration and frequency perception tasks were made using multivariate analysis of variance with group (control, SB lower lesion, SB upper lesion) and task (duration, frequency). *Post hoc* analyses of significant effects were conducted using Bonferroni-corrected pairwise comparisons. Children with SB performed more poorly than controls on the duration perception task [Fig. 2;  $F(2,122) = 5.92$ ,  $P < 0.005$ ], although the two groups performed similarly on the frequency perception task. There was no difference in performance between children with SB and upper spinal lesions and those with lower spinal lesions.

### Motor timing

Many participants in the SB group were unable to achieve six valid trials with each hand (Fig. 3). Children with SB and upper spinal lesions completed significantly fewer valid trials than did controls or children with SB and lower spinal lesions [one-way analysis of variance for group by number valid



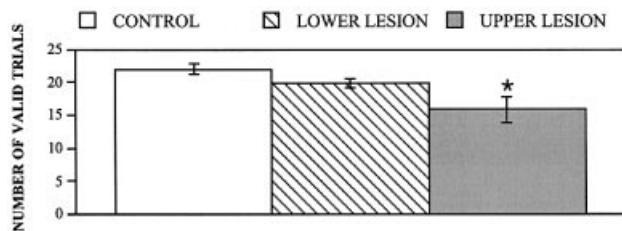
**Fig. 1** Segmented cerebellum of control participant showing demarcations of lateral and medial regions.



**Fig. 2** Perception thresholds in typically developing children ( $n = 37$ ) and in children with SB (lower spinal lesions,  $n = 66$ ; upper spinal lesions,  $n = 22$ ). (Top) Minimum duration above 400 ms detected 80% of the time (mean  $\pm$  SEM). (Bottom) Minimum frequency above 3000 Hz detected 80% of the time (mean  $\pm$  SEM). \*Significant difference between controls and both groups of children with SB ( $P < 0.005$ ; see text).

trials:  $F(2,122) = 6.22$ ,  $P < 0.003$ ]. Whereas 30 out of 32 control subjects and 54 out of 70 children with lower spinal lesions were able to complete six valid trials, only 10 out of 21 children with upper spinal lesions were able to do so.

Only children with six valid trials with each hand were included in the analyses of clock and motor variance. Because of the reduced sample size, we pooled the data from the two SB groups and made subsequent comparisons between SB and control groups. Compared with controls, children with SB showed significantly greater variability in the clock component of the rhythmic tapping task [Fig. 4; interaction



**Fig. 3** Number (mean  $\pm$  SEM) of valid tapping trials in typically developing children ( $n = 32$ ) and in children with SB (lower spinal lesions,  $n = 70$ ; upper spinal lesions,  $n = 21$ ). \*Significant difference between children with upper spinal lesions and both controls and children with lower spinal lesions ( $P < 0.003$ ; see text).

between group and intertap interval variance:  $F(2,91) = 3.503$ ,  $P < 0.05$ ]. This difference was due to the larger clock variance in children with SB compared with controls (Fig. 4; Bonferroni-corrected pair-wise comparisons,  $P < 0.05$ ).

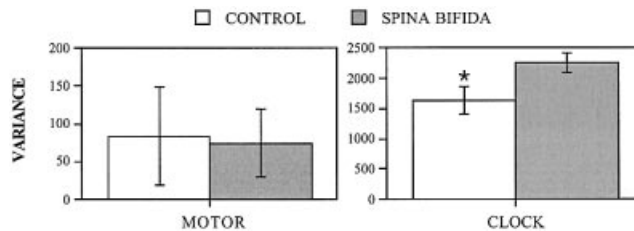
The Wing and Kristofferson (1973) model predicts that adjacent intertap intervals will be negatively correlated, such that longer intertap intervals will be followed by shorter ones. In our sample, the average lag 1 serial covariance across trials was negative in 21 controls and 40 children with SB, and positive in nine controls and 24 children with SB. There were no group differences in lag 1 serial covariance between groups ( $P > 0.5$ ; mean  $\pm$  SEM, negative lag 1, control =  $-0.09 \pm 0.02$ , SB =  $-0.13 \pm 0.02$ ; positive lag 1, control =  $0.08 \pm 0.02$ , SB =  $0.11 \pm 0.02$ ). With the exception of three children with SB, all participants had positive lag 1 covariance on some trials and there were no group differences in average number of trials with a positive lag 1 serial covariance. For those with an overall negative average lag 1, the mean number of trials with a positive lag 1 serial covariance for control and SB groups was  $6.8 \pm 0.6$  and  $6.5 \pm 0.6$ , respectively. For those with an overall positive average lag 1, the number of trials with a positive lag 1 serial covariance for control and SB groups was  $15.2 \pm 0.6$  and  $13.6 \pm 0.6$ , respectively.

The Wing and Kristofferson (1973) model also assumes no reliable correlation between non-adjacent intervals, predicting serial covariance functions for lags greater than 1 to be negligible. In our sample, average lag 2 serial covariance across trials was not significantly different from zero in 18 controls and 36 children with SB. For those with a significant average lag 2 serial covariance, there were no differences between groups ( $P > 0.5$ ; control =  $0.14 \pm 0.01$ ; SB =  $0.15 \pm 0.01$ ); however, lag 2 was negatively correlated with age (Pearson  $r = -0.413$ ,  $P = 0.008$ ).

No effects of hand preference (preferred writing hand versus non-preferred writing hand) or of hand used (right versus left) were found on performance in the tapping task.

### Correlation between perceptual and motor timing

To explore the question of a common mechanism for short duration perceptual and motor timing, we correlated scores on



**Fig. 4** Motor and clock variance of the intertap interval in typically developing children ( $n = 30$ ) and in children with SB ( $n = 64$ ). \*Significant difference between SB and control groups ( $P < 0.05$ ; see text).

the duration and tapping tasks. Significant Spearman correlations were found between duration perception threshold and unpaced clock variance ( $r = 0.38$ ,  $P = 0.02$ ), and duration perception threshold and number of valid tapping trials ( $r = -0.44$ ,  $P = 0.004$ ).

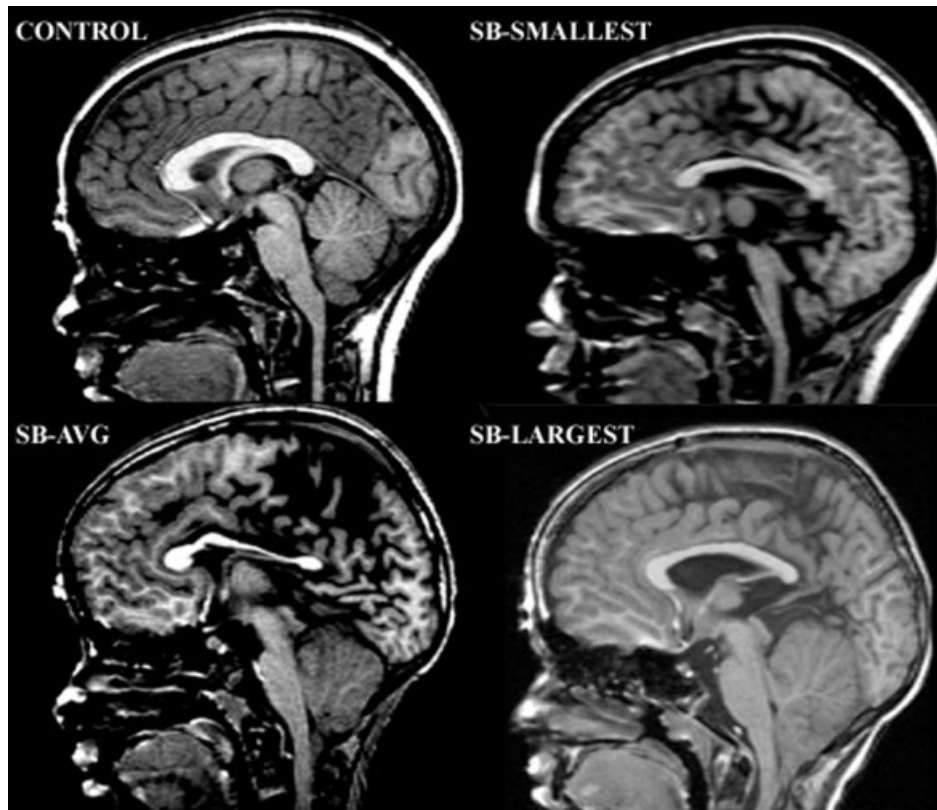
### Comparison of cerebellum volumes in SB and control groups

Cerebellar dysmorphology is evident in children with SB. These children, however, vary widely in the magnitude of the dysmorphology (Fig. 5).

Qualitative coding of the MRI scans confirmed that the cerebellum was grossly abnormal in children with SB, although not in control children. Of the 89 scans from children with SB, the cerebellum was abnormal in 86 (97%), the vermis was abnormal in 77 (86%), the hemispheres were abnormal in 73 (82%), and some form of Arnold–Chiari malformation was evident in 82 (92%). Except for one rating of an abnormal vermis, none of the cerebellum qualitative measures was abnormal on any of these ratings for control brains.

The cubic volume (cubic centimetres) data from the quantitative analyses of the cerebellum are presented in Table 1. Statistical analyses confirmed the reduction of whole cerebellum volume in children with SB. In addition to a one-way analysis of variance comparing the three groups (SB and upper level lesions, SB and lower level lesions, controls) in total cerebellum volume, comparisons were conducted to evaluate the regional distribution of white matter, grey matter, and CSF using repeated-measures multivariate analysis of variance for group, tissue type and region (medial, lateral). *Post hoc* analyses were made using Bonferroni-corrected pairwise comparison procedures.

As shown in Table 1, measures of total cerebellar volumes were significantly smaller in children with SB and upper lesions relative to those with SB and lower level lesions, both SB groups showing significantly smaller volumes than controls [ $F(2,65) = 11.7$ ,  $P < 0.0001$ ]. All pairwise comparisons were significant at the critical level of alpha ( $P < 0.0167$ ), showing that children with SB and upper level spinal lesions had the smallest volumes, followed by those with SB



**Fig. 5** Mid-sagittal view of the brain in representative control and spina bifida cases. (*Top left*) Representative control (volume = 153.2 cm<sup>3</sup>). (*Top right*) SB, smallest cerebellum (volume = 7.8 cm<sup>3</sup>). (*Bottom left*) SB, representative cerebellum (volume = 116.8 cm<sup>3</sup>). (*Bottom right*) SB, largest cerebellum (volume = 152.9 cm<sup>3</sup>).

**Table 1** Cerebellar MRI volumetric data (cubic centimetres; mean  $\pm$  SD)

| Region  | Group   | Total         | CSF        | Grey          | White       |
|---------|---------|---------------|------------|---------------|-------------|
| Whole   | Control | 151.45 (13.1) | 6.36 (1.6) | 109.70 (10.4) | 35.38 (4.5) |
|         | Lower   | 118.16 (19.2) | 4.62 (1.4) | 84.29 (15.6)  | 29.26 (5.1) |
|         | Upper   | 94.86 (30.7)  | 3.81 (1.6) | 67.05 (22.0)  | 24.01 (8.5) |
| Medial  | Control | 19.26 (2.9)   | 2.59 (0.9) | 14.01 (1.9)   | 2.66 (0.8)  |
|         | Lower   | 19.24 (3.6)   | 1.32 (0.5) | 14.60 (2.9)   | 3.32 (1.0)  |
|         | Upper   | 16.64 (5.8)   | 1.11 (0.7) | 12.41 (4.4)   | 3.13 (1.5)  |
| Lateral | Control | 132.19 (10.6) | 3.76 (1.2) | 95.71 (8.8)   | 32.70 (3.9) |
|         | Lower   | 98.93 (16.2)  | 3.30 (1.0) | 69.70 (13.1)  | 25.93 (4.5) |
|         | Upper   | 78.22 (25.1)  | 2.70 (1.0) | 54.67 (17.8)  | 20.86 (7.2) |

and upper level lesions, and then controls. Similar findings were apparent for total grey matter [ $F(2,65) = 26.65$ ,  $P < 0.001$ ] and total white matter [ $F(2,65) = 14.19$ ,  $P < 0.0001$ ], with all pairwise comparisons significant and in the same direction. For CSF, the analysis of variance was significant [ $F(2,65) = 11.73$ ,  $P < 0.0001$ ], but the difference between SB children with upper versus lower lesions did not meet the critical level of alpha. Both groups with SB had less CSF than controls.

Measures of cerebellar volume varied by region and tissue type across groups [region  $\times$  tissue type  $\times$  group interaction:

$F(4,128) = 22.44$ ,  $P < 0.0001$ ], demonstrating that the regional distribution of tissue type varied within region across the groups. Within the lateral region of the cerebellum, the group  $\times$  tissue interaction was significant [ $F(4,128) = 15.74$ ,  $P < 0.0001$ ]. Comparing tissue types within groups showed a significant effect for grey matter [ $F(2,65) = 36.83$ ,  $P < 0.0001$ ]. Pairwise comparisons showed that controls had larger grey matter volume than children with SB and lower lesions, who in turn differed from the group with the least grey matter, SB with upper lesions (Table 1). The same pattern was apparent for white matter [ $F(2,65) = 21.16$ ,

$P < 0.0001$ ]. For CSF, the overall group effect was significant [ $F(2,65) = 3.78, P < 0.03$ ]. However, *post hoc* comparisons showed differences only between the controls and SB and upper lesion group; the comparison of the two groups with SB, or controls and SB and lower lesions, did not meet the critical level of alpha.

In the medial region, the group  $\times$  tissue interaction was significant [ $F(4,128) = 9.67, P < 0.0001$ ]. For grey matter, the group effect was not statistically significant [ $F(2,65) = 2.52, P < 0.09$ ]. A similar pattern was apparent for white matter [ $F(2,65) = 2.06, P < 0.15$ ]. The group differences occurred largely because of the significantly greater amount of CSF in the cerebellum of the controls relative to both groups with SB [ $F(2,65) = 24.32, P < 0.0001$ ]. Overall, these results parallel the analysis for total cerebellum volume, showing more CSF in the controls and less grey and white matter in the two groups with SB. The differences in grey and white matter were especially apparent in the lateral cerebellum measure, the group with SB and upper level lesions showing less grey and white matter than either the controls or the group with SB and lower level lesions, and the latter also showing a significant reduction relative to controls. Most important, the interactions show that the cerebellum differences were not simply overall volume reductions, but instead reflected a different pattern of organization of grey matter, white matter and CSF in the groups with SB.

### **Correlation of timing function and cerebellum volumes**

To evaluate the relationship between cerebellar dysmorphology and short-duration perceptual and motor timing, we compared performance on the timing and tapping tasks with cerebellar volumetric measures using Pearson correlation coefficients with partial correlations with age. Correlations were performed separately for SB and control groups to avoid inflating correlation coefficients because of group differences on the timing data. Because of significant skewedness in the motor and clock variance, the data were subjected to a square-root transformation. Two cases with extreme values ( $< -2000$ ) were dropped from the analyses because they were substantial outliers.

Perceptual timing was related to cerebellar measures. For controls, duration threshold was negatively correlated with several cerebellar measures (whole cerebellum,  $r = -0.58, P < 0.03$ ; lateral cerebellum,  $r = -0.61, P < 0.02$ ; cerebellar whole grey matter,  $r = -0.70, P < 0.005$ ; and cerebellar lateral grey matter,  $r = -0.72, P < 0.005$ ). For the SB group, duration perception threshold was positively correlated with lateral cerebellar CSF volume ( $r = 0.34, P < 0.05$ ). For neither group were there correlations between frequency threshold and cerebellar volumes.

Motor timing was related to cerebellar measures for the SB group. For neither controls nor SB group were there significant correlations between either clock variance or

motor variance and cerebellar volumes. Children with SB showed a significant positive relation between number of valid tapping trials and cerebellar volumes (whole lateral cerebellar volume,  $r = 0.30, P < 0.06$ ; whole cerebellar grey matter,  $r = 0.34, P < 0.03$ ; lateral cerebellar grey matter,  $r = 0.33, P < 0.03$ ). Controls showed no variability in the number of valid tapping trials, so correlations with brain volumes could not be calculated.

There were no correlations between whole brain cerebral volume and any of the perceptual or motor timing measures ( $P > 0.5$ ). This supports the idea that the relationship between brain dysmorphology and performance is specific to the cerebellum, and not a result of a general reduction in brain size.

### **Discussion**

Children with SB show deficits in perceptual and motor timing, as well as significant volume reductions in the cerebellum. Further, there are relations among the two modalities of timing deficits, and between timing deficits and cerebellar volume. The data bear on several issues: the nature of perceptual timing deficits; the specificity of motor timing deficits; the patterns and magnitude of cerebellar volume loss in SB; the relations between timing function and cerebellar volume loss; the relation between perceptual and motor timing and the nature of a possible amodal cerebellar timer; and the more general question of age-based functional plasticity in the cerebellum.

The perceptual timing deficit is relatively specific to timing. It does not represent a generalized auditory discrimination deficit because the SB group could perform a frequency discrimination task with similar task demands to the duration task.

Children with SB have motor timing deficits, which may involve impairment in central timing. Children with SB had no difficulty with paced tapping, so their documented motor problems (Hetherington and Dennis, 1999) do not prevent them performing the basic motor timing task (for a discussion of the fact that adult cerebellar contributions to many tasks cannot readily be explained by motor deficits, see Fiez, 1996). Deficits in unpaced timing were associated with clock variance rather than with motor implementation. The idea of a central timing deficit in the control of precisely timed movements is congruent with recent observations that children with SB have elevated rates of ataxic dysarthria that include impaired temporal regulation of speech (Huber-Okraïnc *et al.*, 2002). In this context, adult stutterers have poor time estimation skills (Ezrati-Vinacour and Levin, 2001).

The mathematics of the standard model for decomposition of variance in isochronous motor tapping tasks (Wing and Kristofferson, 1973) require the motor and clock variance to be serially independent, which will not occur whenever there is a drift in tapping tempo. It has recently been proposed that a slow drift process adds a third source of variance, and

algorithms have been offered to estimate motor variance, drift variance and drift-free clock variance (Collier and Ogden, 2001).

The cerebellum is compromised in children with SB from the time of neurogenesis. The Arnold–Chiari II malformation arises from the neural tube defect and a series of interrelated, time-dependent defects in the development of the ventricular system (McLone and Knepper, 1989). One result is dysgenetic development of the cerebellum in a small posterior fossa (Barkovich, 1995). Reduced cerebellar size has long been observed in children with SB; for example, Variend and Emery (1973) reported that the weight of the cerebellum in autopsied cases of SB was less than that in controls, regardless of age and (Holter valve) treatment.

The present data provide three new pieces of information about cerebellar compromise in the SB condition. Children with SB who are not mentally retarded have significant reductions in cerebellar volume, which confirms the older autopsy data and extends it by MRI volumetric *in vivo* measurements of cerebellar volumes in a large sample. Both grey and white cerebellar volumes are reduced in children with SB, which parallels the observations of both grey and white matter compromises in the cortex of children with this condition (Fletcher *et al.*, 1996). Compared with controls, children with SB have different patterns of grey matter, white matter and CSF.

To date, neither child nor adult studies of timing have analysed structure–function relations between timing and cerebellar malformations. Asking whether the extent of timing deficit was related to the extent of cerebellar compromise, we identified structure–function correlations between timing measures and cerebellar volumes. Duration perception, but not frequency perception, was correlated with whole and lateral cerebellar volumes. On the motor timing task, the number of valid tapping trials was related to whole and lateral cerebellar grey matter, a finding of interest because the implementation of motor response in the cerebellum involves grey matter and the deep cerebellar nuclei. The relations between timing function and the amount of cerebellar volume might be investigated further by functional neuroimaging studies, especially modalities such as magnetic source imaging.

The consequences of deficits in short-duration timing and event timing are considerable. Many eye–hand tasks require the constant modulation of motor timing. For example, throwing a ball requires that the opening of the fingers to release the ball must occur within a narrow temporal window with respect to the extension of the arm (Hore *et al.*, 1999). Rhythm perception requires that the listener use the temporal information in rhythmic patterns, and this is impaired in children with SB, who, unlike age peers, do not capitalize on the presence of a strong meter in discriminating rhythms (Hopyan *et al.*, 2003).

Temporal information must be incorporated into the representation of actions, which probably involves both perceptual and motor timing. It has been proposed that

humans have a dedicated temporal processor that regulates motor events and provides a representation for comparing the duration of different perceptual events (Ivry and Richardson, 2002). Consistent with this proposal, variance measures are correlated over motor tasks that use different effectors (Keele *et al.*, 1985; Franz *et al.*, 1992). More specifically, perceptual and motor timing constitute an integrated internal representation of the target interval (Ivry and Hazeltine, 1995), a view that is consistent with recent theories (e.g. Rao *et al.*, 2001) that the role of the cerebellum is to generate predictions about the sensory consequences of motor acts, a role that would seem to require an amodal, integrated timing mechanism.

In the literature on adult focal cerebellar lesions, dissociations between perceptual and motor timing have been described. Perceptual and motor timing deficits are often uncorrelated, although correlations do emerge with parametric variations in the timing tasks (Ivry and Hazeltine, 1995). In children with SB, perceptual and motor tasks were correlated for the variance specifically associated with timing (e.g. duration discrimination and clock variance), but not for the variance associated with other aspects of task performance (e.g. frequency threshold and motor variance). Perceptual and motor timing deficits may be correlated after major malformations of the cerebellum during embryogenesis; in that event, perceptual and motor timing in children with SB might represent dysfunction in a central, amodal timing mechanism arising from fundamental dysgenesis of cerebellar microarchitecture. To test this idea, it would be of interest to study whether individuals with other neurodevelopmental disorders show more generalized timing deficits than individuals with adult-onset lesions.

Timing has been studied in some other clinical paediatric conditions. Young adults with attention deficit hyperactivity disorder (ADHD) show deficient time estimation for long duration time intervals, such as 15 s and 1 min (Barkley *et al.*, 2001), regardless of the presence of comorbid conditions. For short-duration timing intervals around 400 ms, the results are mixed. Toplak and colleagues (Toplak *et al.*, 2003) found impairment of short-duration timing in ADHD individuals, especially those with comorbid reading difficulties. Radonovich and Mostofsky (2004) found a dissociation between normal short duration timing and impaired long duration timing, which suggested that timing deficits in ADHD represent deficient utilization of temporal information rather than a more basic impairment in timing. Because ADHD is diagnosed by abnormal behaviour rather than an abnormal brain, differences in results of timing studies may reflect differences in diagnostic criteria for the participants, as well as differences in procedures. Individuals with ADHD have difficulty with long-duration timing, and their poor performances on some short-duration timing tasks may be related to comorbid conditions and represent problems in the cognitive utilization of temporal information. Whether and how any short-duration timing deficits in ADHD are correlated with individual differences in cerebellar structure and/or function remains to be studied.

The cerebellum has been shown to modulate the temporal contingencies of eyeblink conditioning (Clark *et al.*, 2002), which requires pairing of a conditioned and an unconditioned stimulus over specific brief time intervals. Lesions of the cerebellum disrupt eyeblink conditioning in humans, rabbits, and rodents (Bracha *et al.*, 1997; for reviews see Kim and Thompson, 1997; Steinmetz, 2000). The observed perceptual timing deficit concerns intervals of 400 ms; it remains to be studied whether children with SB are impaired in functions that require precise temporal contingencies over short-duration ranges, such as eyeblink conditioning (Clark *et al.*, 2002).

For adults, long-duration timing (in the range of several seconds) and short-duration timing (of subsecond intervals) involve different psychophysical (Gibbon *et al.*, 1997) and pharmacological (Rammsayer, 1999) characteristics. Long- and short-duration timing also appear to involve somewhat distinct neural mechanisms (Ivry, 1996), the cerebellum being functionally engaged only for short-duration timing (Lewis and Miall, 2003a). It has been suggested that parts of the motor system are involved in the automatic measurement of brief durations, in contrast to longer intervals, which require the flexible cognitive modules of the anterior cerebral cortex (Lewis and Miall, 2003b). Adult survivors of childhood cerebellar tumours have deficits in short-duration (around 400 ms) but not long-duration timing (Hetherington, *et al.*, 2000).

The involvement of the cerebellum in the neural circuit for short-duration timing appears to exist for both the mature and the immature brain. More broadly, these and other timing data suggest that short-duration timing deficits are ubiquitous after lesions to the cerebellum at several points in the lifespan. The present data add to the emerging information about developmental disorders of the cerebellum, showing that significant timing deficits occur after a range of genetic (Mostofsky *et al.*, 2000) and developmental disorders of the cerebellum, such as childhood astrocytomas and medulloblastomas of the posterior fossa (Hetherington *et al.*, 2000). This is consistent with the idea that the potential for functional plasticity of some core cerebellar operations is severely limited, at best, and is certainly not based on age at lesion onset (Dennis *et al.*, 1999). Cerebellar lesions, whether originating in embryogenesis, in childhood or in adult life, appear to be associated with poor development of perceptual and motor timing.

## Acknowledgements

We thank Joanne Robitaille, Jennifer Janes, Andrea Martin, Amy Boudousquie, Irene Townsend, and Susan Inwood for research assistance. This work was supported by National Institute of Child Health and Human Development Grant P01 HD35946 'Spina Bifida: Cognitive and Neurobiological Variability'.

## References

- Barkley RA, Murphy KR, Bush T. Time perception and reproduction in young adults with attention deficit hyperactivity disorder. *Neuropsychology* 2001; 15: 351–60.
- Barkovich AJ, editor. *Pediatric neuroimaging*. 2nd ed. New York: Raven Press; 1995.
- Bracha V, Zhao L, Wunderlich DA, Morrissy SJ, Bloedel JR. Patients with cerebellar lesions cannot acquire but are able to retain conditioned eyeblink reflexes. *Brain* 1997; 120: 1401–13.
- Brandt ME, Fletcher JM, Bohan TP. Estimation of CSF, white, and gray matter volumes from MRIs of hydrocephalic and HIV-positive subjects. *Proc SimTec/WNN* 1992: 643–50.
- Brandt ME, Bohan TP, Kramer LA, Fletcher JM. Estimation of CSF, white and gray matter volumes in hydrocephalic children using fuzzy clustering of MR images. *Comput Med Imaging Graph* 1994; 18: 25–34.
- Brandt ME, Bohan TP, Thorstad K, McCauley SR, Davidson KC, Francis DJ, et al. Reliability of brain structure morphometry in hydrocephalic children using MR images. *Magn Reson Imaging* 1996; 14: 649–55.
- Clark RE, Manns JR, Squire LR. Classical conditioning, awareness, and brain systems. *Trends Cogn Sci* 2002; 6: 524–31.
- Collier GL, Ogden RT. Variance decomposition of tempo drift in isochronous rhythmic tapping. *Ann NY Acad Sci* 2001; 930: 405–8.
- del Bigio MR. Neuropathological changes caused by hydrocephalus. *Acta Neuropathol (Berl)* 1993; 18: 573–85.
- Dennis M, Hetherington CR, Spiegler BJ, Barnes MA. Functional consequences of congenital cerebellar dysmorphologies and acquired cerebellar lesions of childhood. In: Broman SH, Fletcher JM, editors. *The changing nervous system: neurobehavioral consequences of early brain disorders*. New York: Oxford University Press; 1999: p. 172–98.
- Ezrati-Vinacour R, Levin I. Time estimation by adults who stutter. *J Speech Lang Hear Res* 2001; 44: 144–55.
- Fiez JA. Cerebellar contributions to cognition. *Neuron* 1996; 16: 13–5.
- Fletcher JM, Francis DJ, Thompson NM, Davidson KC, Miner ME. Verbal and nonverbal skill discrepancies in hydrocephalic children. *J Clin Exp Neuropsychol* 1992; 14: 593–609.
- Fletcher JM, Bohan TP, Brandt ME, Kramer LA, Brookshire BL, Thorstad K, et al. Morphometric evaluation of the hydrocephalic brain: relationships with cognitive development. *Childs Nerv Syst* 1996; 12: 192–9.
- Fletcher JM, Dennis M, Northrup H. Hydrocephalus. In: Yeates KO, Ris MD, Taylor HG, editors. *Pediatric neuropsychology: research, theory, and practice*. New York: Guilford Press; 2000. p. 25–46.
- Franz EA, Zelaznik HN, Smith A. Evidence of common timing processes in the control of manual, orofacial, and speech movements. *J Mot Behav* 1992; 24: 281–7.
- Gerig G, Kubler O, Kikinis R, Jolesz FA. Nonlinear anisotropic filtering of MRI data. *IEEE Trans Med Imaging* 1992; 11: 221–232.
- Gibbon J, Malapani C, Dale CL, Gallistel C. Toward a neurobiology of temporal cognition: advances and challenges. *Curr Opin Neurobiol* 1997; 7: 170–84.
- Hetherington R, Dennis M. Motor function profile in children with early onset hydrocephalus. *Dev Neuropsychol* 1999; 15: 25–51.
- Hetherington R, Dennis M, Spiegler B. Perception and estimation of time in long-term survivors of childhood posterior fossa tumors. *J Int Neuropsychol Soc* 2000; 6: 682–92.
- Holmes G. The clinical symptoms of cerebellar disease and their interpretation. *Lancet* 1922; 1: 1177–82, 1231–7; 2: 59–63, 111–5.
- Hopyan T, Schellenberg EG, Dennis M. Perception of rhythms with a strong or weak metric structure in children with spina bifida. *J Int Neuropsychol Soc* 2003; 9: 142.
- Hore J, Ritchie R, Watts S. Finger opening in an overarm throw is not triggered by proprioceptive feedback from elbow extension or wrist flexion. *Exp Brain Res* 1999; 125: 302–12.
- Huber-Okrainec J, Dennis M, Brettschneider J, Spiegler BJ. Neuromotor speech deficits in children and adults with spina bifida and hydrocephalus. *Brain Lang* 2002; 80: 592–602.

- Ivry RB. The representation of temporal information in perception and motor control. *Curr Opin Neurobiol* 1996; 6: 851–57.
- Ivry RB, Hazeltine RE. Perception and production of temporal intervals across a range of durations: evidence for a common timing mechanism. *J Exp Psychol Hum Percept Perform* 1995; 21: 3–18.
- Ivry R, Keele S. Timing functions of the cerebellum. *J Cogn Neurosci* 1989; 1: 136–51.
- Ivry RB, Richardson TC. Temporal control and coordination: the multiple timer model. *Brain Cogn* 2002; 48: 117–32.
- Ivry RB, Keele SW, Diener HC. Dissociation of the lateral and medial cerebellum in movement timing and movement execution. *Exp Brain Res* 1988; 73: 167–80.
- Jancke L, Loose R, Lutz K, Specht K, Shah NJ. Cortical activations during paced finger-tapping applying visual and auditory pacing stimuli. *Brain Res Cogn Brain Res* 2000; 10: 51–66.
- Keele SW, Pokorny RA, Corcos DM, Ivry RB. Do perception and motor production share common timing mechanisms: a correctional analysis. *Acta Psychol (Amst)* 1985; 60: 173–91.
- Kim JJ, Thompson RF. Cerebellar circuits and synaptic mechanisms involved in classical eyeblink conditioning. *Trends Neurosci* 1997; 20: 177–81.
- Lewis PA, Miall RC. Brain activation patterns during measurement of sub- and supra-second intervals. *Neuropsychologia* 2003a; 41: 1583–92.
- Lewis PA, Miall RC. Distinct systems for automatic and cognitively controlled time measurement: evidence from neuroimaging. *Curr Opin Neurobiol* 2003b; 13: 250–5.
- Mangels JA, Ivry RB, Shimizu N. Dissociable contributions of the prefrontal and neocerebellar cortex to time perception. *Brain Res Cogn Brain Res* 1998; 7: 15–39.
- Massaquoi SG, Topka H. Models of cerebellar function. In: Manto MU, Pandolfo M, editors. *The cerebellum and its disorders*. Cambridge: Cambridge University Press, 2002. p. 69–94.
- Mauk MD, Medina JF, Nores WL, Ohyama T. Cerebellar function: coordination, learning or timing? *Curr Biol* 2000; 10: R522–5.
- McLone DG, Knepper PA. The cause of Chiari II malformation: a unified theory. *Pediatr Neurosci* 1989; 15: 1–12.
- Mostofsky SH, Kunze JC, Cutting LE, Lederman HM, Denckla MB. Judgment of duration in individuals with ataxia–telangiectasia. *Dev Neuropsychol* 2000; 17: 63–74.
- Nichelli P, Alway D, Grafman J. Perceptual timing in cerebellar degeneration. *Neuropsychologia* 1996; 34: 863–71.
- Pao YH. *Adaptive pattern recognition and neural networks*. Reading (MA): Addison-Wesley; 1989.
- Park CH, Stewart W, Khoury MJ, Mulinare J. Is there etiologic heterogeneity between upper and lower neural tube defects? *Am J Epidemiol* 1992; 136: 1491–501.
- Penhune VB, Zatorre RJ, Evans AC. Cerebellar contributions to motor timing: a PET study of auditory and visual rhythm reproduction. *J Cogn Neurosci* 1998; 10: 752–65.
- Radonovich KJ, Mostofsky SH. Duration judgments in children with ADHD suggest deficient utilization of temporal information rather than general impairment in timing. *Child Neuropsychol*. In press 2004.
- Rammsayer TH. Neuropharmacological evidence for different timing mechanisms in humans. *Q J Exp Psychol B* 1999; 53: 273–86.
- Rao SM, Harrington DL, Haaland KY, Bobholz JA, Cox RW, Binder JR. Distributed neural systems underlying the timing of movements. *J Neurosci* 1997; 17: 5528–35.
- Rao SM, Mayer AR, Harrington DL. The evolution of brain activation during temporal processing. *Nat Neurosci* 2001; 4: 317–23.
- Rosenbaum DA, Collyer CE, editors. *Timing of behavior. Neural, psychological, and computational perspectives*. Cambridge (MA): MIT Press; 1998.
- Steinmetz JE. Brain substrates of classical eyeblink conditioning: a highly localized but also distributed system. *Behav Brain Res* 2000; 110: 13–24.
- Swanson JM. *School-based assessments and interventions for ADD students*. Irvine (CA): KC Publishing; 1992.
- Théoret H, Haque J, Pascual-Leone A. Increased variability of paced finger tapping accuracy following repetitive magnetic stimulation of the cerebellum in humans. *Neurosci Lett* 2001; 306: 29–32.
- Thorndike RL, Hagen EP, Sattler JM. *The Stanford-Binet intelligence scale*. 4th ed. Itasca (IL): Riverside; 1986.
- Toplak ME, Rucklidge JJ, Hetherington R, John SCF, Tannock R. Time perception deficits in attention-deficit/hyperactivity disorder and comorbid reading difficulties in child and adolescent samples. *J Child Psychol Psychiatry* 2003; 44: 888–903.
- Tracy JI, Faro SH, Mohamed FB, Pinsk M, Pinus A. Functional localization of a ‘time keeper’ function separate from attentional resources and task strategy. *Neuroimage* 2000; 11: 228–42.
- van der Put NM, van Straaten HW, Trijbels FJ, Blom HJ. Folate, homocysteine and neural tube defects: an overview. *Exp Biol Med (Maywood)* 2001; 226: 243–70.
- Variend S, Emery JL. The weight of the cerebellum in children with myelomeningocele. *Dev Med Child Neurol* 1973; Suppl 29: 77–83.
- Volcik KA, Blanton SH, Northrup H. Examinations of methylenetetrahydrofolate reductase C677T and A1298C mutations—and in utero viability. *Am J Hum Genet* 2001; 69: 1150–3.
- Wetherill GB, Levitt H. Sequential estimation of points on a psychometric function. *Br J Math Stat Psychol* 1965; 18: 1–10.
- Wing AM, Kristofferson AB. Response delays and the timing of discrete motor responses. *Percept Psychophys* 1973; 14: 5–12.