NeuroImage 86 (2014) 392–403

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Genetic effects on the cerebellar role in working memory: Same brain, different genes?

Gabriëlla A.M. Blokland [a,b,c,*], Katie L. McMahon [b], Paul M. Thompson [d], Ian B. Hickie [e], Nicholas G. Martin [a], Greig I. de Zubicaray [c], Margaret J. Wright [a,c]

[a] Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Brisbane, Australia
[b] Centre for Advanced Imaging, University of Queensland, Brisbane, Australia
[c] School of Psychology, University of Queensland, Brisbane, Australia
[d] Laboratory of Neuro Imaging, Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
[e] Brain & Mind Research Institute, The University of Sydney, Sydney, Australia

ARTICLE INFO

Article history:
Accepted 7 October 2013
Available online 12 October 2013

Keywords:
Cerebellum
Heritability
Genetics
Functional MRI
Working memory
Twin study

ABSTRACT

Over the past several years, evidence has accumulated showing that the cerebellum plays a significant role in cognitive function. Here we show, in a large genetically informative twin sample ($n = 430$; aged 16–30 years), that the cerebellum is strongly, and reliably ($n = 30$ rescans), activated during an *n*-back working memory task, particularly lobules I–IV, VIIa Crus I and II, IX and the vermis. Monozygotic twin correlations for cerebellar activation were generally much larger than dizygotic twin correlations, consistent with genetic influences. Structural equation models showed that up to 65% of the variance in cerebellar activation during working memory is genetic (averaging 34% across significant voxels), most prominently in the lobules VI, and VIIa Crus I, with the remaining variance explained by unique/unshared environmental factors. Heritability estimates for brain activation in the cerebellum agree with those found for working memory activation in the cerebral cortex, even though cerebellar cyto-architecture differs substantially. Phenotypic correlations between BOLD percent signal change in cerebrum and cerebellum were low, and bivariate modeling indicated that genetic influences on the cerebellum are at least partly specific to the cerebellum. Activation on the voxel-level correlated very weakly with cerebellar gray matter volume, suggesting specific genetic influences on the BOLD signal. Heritable signals identified here should facilitate discovery of genetic polymorphisms influencing cerebellar function through genome-wide association studies, to elucidate the genetic liability to brain disorders affecting the cerebellum.

Introduction

Traditionally, the cerebellum has been associated primarily with the coordination of movement. However, in many human lesion and functional neuroimaging studies, the cerebellum clearly also subserves emotional control and higher cognitive operations, such as working memory (Chen and Desmond, 2005; Gottwald et al., 2004; Hokkanen et al., 2006; Ravizza et al., 2006; Scott et al., 2001). Recently, a meta-analysis of working memory neuroimaging studies reported significant activation in the cerebellum, in bilateral lobules VI and VIIa Crus I, and right lobule VIIIa (Stoodley and Schmahmann, 2009). The cerebellar role in working memory and other non-motor functions is attributed to cerebro-cerebellar pathways that link the cerebellum with motor, association, and paralimbic cortices (Stoodley and Schmahmann, 2009). Histological studies and investigations in non-human primates reveal connections from vermal and hemispheral parts of cerebellar lobule VII Crus II to mainly lateral portions of dorsolateral prefrontal cortex (DLPFC). Their co-activation is consistent with the operation of a functional network: the 'prefrontal' cortico-cerebellar loop (Kelly and Strick, 2003; Middleton and Strick, 2001; Ramnani, 2006). Cerebellar activation during working memory may reflect the automated simulation of cognitive operations that initially rely on interactions between prefrontal areas (Hayter et al., 2007).

Abnormal cerebellar activation and disruption of cortico-cerebellar circuits, in addition to abnormal cerebral activation, have been observed in neuropsychiatric disorders such as schizophrenia and attention-deficit hyperactivity disorder (Andreasen et al., 1996; Bor et al., 2011; Massat et al., 2012; Valera et al., 2005). This disruption may be an early diagnostic indicator of psychopathology. As susceptibility to these disorders is strongly genetically determined, finding the genetic influences on the underlying brain processes may greatly increase our understanding of the etiology of these disorders. Hypo-activation of the fronto-cerebellar loop observed in these disorders may actually be due to specific genetic risk factors affecting the cerebellum, and not the frontal cortex. Although it is reasonable to assume that if genetic effects on cerebral working memory blood oxygenation level dependent (BOLD) signal exist (~33%; Blokland et al., 2011), they would also exist for the cerebellar involvement in working memory, it

* Corresponding author at: Genetic Epidemiology Laboratory, Queensland Institute of Medical Research, Locked Bag 2000, Royal Brisbane Hospital, Herston, Qld 4029, Australia.
E-mail address: gabriella.blokland@uqconnect.edu.au (G.A.M. Blokland).

1053-8119/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.10.006

is of interest to study the cerebellum separately because of its unique cyto-architecture. The cerebellum may be influenced by genes specific to this unique cyto-architecture, and/or may be influenced by genes to a different extent.

Even though some twin studies have shown high heritability for cerebellar gray matter (GM) volume (~64–93%; Kremen et al., 2010; van Soelen et al., 2013), so far no functional MRI (fMRI) twin studies have examined the heritability of task-related neural activity in the cerebellum (Blokland et al., 2008, 2011; Côté et al., 2007; Koten et al., 2009; Matthews et al., 2007; Park et al., 2012; Polk et al., 2007). The focus of imaging genetics studies using working memory fMRI as an endophenotype has been on the prefrontal cortex (e.g., Bertolino et al., 2006, 2010; Egan et al., 2001; Krug et al., 2008), but if cerebellar activation is heritable, and particularly if different sets of genes influence the BOLD signal of the cerebrum and cerebellum, using activation of the cerebellum as an endophenotype in gene finding studies, provides an opportunity to detect novel gene variants that may be relevant to brain (dys)function, whose association would not be detected when using activation of the cerebrum as an endophenotype. This could significantly increase our knowledge of the brain, and these gene variants could be of critical importance to brain disorders.

Therefore, the goal of this study was two-fold: First, we investigated the usefulness of working memory cerebellar activation as an endophenotype by computing voxel-wise heritability maps. Even with a rigidly standardized *n*-back task, the extent and intensity of cerebellum activation differs greatly between individuals. To disentangle genetic and environmental contributions to this individual variability in cerebellar BOLD response, we measured cerebellar response to an established spatial *n*-back task (Callicott et al., 2003), using fMRI in a large sample that includes both monozygotic (MZ, who share all their genes) and dizygotic (DZ, who share 50% of their genes, on average) twin pairs. We improved the localization of cerebellar activation in response to a working memory task by normalizing the individual functional scans to the spatially unbiased infra-tentorial template (Diedrichsen, 2006).

Second, we investigated the phenotypic and genetic relationship between BOLD percent signal change in the cerebellum and cerebrum on a region-of-interest (ROI) basis. Using bivariate genetic modeling we investigated whether the same set of genes influences BOLD percent signal change in both the cerebrum and the cerebellum, i.e., whether there is genetic overlap between cerebellar areas of peak BOLD response and cerebral areas of peak BOLD response. In line with the existence of a cortico-cerebellar loop, we expected to find significant phenotypic correlations between prefrontal BOLD signal and cerebellar BOLD signal, but given the different cyto-architecture in the cerebral and cerebellar cortex, we expected partly specific genetic influences on the cerebellar BOLD signal.

Materials and methods

Participants

Twins between 16 and 30 years of age participated in the present study. The large majority of these individuals also participated in the Brisbane Twin Cognition study at age 16 (Wright and Martin, 2004). Thus, additional information was available on general intellectual ability (Full-scale intelligence quotient; FIQ; mean interval between cognitive testing and MRI scanning = 4.4 years; range 0–14 years), gestational duration, birth weight, and parental socio-economic status (rated according to the Australian Socioeconomic Index (SEI) 2006; McMillan et al., 2009). Gestational duration, birth weight, and parental socio-economic status were obtained from parental report, usually the mother. Prior to inclusion, twins were assessed for handedness, and screened (by self-report) for their suitability for imaging. Individuals with significant medical, psychiatric or neurological conditions, including head injuries, a current or past diagnosis of substance abuse, or current use of medication that could affect cognition were excluded. Zygosity was determined by genome-wide single nucleotide polymorphism genotyping (Illumina 610K chip) in ~90% of participants. If this was not available zygosity was established objectively by typing nine independent DNA microsatellite polymorphisms by polymerase chain reaction, and cross-checked with blood group results and phenotypic data, as described elsewhere (Wright and Martin, 2004).

A sample of 430 twins, which included 60 MZ pairs (47 female, 13 male pairs), 76 DZ pairs (39 female, 12 male, 25 opposite sex pairs), and 158 unpaired twins (114 female, 44 male pairs), aged 20.8 ± 3.1 years (mean ± s.d.), and all right handed, was included in our analyses. Unpaired twins did not contribute to the estimation of the genetic and environmental parameters, but they did contribute to the estimation of mean and variance effects, allowing more accurate estimation of phenotypic correlations and phenotypic effects. The majority of twin pairs (both MZ and DZ) were scanned on the same day (86%), with the remainder, on average, within 13 days of each other. A subset of this sample was included in our previous study on working memory brain activation in the cerebrum (60 of 430, 14%) (Blokland et al., 2011). However, in that study we did not explicitly examine the cerebellum. That is, the sample was not selected according to cerebellar coverage in their fMRI scans, whereas the sample here is a select subset of the cohort with full cerebellar coverage.

Human Research Ethics Committees of the Queensland Institute of Medical Research, University of Queensland, and Uniting Health Care approved the study. Written informed consent was obtained from each participant, and from a parent or legal guardian if the participant was under 18. Each participant received a $100 gift voucher in appreciation of their time.

Experimental procedure

Imaging was conducted on a 4 Tesla Bruker Medspec whole body scanner (Bruker, Germany) in Brisbane, Australia. Functional images were acquired using a T2*-weighted gradient echo planar imaging (EPI) sequence, sensitive to blood oxygen level-dependent (BOLD) contrast (interleaved; repetition time, TR = 2100 ms; echo time, TE = 30 ms; flip angle = 90°; field of view, FOV = 230 × 230 mm). Geometric distortions in the EPI images caused by magnetic field inhomogeneities at high-field were corrected using a point-spread mapping approach (Zeng and Constable, 2002). Over a continuous imaging run, 127 axial brain volumes were acquired, with 36 coronal slices of 3 mm thickness (64 × 64 matrix; voxel size 3.6 × 3.6 × 3.0 mm), and with a 0.6 mm slice gap. In addition to the functional scans, 3D T1-weighted anatomical images were acquired (MP-RAGE; TR = 1500 ms; TE = 3.35 ms; TI = 700 ms; pulse angle = 8°; coronal orientation; FOV = 230 mm; 256 × 256 × 256 matrix; slice thickness = 0.9 mm).

During functional imaging, participants performed the 0-back and 2-back versions of a block design spatial, numerical *n*-back working memory task based on Callicott et al. (2003). In this task, a number (1–4, randomized) was presented in a fixed position in one of the four corners of a diamond-shaped square. For the 0-back condition, the task required a simple button press in response to the number displayed, using a response box with four buttons arranged in the same configuration as the numbers presented on the screen. For the 2-back condition, participants pressed the key corresponding to the number presented two trials before the current one, thus requiring both the maintenance of the last 2 numbers in memory and the updating of these remembered stimuli as each new stimulus was presented. See Blokland et al. (2008, 2011) for a full task description. Participants were fully trained on the task, prior to being positioned in the scanner. The importance of effort and commitment to the task was emphasized. Task performance was measured as the percentage of correct responses (accuracy) and average response time (RT; across correct trials) for each of the task conditions separately.