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Chapter 8

Anxiety modulates the relation between ADHD severity and working memory-related brain activity

Under review as: Dennis van der Meer, Pieter J. Hoekstra, Daan van Rooij, Anderson M. Winkler, Hanneke van Ewijk, Dirk J. Heslenfeld, Jaap Oosterlaan, Stephen V. Faraone, Barbara Franke, Jan K. Buitelaar & Catharina A. Hartman. Anxiety modulates the relation between attention-deficit/hyperactivity disorder severity and working memory-related brain activity.

Abstract

Introduction: Individuals with attention-deficit/hyperactivity disorder (ADHD) often have heightened levels of anxiety, which has been associated with worse performance on working memory tasks. Knowledge of the neural pathways underlying the combined presence of ADHD and anxiety may aid in a better understanding of their co-occurrence. Therefore, we investigated how anxiety modulates the effect of ADHD severity on neural activity during a visuospatial working memory (VSWM) task.

Methods: Neuroimaging data was available for 371 adolescents and young adults participating in the multicentre cohort study NeuroIMAGE (average age 17.1 years). We analysed the effects of ADHD severity, anxiety severity, and their interaction on task accuracy, and on neural activity associated with working memory (VSWM trials minus baseline), and memory load (high memory load trials minus low load trials).

Results: Anxiety significantly modulated the relation between ADHD severity and neural activity in the cerebellum for the working memory contrast, and bilaterally in the striatum and thalamus for the memory load contrast.

Discussion: We found that ADHD with co-occurring anxiety is associated with lowered neural activity during a VSWM task in regions important for information gating. This fits well with previous theorizing on ADHD with co-occurring anxiety, and illustrates the neurobiological heterogeneity of ADHD.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is associated with heightened levels of anxiety; up to 50 percent of individuals with ADHD have one or more comorbid anxiety disorders (Schatz & Rostain, 2006, Yoshimasu *et al.*, 2012). Higher rates of anxiety disorders are found in both clinical and epidemiological samples of individuals with ADHD, even when controlling for overlap in symptomatology (Angold *et al.*, 1999, Pliszka, 2000). Given reports of significant effects of co-occurring anxiety on the cognitive functioning of individuals with ADHD (Bloemsma *et al.*, 2013), their clinical presentation (March *et al.*, 2000), and response to medication (M. T. A. Cooperative Group, 1999, Bedard & Tannock, 2008, Tannock *et al.*, 1995), some authors have suggested that ADHD with comorbid anxiety represents a distinct subtype of ADHD (Jensen *et al.*, 1997).

Visuospatial working memory (VSWM) tasks are well suited to investigate how co-occurring anxiety may influence ADHD-related cognitive impairments. Impaired working memory is a core cognitive deficit in ADHD, with strongest deficits in VSWM (Martinussen *et al.*, 2005). Working memory is also a strong determinant of functional outcomes such as academic achievement (Rennie *et al.*, 2014), which makes it an important research target. Cognitive interference theories predict that high levels of anxiety have adverse effects on the performance of cognitive tasks, as irrelevant thoughts may interfere with information processing (Sarason, 1984). Anxiety has been rather inconsistently associated with lowered VSWM performance (Shackman *et al.*, 2006, Aronen *et al.*, 2005, Staugaard, 2010), but has also been reported to interact with working memory capacity, such that only individuals with low capacity showed a negative relation between anxiety level and task performance (Owens *et al.*, 2014). Given that ADHD is associated with impaired working memory, such findings suggest that anxiety may be particularly detrimental if co-occurring with ADHD. This is in accordance with several reports that individuals with ADHD and comorbid anxiety are less accurate on working memory tasks than those who have ADHD without anxiety (Jarrett *et al.*, 2012, Bedard & Tannock, 2008, Skirbekk *et al.*, 2011, Pliszka, 1989), although these findings have not always been replicated (Vance *et al.*, 2013, Manassis *et al.*, 2007).

Neuroimaging data may provide a more sensitive and informative measure of working memory impairments than data on behavioural performance. VSWM is primarily associated with activity of frontal-parietal brain regions, in line with the role of these regions in executive functioning and spatial awareness (Curtis, 2006). Longitudinal studies of VSWM capacity have further shown that its development is associated with increased neural activity and connectivity of these frontal and parietal, as well as striatal, regions (Darki & Klingberg, 2014). The few functional magnetic resonance imaging (fMRI) studies on the relation between ADHD and VSWM have not produced a clear pattern of results, as they reported either higher or lower activity of these neural circuits in individuals with ADHD compared to controls, or no differences at all (Li *et al.*, 2014, Vance *et al.*, 2007, Silk *et al.*, 2005, van Ewijk *et al.*, 2015). Anxiety has been associated mostly, though also inconsistently, with heightened activity in frontal regions during working memory, particularly the dorsolateral prefrontal cortex (DLPFC; Fales *et al.*, 2008; Park *et al.*,

2016, Denkova *et al.*, 2010, Moon & Jeong, 2015, Basten *et al.*, 2012). To our knowledge, no study to date has investigated whether ADHD and anxiety interact on working-memory related brain activity.

We hypothesized that the neurobiological pathways underlying ADHD with co-occurring anxiety may differ from those that have been found for ADHD or anxiety individually, thereby contributing to the large heterogeneity observed in behavioural and neuroimaging studies. Because of these possible differences, we did not restrict our analyses to brain regions commonly reported to be associated with ADHD or anxiety but employed a whole-brain approach. We carried out the analyses in an adolescent and young adult sample (mean age 17.1 years) consisting of individuals with ADHD, individuals with subthreshold ADHD, and healthy comparison subjects, thus enabling analysis within a wide range of ADHD severity in accordance with the continuous distribution of ADHD within the population (Levy *et al.*, 1997).

Methods

Participants were selected from the NeuroIMAGE study, a follow-up of the Dutch part of the International Multicentre ADHD Genetics (IMAGE) study (von Rhein *et al.*, 2014). NeuroIMAGE included 365 families with at least one child with ADHD and at least one biological sibling (regardless of ADHD diagnosis) and 148 control families with at least one child without any formal or suspected ADHD diagnosis in any of the family members or their first-degree relatives. To be included in NeuroIMAGE, participants had to be of European Caucasian descent, between ages 5 and 30 years, have an IQ ≥ 70, and have no diagnosis of autism, epilepsy, general learning difficulties, brain disorders, or known genetic disorders. The study was approved by the regional ethics committee (CMO Regio Arnhem – Nijmegen; 2008/163; ABR: NL23894.091.08) and the medical ethical committee of the VU University Medical Centre. All participants, and their parents (if the participant was younger than 18 years), signed informed consent; parents signed informed consent for participants under twelve years of age.

For the analyses reported in this paper, 371 participants, from 234 families, had complete data. Of the 371 participants, 122 participants had an ADHD diagnosis, 49 participants had subthreshold ADHD (i.e., had elevated levels of ADHD symptoms without meeting the full criteria for an ADHD diagnosis), and 200 participants were healthy controls. ADHD diagnoses were made in accordance with DSM IV-TR criteria, on the basis of a combination of a semi-structured interview and the Conners Rating Scales (Conners *et al.*, 1998). Of those with an ADHD diagnosis, 93 (76%) had a history of treatment with stimulant medication. Sixteen (33%) of those with subthreshold ADHD and one healthy control (0.5%) had a history of receiving stimulant medication. Participants were asked to withhold use of their stimulant medication or other psychoactive drugs for 48 hours before measurement. Mean age of this sample was 17.1 years (standard deviation (SD) 3.4; range 7.7 to 29.2 years) and 52.3% were males. More information on the NeuroIMAGE study, its diagnostic algorithm, and its participants, is presented in the Supplementary Information (SI) and in von Rhein *et al.*, (2014).

ADHD outcome measure

In order to retain as much information as possible, we used a continuous measure of ADHD severity, the raw score on subscale N of the Conners Parent Rating Scale (CPRS; Conners *et al.*, 1998). This score ranged from 0 to 53, with an average of 11.3 (SD 11.5). This measure was chosen because it was available for all participants, from both ADHD families and control families.

Anxiety outcome measure

Anxiety severity was measured with the emotion subscale of the Strengths and Difficulties Questionnaire (SDQ-E; Goodman, 2001). The SDQ is well validated, has good internal consistency, and is reliable across informants and time (Achenbach *et al.*, 2008). It has been shown to be a good dimensional measure of psychopathology in children, with the odds of a psychiatric disorder increasing at a constant rate across the range of scores (Goodman & Goodman, 2009). The emotion subscale

contains the following items: “Many worries, often seems worried”, “Often unhappy, down-hearted or tearful”, “Often complains of headaches, stomach-aches or sickness”, “Nervous or clingy in new situations, easily loses confidence”, and “Many fears, easily scared”. Ratings on the emotion subscale have been shown to be associated with the presence of common comorbid anxiety disorders, but not depression, in an ADHD sample (Bekker *et al.*, 2013). Each item was rated both by the participant (if older than 12 years of age) and a parent on a three-point scale (“not true” (0), “somewhat true” (1), “certainly true” (2)). In order to gain maximum sensitivity to often underreported anxiety symptoms (Pliszka, 1992), we selected the highest of either of the two informants’ scores on each item and subsequently summed these five item scores together, for a minimum of 0 and a maximum of 10. The average score for the whole sample was 4.16 (SD 2.03). Additional information on the composition of this measure is given in SI, as well as an overview of the scores per item and per informant.

Socio-economic status

As a measure of socio-economic status (SES), the highest, successfully completed education level of the parents was re-coded into a measure reflecting years of education. This scale contained nine levels, ranging from 0 (no formal education) to 17 (university) years of education (Buis, 2010). The average of both parents was used, which, in this sample, ranged from 5 to 17 with an average of 12.2 (SD 2.47).

Visuospatial working memory task

To measure VSWM, we used a spatial span task (Klingberg *et al.*, 2002) described more fully elsewhere (van Ewijk *et al.*, 2014). The task consisted of two trial types (baseline and working memory) and two memory loads (low and high). In working memory trials, either three (low load) or six (high load) yellow circles were sequentially displayed on a 4x4 grid for 500ms, with a 500ms inter-stimulus interval. This was followed by a 2000ms response window, during which the participant was asked whether a probe, consisting of a question mark and a number, was presented in the correct spatial location and the right temporal order of the presentation of cues, as indicated by the number. Participants answered by pressing one of two buttons representing ‘yes’ and ‘no’. We also included baseline trials for the low and high memory load conditions, both consisting of sequentially presented red circles in the four corners of the grid, followed by a probe (always the number 8). Participants were asked to pay attention but not to try to remember the sequence, and to press the ‘no’ button. Feedback on all trials was presented after the response in the form of a green or red coloured bar below the probe (for correct and incorrect responses, respectively). Accuracy on all trial types was determined as percentage correct responses. The task consisted of 4 blocks of 24 trials each, presented in fixed random order, for a total time of approximately 16 minutes.

fMRI analysis

Details on fMRI data acquisition, handling of runs with excessive head motion, and pre-processing are given in the SI.

For the within-run analyses, we constructed a general linear model containing regressors for each of the four types of trial (i.e. low and high memory load, for both working memory and baseline trials), modelled from the start of the trial to the onset of the probe, in accordance with the approach by Klingberg *et al.* (2002). We also included regressors for feedback on correct and incorrect trials in the model, to remove unexplained variance. Further, global signal from cerebral spinal fluid and white matter, and standard and extended motion parameters were included as nuisance covariates. Beta-maps from all runs were concatenated using a single-subject fixed effects model, and the resulting maps were transformed to a study-specific template. Our contrasts of interest were *mean working memory* (both memory loads averaged, working memory trials minus baseline trials) and *memory load difference* (high minus low memory load trials), both consisting of the regressors for working memory trials corrected for regressors of the baseline trials of the same load.

Whole-brain analysis was conducted with the use of a variant of FSL's 'randomise' algorithm, which employs nonparametric permutation inference (Winkler *et al.* 2015). To take into account the family relatedness present in the sample, we specified exchangeability blocks based on family membership, which ensured that only the rearrangements of the data that respect exchangeability were used (Winkler *et al.*, 2015). The main model consisted of ADHD severity, anxiety severity, their interaction, and age, sex, socio-economic status, and scanning location as additional covariates. All regressors were mean-centred. The data were permuted 5000 times. We used cluster mass-based inference, with an initial cluster-forming threshold of $z=2.3$. We set the whole brain family-wise error rate corrected p-value at $p < .01$ to correct for the fact that we were looking at whole-brain maps for both the working memory contrast and memory load contrast.

Analysis of task accuracy and cluster data

All additional analyses were performed in R, v3.1.1, with linear mixed effects models. To account for the non-independence of the observations, due to the inclusion of siblings in the sample, we estimated a random intercept for family. We used a repeated measures model, with memory load (high and low load) as a within-subject factor, and accuracy on baseline trials as a covariate to control for basic processing or motivational effects. All models had age, sex, socio-economic status, and scanner location included as covariates. All continuous variables were standardized, because they were measured on widely differing scales.

We ran extended models on the task accuracy data to assess the effects of memory load. These models included additional interaction terms of memory load condition (low or high load) with ADHD, anxiety, and their interaction (i.e. a three-way interaction).

We further extracted the mean signal from significant clusters from the whole-brain analysis and calculated Cohen's f^2 as a measure of effect size for the significant predictors, as shown in Table 1. As a post-hoc analysis, we also ran a mixed effects repeated measures model where working memory task accuracy was regressed on

the cluster data, corrected for baseline accuracy, in order to couple our results at the neural level to task performance.

Sensitivity analyses

Because of our dimensional approach to studying ADHD and its interaction with anxiety, we report demographic information on the participants split by ADHD diagnostic status in the SI. We further checked whether the findings were influenced by scanning location, by adding an interaction term between testing location and our predictors of interest and rerunning the analysis on the data from clusters found to be significant in the main analysis. In addition, while stimulant medication was discontinued 48h before measurement, we also reran the analyses with a covariate for duration of stimulant medication use, in the subset of individuals with an ADHD diagnosis.

Results

Task accuracy

ADHD severity was associated with lower accuracy on the VSWM task, independent of anxiety (B=-0.10, SE=0.05, p=.04), in accordance with a previous report on a subsample used in this study (van Ewijk *et al.*, 2014). There was no conditional effect of anxiety severity (B=-0.05, SE=0.04, p=.26), nor did anxiety severity modulate the effect of ADHD severity on working memory task accuracy (B=0.01, SE=0.03, p=.77).

Memory load was a highly significant predictor of accuracy (B=-0.45, SE=0.06, p<.001), i.e., the harder the task the worse participants performed, confirming the success of the task manipulation. Memory load did not significantly modulate the effect of ADHD severity, anxiety severity, or their interaction, on task accuracy (all p>.19).

Working memory related neural activation

The mean working memory contrast was associated with widespread brain activity across superior and middle frontal gyri, superior parietal cortex, thalamus, striatum, and cerebellum. Further information on these clusters is provided in SI.

We found ADHD severity to be negatively correlated with working memory-associated brain activity in the left frontal pole. There was no conditional effect of anxiety severity. Anxiety did modulate the relation between ADHD severity and brain activity in the right cerebellum, such that it correlated positively with ADHD severity for individuals with low levels of anxiety and negatively for individuals with high levels of anxiety, see Figure 1. More information on these clusters is reported in Table 1. Figure 2 shows the location of these clusters overlaid on the sample's average anatomical image.

Table 1. Summary of the clusters that showed a significant correlation (PFWE < 0.01) between brain activity and the predictors. The upper part of the table shows the clusters correlating with activity for the mean working memory contrast, the bottom part of the table shows those associated with memory load.

Contrast	*Predictor*	*Direction*	*Region*	*X*	*Y*	*Z*	*Size*	Z_{max}	*Cohen's* f^2
Working memory	ADHD	Negative	L Frontal Pole, Superior Frontal Gyrus	-4	42	56	918	4.09	.011
	Anxiety	N/A							
	ADHD * Anxiety	Negative	R Cerebellar Crus I, Crus II VIIIa	18	-88	-34	762	3.54	.028
Memory load	ADHD	N/A							
	Anxiety	N/A							
	ADHD * Anxiety	Negative	R Thalamus, Caudate, Putamen	12	-18	16	682	4.46	.047
		Negative	L Thalamus, Caudate, Putamen, Pallidum	-20	0	0	516	3.93	.055

Note: X, Y, Z coordinates are in MNI-space in mm, and represent the peak of the cluster. The anatomical labels are according to the Harvard-Oxford atlas. MNI=Montreal Neurological Institute; Z_{max}*= Z-score at the peak of the cluster.*

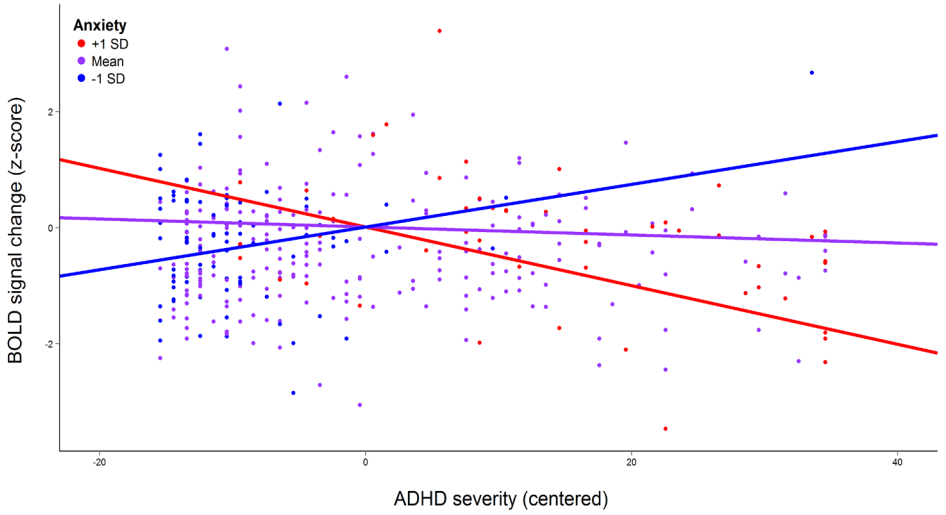

Figure 1. Modulating effect of anxiety severity on the association between ADHD severity and blood-oxygen dependent level (BOLD) signal change in the cerebellum while subjects performed the visuospatial working memory task. Regression lines indicate effect of ADHD severity for average anxiety severity (purple line), as well as one standard deviation (SD) above (blue line) and one SD below average anxiety severity (red line).

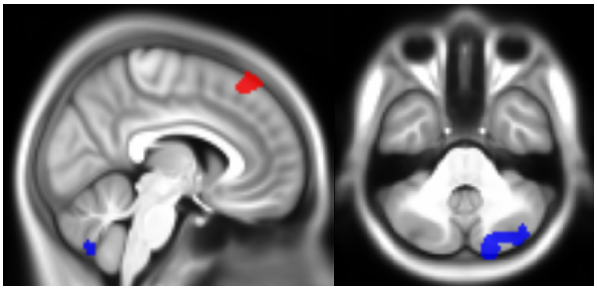

Figure 2. Location of the clusters where there was an effect of ADHD severity (red) and the interaction between anxiety and ADHD (blue) on brain activity for the mean working memory contrast, overlaid onto the sample's average anatomical image at x=6, y= -60, z=-36).

Memory load-related neural activation

Anxiety severity modulated the association of ADHD severity with brain activity bilaterally in the dorsal striatum and thalamus for the memory load difference contrast. Individuals with high anxiety had a negative correlation between ADHD severity and task difficulty-associated brain activity, whereas those with low anxiety displayed a positive slope (Figure 3). Further information on these clusters is reported in Table 1 and Figure 4. Post-hoc analysis showed that task accuracy was significantly positively correlated with the mean values of these clusters for the memory load difference contrast (B=0.17, SE=0.05, p<.001). In other words, an increase of basal ganglia activity when the task became more difficult was associated with higher task accuracy.

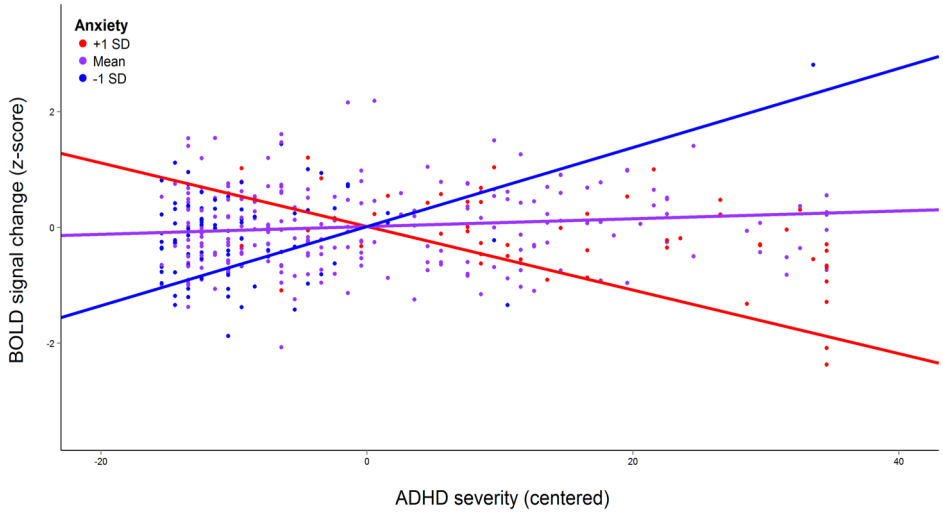

Figure 3. Graph of the mean blood-oxygen level dependent (BOLD) signal change at the clusters in the striatum and thalamus (clusters combined), for the memory load contrast. Regression lines indicate effect of ADHD severity for average anxiety severity (purple line), as well as one standard deviation above (blue line) and below (red line) average anxiety severity.

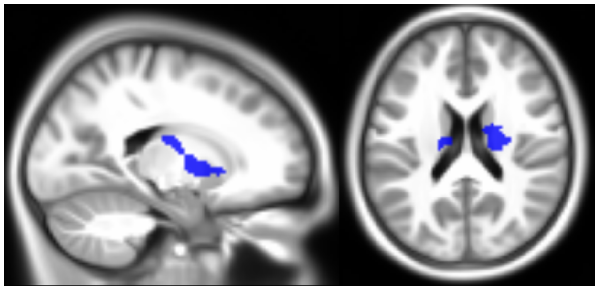

Figure 4. Location of the clusters where there was an interaction effect between anxiety and ADHD (blue) on brain activity for the memory load difference contrast, overlaid onto the sample's average anatomical image z=-20, y=-10, z=20.

Sensitivity analyses

Results from the sensitivity analyses can be found in SI. There were no interaction effects between the predictors of interest and testing location on brain activity in the significant clusters. Further, the predictors found to be significant in the main analyses remained significant when treatment duration was added as a covariate.

Discussion

We aimed to investigate whether anxiety modulates the association between ADHD severity and neural correlates of VSWM. Such interaction effects would indicate that previously reported differences in working memory performance between people with ADHD with and without comorbid anxiety (Jarrett *et al.*, 2012; Bedard & Tannock, 2008, Skirbekk *et al.*, 2011, Pliszka, 1989) cannot be attributed solely to additive effects of ADHD and anxiety, and illustrate that their co-occurrence carries significant information that may aid in explaining some of the etiological and phenotypic heterogeneity typical of ADHD research. We found that anxiety severity modulates the association between ADHD severity and brain activity in the cerebellum, basal ganglia and thalamus.

In our sample, ADHD was associated with lowered accuracy on VSWM task performance (van Ewijk *et al.*, 2014), in accordance with meta-analyses (Martinussen *et al.*, 2005, Willcutt *et al.*, 2005). We also replicated a previous finding of a negative relation between ADHD severity and activity in frontal brain regions underlying VSWM (Silk *et al.*, 2005). Specifically, we found less activity in the left frontal pole and superior frontal gyrus, which are known to respond more to spatial tasks than to object-based tasks (Courtney *et al.*, 1996), when working memory must be continuously updated, and when temporal order must be maintained (Wager & Smith, 2003), as was the case in our task. Further, these effects were independent of co-occurring anxiety.

While cognitive interference theories state that anxiety causes irrelevant information to interfere with task performance (Sarason, 1984), we did not find effects of anxiety on VSWM brain activation or performance. Eysenck *et al.* theorized that the worrying of anxious individuals can lead to lowered performance on cognitive tasks by occupying resources, but that this is partly compensated by greater on-task effort (Eysenck & Calvo, 1992). This may cause the negative effect of anxiety on working memory performance to only become apparent at higher difficulty levels, or in individuals with low working memory capacity, as has been previously reported (Owens *et al.*, 2014), rather than there being a direct relation between anxiety and cognitive functioning (Eysenck *et al.*, 2007). The inconsistent results from previous behavioural and neuroimaging studies, as well as our null findings, attest to a complex relation between anxiety and working memory that is dependent on task and sample characteristics (Jarrett, 2016).

Consistent with this reasoning, we did find that anxiety interacts with ADHD severity on neural activity. During the working memory task, higher levels of anxiety were associated with a more negative relation between ADHD severity and neural activity in the cerebellum. The cerebellum is increasingly recognized to be important for the development of cognitive functions (Riva & Giorgi, 2000), including working memory (Ford *et al.*, 2014), and has been previously shown to be less active in individuals with ADHD compared to controls during working memory tasks (Valera *et al.*, 2005, Massat *et al.*, 2012). Its specific function during working memory has been characterized as that of a gatekeeper, based on findings that individuals with lesions in the cerebellum are more susceptible to interference by irrelevant

information during tasks reliant on VSWM (Baier *et al.*, 2014). Less cerebellar activity therefore could indicate that ADHD co-occurring anxiety is associated with lowered information filtering.

The interaction between ADHD and anxiety was further associated with less task difficulty-related brain activity in the caudate nucleus, putamen, and thalamus, supporting the notion that ADHD with co-occurring anxiety may involve lowered information filtering. Activity in the basal ganglia and thalamus during working memory has been shown to predict the extent to which only relevant information is stored, a strong determinant of working memory capacity (McNab & Klingberg, 2008). Activation of the basal ganglia allows for the disinhibition of the thalamus and subsequent processing of stimuli for working memory (Gisiger & Boukadoum, 2011). Information filtering should become more important with increasing task difficulty, as higher amounts of information may become overwhelming and reduce performance. Results from our post hoc analysis are consistent with this reasoning, as basal ganglia activity due to increased memory load was associated with higher task accuracy. Further, it is also in line with Levy's theory of ADHD with comorbid anxiety, which states that divergent gating of the basal ganglia leads to increased detection of threatening stimuli, contributing to feelings of anxiety (Levy, 2004). The important role of dopamine in this process (Grace *et al.*, 2007) conceivably plays a role in the repeatedly reported finding that individuals with ADHD with comorbid anxiety respond less well to stimulant medications than those without comorbid anxiety (Pliszka, 1989, M.T.A. Cooperative Group, 1999, Tannock *et al.*, 1995).

We did not find evidence that anxiety modulates the relation between ADHD severity and task performance. Findings from studies into the relation between working memory performance and ADHD with comorbid anxiety have been mixed, with some reporting significant differences (Jarrett *et al.*, 2012; Bedard & Tannock 2008, Skirbekk *et al.* 2011, Pliszka, 1989) and others reporting null findings (Vance *et al.*, 2013, Manassis *et al.*, 2007). This inconsistency may be partly due to differences in task characteristics between studies; our findings on brain activity associated with varying memory load described above suggest that particularly task difficulty may be an important factor in outcomes of neuropsychological research of ADHD with co-occurring anxiety; problems with information gating may only be captured by measures of task performance when the stimulus rate increases and the information flow gets too high to adequately process. We therefore speculate that at higher task difficulties than present in the current study, less activity of the basal ganglia and cerebellum associated with the interaction between anxiety and ADHD severity may also be associated with overt performance difficulties on working memory tasks.

Strengths of this study include a large sample size and use of dimensional measures, allowing for optimal detection of effects. Furthermore, to our knowledge, we are the first to investigate the effects of ADHD co-occurring anxiety at the neural level, thereby providing information on its neurobiological correlates. The foremost limitation is the observational, cross-sectional design of our study, which prevents strong inferences about any causal relationship between the developmental course of

ADHD, anxiety, and the brain. One might also argue that our measure of anxiety is not entirely specific to anxiety, but also measures depression. However, in ADHD samples, this instrument was found to only significantly relate to commonly occurring comorbid anxiety disorders, and not to mood disorders (Bekker *et al.*, 2013), see also SI. Given that we are the first to report on the neural correlates of ADHD co-occurring anxiety, future studies are needed to determine whether our findings of involvement of the cerebellum and basal ganglia replicate in independent samples.

In conclusion, we found that ADHD with co-occurring anxiety is associated with patterns of brain activity beyond the additive effects of ADHD and anxiety. This indicates that the combined presence of symptoms of ADHD and anxiety may be due to partly different neurobiological correlates than either separately. Given their common co-occurrence, these interaction effects may form a source of heterogeneity that contributes to inconsistent findings in studies of ADHD and anxiety. The involvement of brain regions important for gating of information could suggest that measures of the combined presence of ADHD and anxiety may capture problems with filtering of information, in line with Levy's biological theory of ADHD with comorbid anxiety (Levy, 2004). Further research on the neurobiological correlates of ADHD and co-occurring anxiety may not only aid in resolving some of the heterogeneity of ADHD, but ultimately also have clinical implications by enabling a more effective treatment tailored to the individual's profile (M.T.A. Cooperative Group 1999).

References

Achenbach, T. M., A. Becker, M. Dopfner, E. Heiervang, V. Roessner, H. C. Steinhausen, and A. Rothenberger. 2008. "Multicultural assessment of child and adolescent psychopathology with ASEBA and SDQ instruments: research findings, applications, and future directions." *Journal of child psychology and psychiatry, and allied disciplines* 49 (3):251-75. doi: 10.1111/j.1469-7610.2007.01867.x; 10.1111/j.1469-7610.2007.01867.x.

Angold, A., E. J. Costello, and A. Erkanli. 1999. "Comorbidity." *Journal of child psychology and psychiatry, and allied disciplines* 40 (1):57-87.

Aronen, ET, Virve Vuontela, M-R Steenari, Juha Salmi, and Synnöve Carlson. 2005. "Working memory, psychiatric symptoms, and academic performance at school." *Neurobiol Learn Mem* 83 (1):33-42.

Baier, B., N. G. Muller, and M. Dieterich. 2014. "What part of the cerebellum contributes to a visuospatial working memory task?" *Annals of Neurology*. doi: 10.1002/ana.24272 [doi].

Basten, U., C. Stelzel, and C. J. Fiebach. 2012. "Trait anxiety and the neural efficiency of manipulation in working memory." *Cognitive, affective & behavioural neuroscience* 12 (3):571-88. doi: 10.3758/s13415-012-0100-3 [doi].

Bedard, A. C., and R. Tannock. 2008. "Anxiety, methylphenidate response, and working memory in children with ADHD." *Journal of Attention Disorders* 11 (5):546-57. doi: 1087054707311213 [pii].

Bekker, J., D. Bruck, and E. Sciberras. 2013. "Congruent Validity of the Strength and Difficulties Questionnaire to Screen for Comorbidities in Children With ADHD." *Journal of Attention Disorders*. doi: 1087054713496462 [pii].

Bloemsma, J. M., F. Boer, R. Arnold, T. Banaschewski, S. V. Faraone, J. K. Buitelaar, J. A. Sergeant, N. Rommelse, and J. Oosterlaan. 2013. "Comorbid anxiety and neurocognitive dysfunctions in children with ADHD." *European child & adolescent psychiatry* 22 (4):225-34. doi: 10.1007/s00787-012-0339-9 [doi].

Buis, Maarten L. 2010. "Scaling levels of education." Faculty of Social Sciences, VU-University Amsterdam.

Conners, C. K., G. Sitarenios, J. D. Parker, and J. N. Epstein. 1998. "The revised Conners Parent Rating Scale (CPRS-R): factor structure, reliability, and criterion validity." *Journal of abnormal child psychology* 26 (4):257-68.

Courtney, S. M., L. G. Ungerleider, K. Keil, and J. V. Haxby. 1996. "Object and spatial visual working memory activate separate neural systems in human cortex." *Cerebral cortex (New York, N.Y.: 1991)* 6 (1):39-49.

Curtis, C. E. 2006. "Prefrontal and parietal contributions to spatial working memory." *Neuroscience* 139 (1):173-80. doi: http://dx.doi.org/10.1016/j.neuroscience.2005.04.070.

Darki, F., and T. Klingberg. 2014. "The Role of Fronto-Parietal and Fronto-Striatal Networks in the Development of Working Memory: A Longitudinal Study." *Cerebral cortex (New York, N.Y.: 1991)*. doi: bht352 [pii].

Denkova, Ekaterina, Gloria Wong, Sanda Dolcos, Keen Sung, Lihong Wang, Nicholas Coupland, and Florin Dolcos. 2010. "The impact of anxiety-inducing distraction on cognitive performance: a combined brain imaging and personality investigation." *PloS one* 5 (11):e14150.

Eysenck, Michael W, Nazanin Derakshan, Rita Santos, and Manuel G Calvo. 2007. "Anxiety and cognitive performance: attentional control theory." *Emotion* 7 (2):336.

Eysenck, Michael W., and Manuel G. Calvo. 1992. "Anxiety and performance: The processing efficiency theory." *Cognition & Emotion* 6 (6):409-34.

Fales, C. L., D. M. Barch, G. C. Burgess, A. Schaefer, D. S. Mennin, J. R. Grey, and T. S. Braver. 2008. "Anxiety and cognitive efficiency: differential modulation of transient and sustained neural activity during a working memory task." *Cognitive, affective & behavioural neuroscience* 8 (3):239-53.

Ford, B. Q., I. B. Mauss, A. S. Troy, A. Smolen, and B. Hankin. 2014. "Emotion Regulation Moderates the Risk Associated With the 5-HTT Gene and Stress in Children." *Emotion (Washington, D.C.)*. doi: 2014-21029-001 [pii].

Gisiger, T., and M. Boukadoum. 2011. "Mechanisms Gating the Flow of Information in the Cortex: What They Might Look Like and What Their Uses may be." *Frontiers in computational neuroscience* 5:1. doi: 10.3389/fncom.2011.00001 [doi].

Goodman, A., and R. Goodman. 2009. "Strengths and difficulties questionnaire as a dimensional measure of child mental health." *Journal of the American Academy of Child and Adolescent Psychiatry* 48 (4):400-3. doi: 10.1097/CHI.0b013e3181985068 [doi].

Goodman, R. 2001. "Psychometric properties of the strengths and difficulties questionnaire." *Journal of the American Academy of Child and Adolescent Psychiatry* 40 (11):1337-45. doi: S0890-8567(09)60543-8 [pii].

Grace, A. A., S. B. Floresco, Y. Goto, and D. J. Lodge. 2007. "Regulation of firing of dopaminergic neurons and control of goal-directed behaviours." *Trends Neurosci* 30 (5):220-7. doi: 10.1016/j.tins.2007.03.003.

Jarrett, M. A. 2016. "Attention-deficit/hyperactivity disorder (ADHD) symptoms, anxiety symptoms, and executive functioning in emerging adults." *Psychol Assess* 28 (2):245-50. doi: 10.1037/pas0000190.

Jarrett, M. A., J. C. Wolff, T. E. Davis, 3rd, M. J. Cowart, and T. H. Ollendick. 2012. "Characteristics of Children With ADHD and Comorbid Anxiety." *Journal of Attention Disorders*. doi: 1087054712452914 [pii].

Jensen, P. S., D. Martin, and D. P. Cantwell. 1997. "Comorbidity in ADHD: implications for research, practice, and DSM-V." *Journal of the American Academy of Child and Adolescent Psychiatry* 36 (8):1065-79. doi: 10.1097/00004583-199708000-00014.

Klingberg, T., H. Forssberg, and H. Westerberg. 2002. "Increased brain activity in frontal and parietal cortex underlies the development of visuospatial working memory capacity during childhood." *Journal of cognitive neuroscience* 14 (1):1-10. doi: 10.1162/089892902317205276 [doi].

Levy, F. 2004. "Synaptic gating and ADHD: a biological theory of comorbidity of ADHD and anxiety." *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 29 (9):1589-96. doi: 10.1038/sj.npp.1300469 [doi].

Levy, F., D. A. Hay, M. McStephen, C. Wood, and I. Waldman. 1997. "Attention-deficit hyperactivity disorder: a category or a continuum? Genetic analysis of a large-scale twin study." *Journal of the American Academy of Child and Adolescent Psychiatry* 36 (6):737-44. doi: 10.1097/00004583-199706000-00009.

Li, Y., F. Li, N. He, L. Guo, X. Huang, S. Lui, and Q. Gong. 2014. "Neural hyperactivity related to working memory in drug-naive boys with attention deficit hyperactivity disorder." *Prog Neuropsychopharmacol Biol Psychiatry* 53:116-22. doi: 10.1016/j.pnpbp.2014.03.013.

Manassis, Katharina, Rosemary Tannock, Arlene Young, and Shonna Francis-John. 2007. "Cognition in anxious children with attention deficit hyperactivity disorder: a comparison with clinical and normal children." *Behavioural and Brain Functions* 3 (1):1-10. doi: 10.1186/1744-9081-3-4.

March, J. S., J. M. Swanson, L. E. Arnold, B. Hoza, C. K. Conners, S. P. Hinshaw, L. Hechtman, *et al.* 2000. "Anxiety as a predictor and outcome variable in the multimodal treatment study of children with ADHD (MTA)." *Journal of abnormal child psychology* 28 (6):527-41.

Martinussen, R., J. Hayden, S. Hogg-Johnson, and R. Tannock. 2005. "A meta-analysis of working memory impairments in children with attention-deficit/hyperactivity disorder." *Journal of the American Academy of Child and Adolescent Psychiatry* 44 (4):377-84. doi: S0890-8567(09)61489-1 [pii].

Massat, Isabelle, Hichem Slama, Martin Kavec, Sylvie Linotte, Alison Mary, Daniele Baleriaux, Thierry Metens, Julien Mendlewicz, and Philippe Peigneux. 2012. "Working Memory-Related Functional Brain Patterns in Never Medicated Children with ADHD." *PloS one* 7 (11):e49392. doi: 10.1371/journal.pone.0049392.

McNab, F., and T. Klingberg. 2008. "Prefrontal cortex and basal ganglia control access to working memory." *Nature neuroscience* 11 (1):103-7. doi: nn2024 [pii].

Moon, Chung-Man, and Gwang-Woo Jeong. 2015. "Functional neuroanatomy on the working memory under emotional distraction in patients with generalized anxiety disorder." *Psychiatry and clinical neurosciences* 69 (10):609-19.

M. T. A. Cooperative Group. 1999. "Moderators and mediators of treatment response for children with attention-deficit/hyperactivity disorder: the Multimodal Treatment Study of children with Attention-deficit/hyperactivity disorder." *Archives of General Psychiatry* 56 (12):1088-96.

Owens, M., J. Stevenson, J. A. Hadwin, and R. Norgate. 2014. "When does anxiety help or hinder cognitive test performance? The role of working memory capacity." *British journal of psychology (London, England: 1953)* 105 (1):92-101. doi: 10.1111/bjop.12009 [doi].

Park, Jong-Il, Gwang-Won Kim, Gwang-Woo Jeong, Gyung Ho Chung, and Jong-Chul Yang. 2016. "Brain Activation Patterns Associated with the Effects of Emotional Distracters during Working Memory Maintenance in Patients with Generalized Anxiety Disorder." *Psychiatry investigation* 13 (1):152-6.

Pliszka, S. R. 1992. "Comorbidity of attention-deficit/hyperactivity disorderand overanxious disorder." *Journal of the American Academy of Child and Adolescent Psychiatry* 31 (2):197-203. doi: S0890-8567(09)64439-7 [pii].

———. 2000. "Patterns of psychiatric comorbidity with attention-deficit/hyperactivity disorder." *Child and adolescent psychiatric clinics of North America* 9 (3):525-40, vii.

Pliszka, Steven R. 1989. "Effect of anxiety on cognition, behaviour, and stimulant response in ADHD." *Journal of the American Academy of Child & Adolescent Psychiatry* 28 (6):882-7.

Rennie, B., M. Beebe-Frankenberger, and H. L. Swanson. 2014. "A longitudinal study of neuropsychological functioning and academic achievement in children with and without signs of attention-deficit/hyperactivity disorder." *Journal of clinical and experimental neuropsychology* 36 (6):621-35. doi: 10.1080/13803395.2014.921284 [doi].

Riva, Daria, and Cesare Giorgi. 2000. "The cerebellum contributes to higher functions during development." *Brain* 123 (5):1051-61.

Sarason, Irwin G. 1984. "Stress, anxiety, and cognitive interference: Reactions to tests." *Journal of personality and social psychology* 46 (4):929-38. doi: 10.1037/0022-3514.46.4.929.

Schatz, D. B., and A. L. Rostain. 2006. "ADHD with comorbid anxiety: a review of the current literature." *Journal of Attention Disorders* 10 (2):141-9. doi: 10/2/141 [pii].

Shackman, Alexander J., Issidoros Sarinopoulos, Jeffrey S. Maxwell, Diego A. Pizzagalli, Aureliu Lavric, and Richard J. Davidson. 2006. "Anxiety selectively disrupts visuospatial working memory." *Emotion* 6 (1):40-61. doi: 10.1037/1528-3542.6.1.40.

Silk, T., A. Vance, N. Rinehart, G. Egan, M. O'Boyle, J. L. Bradshaw, and R. Cunnington. 2005. "Fronto-parietal activation in attention-deficit hyperactivity disorder, combined type: functional magnetic resonance imaging study." *The British Journal of Psychiatry* 187 (3):282-3. doi: 10.1192/bjp.187.3.282.

Skirbekk, Benedicte, Berit Hjelde Hansen, Beate Oerbeck, and Hanne Kristensen. 2011. "The relationship between sluggish cognitive tempo, subtypes of attention-deficit/hyperactivity disorder, and anxiety disorders." *Journal of abnormal child psychology* 39 (4):513-25.

Staugaard, Søren Risløv. 2010. "Threatening faces and social anxiety: a literature review." *Clinical psychology review* 30 (6):669-90.

Tannock, R., A. Ickowicz, and R. Schachar. 1995. "Differential effects of methylphenidate on working memory in ADHD children with and without comorbid anxiety." *Journal of the American Academy of Child and Adolescent Psychiatry* 34 (7):886-96. doi: 10.1097/00004583-199507000-00012.

Valera, E. M., S. V. Faraone, J. Biederman, R. A. Poldrack, and L. J. Seidman. 2005. "Functional neuroanatomy of working memory in adults with attention-deficit/hyperactivity disorder." *Biological psychiatry* 57 (5):439-47. doi: S0006-3223(04)01257-0 [pii].

van Ewijk, H., D. J. Heslenfeld, M. Luman, N. N. Rommelse, C. A. Hartman, P. Hoekstra, B. Franke, J. K. Buitelaar, and J. Oosterlaan. 2014. "Visuospatial working memory in ADHD patients, unaffected siblings, and healthy controls." *Journal of Attention Disorders* 18 (4):369-78. doi: 10.1177/1087054713482582 [doi].

van Ewijk, H., W. D. Weeda, D. J. Heslenfeld, M. Luman, C. A. Hartman, P. J. Hoekstra, S. V. Faraone, B. Franke, J. K. Buitelaar, and J. Oosterlaan. 2015. "Neural correlates of visuospatial working memory in attention-deficit/hyperactivity disorder and healthy controls." *Psychiatry Res* 233 (2):233-42. doi: 10.1016/j.pscychresns.2015.07.003.

Vance, A., M. Ferrin, J. Winther, and R. Gomez. 2013. "Examination of spatial working memory performance in children and adolescents with attention deficit hyperactivity disorder, combined type (ADHD-CT) and anxiety." *Journal of abnormal child psychology* 41 (6):891-900. doi: 10.1007/s10802-013-9721-4 [doi].

Vance, A., T. J. Silk, M. Casey, N. J. Rinehart, J. L. Bradshaw, M. A. Bellgrove, and R. Cunnington. 2007. "Right parietal dysfunction in children with attention deficit hyperactivity disorder, combined type: a functional MRI study." *Molecular psychiatry* 12 (9):826-32.

von Rhein, D., M. Mennes, H. van Ewijk, A. P. Groenman, M. P. Zwiers, J. Oosterlaan, D. Heslenfeld, *et al.* 2014. "The NeuroIMAGE study: a prospective phenotypic, cognitive, genetic and MRI study in children with attention-deficit/hyperactivity disorder. Design and descriptives." *European child & adolescent psychiatry*. doi: 10.1007/s00787-014-0573-4 [doi].

Wager, T. D., and E. E. Smith. 2003. "Neuroimaging studies of working memory: a meta-analysis." *Cognitive, affective & behavioural neuroscience* 3 (4):255-74.

Willcutt, Erik G, Alysa E Doyle, Joel T Nigg, Stephen V Faraone, and Bruce F Pennington. 2005. "Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review." *Biological psychiatry* 57 (11):1336-46.

Winkler, A. M., M. A. Webster, D. Vidaurre, T. E. Nichols, and S. M. Smith. 2015. "Multi-level block permutation." *NeuroImage*. doi: S1053-8119(15)00508-X [pii].

Yoshimasu, K., W. J. Barbaresi, R. C. Colligan, R. G. Voigt, J. M. Killian, A. L. Weaver, and S. K. Katusic. 2012. "Childhood ADHD is strongly associated with a broad range of psychiatric disorders during adolescence: a population-based birth cohort study." *Journal of child psychology and psychiatry, and allied disciplines* 53 (10):1036-43. doi: 10.1111/j.1469-7610.2012.02567.x [doi].