Neuronal activity during working memory performance in the developing brain of children and adolescents with Neurofibromatosis Type I

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Preface

"It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change".

- Charles Darwin -

This thesis would not have been possible without the help, support and knowledge of some important persons around me. First of all, I want to thank my supervisor, Stephan. Thank you for guiding me in this research project. I feel privileged that I was a part of your study. With the valuable feedback and ideas, I was able to grow and learn. I am grateful that you gave me the confidence to develop myself in my areas of interest within this study. In the second place, I want to thank my second reader, Serge. You enriched me with the indispensable knowledge for performing the analyses and helped me with this in a pleasant way. Also, I would like to thank the parents and children who participated in this study. The hospitality with which they welcomed me at their homes made the data collection almost effortless. Next, I would like to thank my family and friends for their encouragement and support during this project. In particular, I want to warmly thank my parents, Ton and Anja, for enabling me to study in a care-free situation. I am thankful for your unconditional support, enthusiasm and heartening words when I struggled. I want to thank Gemma and Anne, my "research matties", for making this research master so much fun. I will never forget the many hilarious moments and statements we had. Also, thank you for new textual insights and always having a listening ear. Last, I would like to thank Jelle. You were always able to make me laugh when I thought there was nothing to laugh about and enriched me with enlightening perspectives. I am grateful for your unrestricted love and support.

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Abstract

This study investigated an aspect of cognitive functioning or more specifically of executive functioning, that appears to be strongly affected in NF1: working memory. The primary goal of this functional MRI study was to investigate whether or not the neuronal activity during working memory performance differs between NF1 children and controls. A second aim was to investigate the working memory performance outside the scanner. Participants included children with NF1 (*N*=21, 7 female), and controls (*N*=18, 10 female). Ages ranged between 8.2 and 19.1 *(M_{age}*= 13.12, *SD*=3.17). Neuronal activity was measured during the N-back task, and working memory performance outside the scanner was measured with the Memory Search 2D task of the ANT program. With respect to the main aim, the group means comparisons revealed non-significant differences. Though, the participants with NF1 had greater activity in the prefrontal cortex, and less activation in the posterior brain regions compared with controls. Overall, the NF1 children performed poorer on the working memory task outside the scanner. They performed even worse on the second, more demanding condition than the controls. These results may be explained by the dysfunction of the protein neurofibromin and a possible compensatory function of brain regions in individuals with NF1. These insights in brain functioning of individuals with NF1 might contribute to the development of intervention or treatment programs, medication and gene therapy.

Keywords: Neurofibromatosis Type 1, Cognitive functioning, Working memory, fMRI, Neuronal activity

Introduction

Every child is born with a set of DNA which includes chromosomes and genes. All the genes together determine how an individual functions. In some cases deletion or mutation of a gene or part of a gene has harmful consequences for a person's functioning. Neurofibromatosis type 1 (NF1), also known as Von Recklinghausen's disease, is a disorder caused by a single gene mutation. NF1 is the most common autosomal dominant disorder, with a prevalence of 1:2500 to 1:3300. Approximately fifty percent of the individuals with NF1 have no affected parent or first-degree relative, which indicates new mutations. The remaining fifty percent have a family member with NF1 (Williams et al., 2009). From all known single-gene disorders NF1 has the highest rate of new spontaneous mutations (Theos & Korf, 2006). The broad variety of mutations makes the clinical expression of NF1 diverse, even across several family members with NF1.

The gene that is responsible for NF1 is located on the long arm of chromosome 17, specifically 17q11.2 (Viskochil, 2002). The gene encodes a protein called neurofibromin, which is a negative inhibitory regulator of cellular proliferation and differentiation. The only function of this protein that has been demonstrated is to regulate the conversion of Ras-GTP into its inactive form Ras-GDP. Identified mutations of the NF1 gene predict inactivity and haploinsufficiency of neurofibromin, which means that the mutation has left only one functional copy of the gene which produces little or no protein (Viskochil, 2002). The loss of function of neurofibromin results in abnormal cell growth and differentiation (Boyd, Korf, & Theos, 2009). The abnormal cell growth can lead to benign neurofibromas and tumors, the reason why this gene is known for its function as tumor suppressor (Riccardi, 2009). Neurofibromin shows up in a wide variety of cell types, primarily in neurons, glial cells, and Schwann cells. Besides, neurofibromin shows up early in melanocyte development (Stocker et al., 1995).

NF1 is characterized by several clinical features and is classified by the diagnostic criteria of the National Institute of Health (1987). These criteria consist of six clinical features, two or more of which have to be present for NF1 to be diagnosed. These characteristics include

six or more cafe-au-lait spots, two or more neurofibromas or one plexiform neurofibroma, skinfold freckling, two or more Lisch nodules, a distinctive osseous lesion (e.g., scoliosis with or without pseudoarthrosis), or a first-degree relative with NF1. Alongside these criteria, individuals with NF1 often exhibit other clinical features as well, such as macrocephaly, optic nerve gliomas, a short stature, and pseudoarthrosis (Boyd, Korf, & Theos, 2009; Tonsgard, 2006; Kayl & Moore, 2000). The penetrance of NF1 is almost 100% at the age of six years (De Goede-Bolder, Cnossen, Dooijes, Van den Ouweland, & Niermeijer, 2001). The penetrance is reflected in the occurrence of an aberrant phenotype by an aberrant genotype. NF1 as a genetic condition is completely penetrant, but some manifestations of NF1 are incompletely penetrant or demonstrate an age-dependent expression of clinical features. For instance, infants often have multiple café-au-lait spots as sole manifesting sign, but Lisch nodules tend to appear around the age of twenty (Viskochil, 2002)

Furthermore, NF1 is a disorder not limited to only clinical features. Individuals with NF1 often have social problems (Johnson, Saal, Lovell, & Schorry, 1999; Noll et al., 2007), learning problems (Descheemaeker, Ghesuière, Symons, Fryns, & Legius, 2005; Hyman, Shores, & North, 2006; Levine et al., 2006), difficulties with executive functioning (Payne, Hyman, Shores, & North, 2010; Roy et al., 2010), and attention deficit and hyperactivity disorder (AD/HD; Barton & North, 2004; Kayl & Moore, 2000). In fact, impaired cognitive functioning is the most commonly reported problem in NF1 (Hyman, Shores, & North, 2006).

The aim of this study was to investigate an aspect of cognitive functioning or more specifically of executive functioning, that appears to be strongly affected in NF1: working memory. Working memory is proposed to be an important function which is impaired in children and adolescents with NF1 (Huijbregts, Swaab, & De Sonneville, 2010; Sangster, Shores, Watt, & North, 2011; Rowbotham, Pit-ten-Cate, Sonuga-Barke, & Huijbregts, 2009). More knowledge about the functioning of working memory would be useful, because working memory may very well be associated with or could even underlie many other cognitive, social, or behavioral problems experienced by individuals with NF1.

The terms ‘cognitive control’ and ‘executive functioning’ are used interchangeably in the literature on cognitive functioning. Executive functioning is not easy to define, mainly due to the variety of different subfunctions it encompasses. Some examples of these subfunctions are planning, organizing, attention, inhibition, and working memory. The role of these executive functions is to organize and integrate other streams of cognitive processing during behavior in which the frontal cortico-stratial networks seem to have a major mediating function (Shilyansky, 2009). Several researchers have tried to establish a cognitive profile for NF1, which has proven to be difficult as children and adults with NF1 appear to suffer from many different cognitive difficulties (Hyman, Shores, & North, 2005; Theos & Korf, 2006; Zöller, Rembeck, & Bäckman, 1997). NF1 is a multifaceted disease with both physical and cognitive manifestations, influenced by a genetic component that causes these manifestations (Levine et al., 2006). In previous research focusing on unraveling the cognitive profile of individuals with NF1 methodological variations such as a variety of comparisons groups and intentions varied per study. This makes it difficult to find a cognitive trend or cognitive profile (Levine et al., 2006). Furthermore, the maturation of the brain is a lifelong process, which runs from posterior brain regions to frontal brain regions. Higher-order cognitive functions may reach adult levels only later in the development. The phenomenon “growing into deficit” may play a role, since the frontal brain regions mature later and difficulties with these functions might not come to light until later in the development. Evidence for this phenomenon comes from a study by Ciesielski, Lesnik, Savoy, Grant, and Ahlfors (2006), who investigated the activated neural networks of children and adults during a categorical N-back task. Their findings suggest that an increase in proficiency and speed on the working memory task and increasing engagement of the inferior/prefrontal cortex come with age. Increased activation in these regions is associated with an age-related increase in working memory performance. A reduced activity or absence of the protein neurofibromin influences the maturation of the brain and may cause brain abnormalities. Because this is different in every individual with NF1, it might be relevant to unravel the

cognitive profile of individuals with NF1, and this should be incorporated in future studies (Levine et al., 2006).

With regard to the subfunctions of executive functioning, the focus is on working memory. The human memory, including working memory, depends on a complex mental system which has been a topic of research for quite some decades now. In their information-processing model Atkinson and Shiffrin (1968) described three components; the sensory register, the short-term store, and the long-term store. They assumed that the short-term store is necessary for long-term learning and other activities, and that there is a serial relation between the short-term store and the long-term store. However, a clinical case study by Shallice and Warrington (1970), of an individual with a greatly reduced short-term capacity and a normal performance on long-term memory (LTM) tasks, provided evidence that the short-term memory (STM) and the LTM do not work serially but have distinguishable functions. Baddeley and Hitch (1974) tackled this issue by developing a model of the working memory. In this model the working memory is a more dynamic system than the sensory register, short-term store, and long-term store. The STM is actually a component of the working memory, which allows us to mentally work with and manipulate the information being held 'online' in STM (Bernstein, Penner, Clarke-Stewart, & Roy, 2003). A distinction can be made between two functions, online maintenance and manipulation of information. Maintenance refers to the simple storage, mental processing, and rehearsal of information in STM, whereas manipulation involves complex operations on the information held 'online' (Purves et al., 2008). Baddeley and Hitch (1974) identified a three-component model of working memory. These three components are the central executive, the visuospatial sketch path, and the phonological loop. Over the course of several years the model was expanded by a fourth component, the episodic buffer (Baddeley, 2000). The central executive serves as supervisory system, and controls the flow of information from and to its subordinate slave systems: the phonological loop and the visuospatial sketch path. The two slave systems are responsible for retaining the information until it is removed from STM, and can be maintained as an online scratch path dedicated to a content domain. The visuospatial sketch path retains

visuospatial representations and the phonological loop retains the phonological (sound-based) representations (Purves et al., 2008). The episodic buffer is assumed to be a limited–capacity temporary storage system that is controlled by the central executive. The buffer is capable of integrating information from a variety of sources, and it retains episodes in which information is integrated across space and potentially across time (Baddeley, 2000). Working memory and LTM involve different representations. In working memory, for instance, rehearsal plays a role in remembering a telephone number, whereas in LTM a telephone number is already stored. The idea that working memory is limited regarding both duration and capacity is generally accepted. Many working memory representations only persist for a small amount of time: approximately twenty seconds (Purves et al., 2008). The capacity is relatively small, approximately four to nine items. The number of items held in the working memory is called the working memory load. This contrasts with the large capacity of the LTM, where other representations may be stored for decades (Purves et al., 2008). Whether a particular memory is stored in the short-term store depend on many factors, such as emotional importance, newness, and the effort required to remember (Carter, Aldridge, Page, & Parker, 2011).

The more modern theorists adopted a different approach concerning working memory. The theorists mentioned in the previous paragraph believed in the role of a central executive that controls the working of the memory. More recently, working memory has come to considered not as one of the executive functions but as a central construct facilitating the other executive functions, such as planning, abstraction, reasoning, and problem solving. Working memory is thought to be involved in the most complex cognitive behaviors and has become a central construct. This central construct consists of multiple components, or a collection of unified processes that carry out several important cognitive functions (Conway, Jarrold, Kane, Miyake, & Towse, 2007).

Elaborating on the theoretical frameworks concerning working memory, researchers investigated neuropsychological functions in an attempt to understand the cognitive profile of children with NF1. An area frequently studied within the neuropsychological functions is that of

working memory, where a distinction can be made between visual working memory and verbal working memory. Verbal working memory is used to manipulate and mentally work with numbers and other symbolic representations (Purves et al., 2008). Maintaining verbal information in working memory is essential for language production and comprehension. Hyman, Shores, and North (2005) investigated 81 children with NF1 and 49 unaffected siblings on a wide range of cognitive tasks including verbal and visual working memory. Verbal working memory was assessed by the Digit Span Forwards task. NF1 patients were able to mentally manipulate the information in their working memory just as well as their siblings. However, the children with NF1 did have a reduced attention span (measured with the Digit Span Forwards minus Digit Span backwards). With regard to the visual working memory, Hyman and colleagues reported that visuospatial deficits are common and consistently reported in children with NF1. Rowbotham, Pit-Ten Cate, Sonuga-Barke, and Huijbregts (2009) studied a sample of 16 children with NF1 and 16 controls. They expected that the children with NF1 to show overall deficient task performance. Differences in performance could be explained by the amount of cognitive control required for the task. Their hypothesis was confirmed by the results of the visual working memory task, during which the children with NF1 performed significantly slower and less accurately than the controls. Huijbregts, Swaab, and De Sonneville (2010) assessed working memory and the amount of cognitive control required in another sample of children and adolescents with NF1. Consistent with their hypothesis, their results showed that during the transition into adolescence children with NF1 draw level with their non-NF1 peers regarding more basic cognitive abilities which require less cognitive control, but these effects were not established when more cognitive control was required. Huijbregts et al. (2010) investigated social information processing in relation to cognitive control (measured on working memory) in a sample of 32 children and adolescents with NF1 and 32 controls. The NF1 children and adolescents had problems with social information processing, and the results indicated that cognitive control deficits also contributed to impaired social functioning. The authors proposed that this might be due to a lack of structural and functional connectivity in the brains of these

individuals, including cortico-subcortical tracts, but this explains only partly the problems with social information processing. These findings indicate the importance of cognitive control in daily functioning, especially for individuals with NF1. Sangster et al. (2011) took a different approach to assess working memory in preschool children with NF1. They asked the parents to fill out a questionnaire, the Behavior Rating Inventory of Executive Functioning (BRIEF-P). Parents reported significantly more problems on the working memory subscale than on the other subscales, even after IQ and SES had been controlled for.

Imaging studies concerning the brains of individuals with NF1 have shed light on frequently occurring brain abnormalities, and researchers sometimes tried to relate these findings to cognitive or physiological outcomes. Among these abnormalities are the so-called Unidentified Bright Objects (UBOs), which are focal areas of high signal intensity on T2-weighted magnetic resonance imaging (MRI) images (Feldmann et al., 2010). The origin of the UBOs is still unknown, but research has focused on those regions in the brain where they light up and on possible relations with cognitive functioning and physiological outcomes. DiPaolo et al. (1995), for instance, investigated the correlation between pathologic physiology and radiologic findings in individuals with NF1. They did indeed find a correlation between pathologic dysplasia and deviations in the cellular and neuronal development. More specifically, these deviations are spongiform myelinopathy or vacuolar change of myelin. The myelinopathy is due to a loss of myelin or of the Schwann cells that produce myelin, which results in a slower or completely blocked conduction of an action potential. With respect to the vacuolar change, DiPaolo and colleagues found vacuoles which they suggest are filled with water. This would explain the brightness of the lesions on T2-weighted images. In a prospective longitudinal study Hyman et al. (2003) investigated the development of UBOs in relation to cognition. They found that the presence or absence of UBOs was not related to cognitive abilities. In addition, a significant decrease in size, number, and intensity of UBOs was not associated with changes in cognitive ability. At a younger age, the NF1 children commonly had UBOs in the basal ganglia and brain stem that disappeared with age, whereas UBOs in the cerebral cortex and deep white matter did

not disappear (Hyman et al., 2003). The UBOs are located in focal, heterogeneous, and blurry shaped areas where grey and white matter regularly overlaps, such as the thalamus and basal ganglia (Barbier et al., 2011). The basal ganglia and thalamus were investigated in order to identify their metabolic characteristics, and to correlate those findings with observed UBOs in a study of Barbier et al. (2011). They found a metabolic change in the right lateral thalamus, independent of the presence of UBOs. Chabernaud et al. (2009) investigated whether or not thalamo-striatal UBOs were correlated with cognitive disturbances. They concluded that cognitive impairments in individuals with NF1 were associated with UBOs contributing to thalamo-cortical dysfunction. Because the thalamus is an important structure that controls the constant flow of information from the senses, and forwards this information to the cerebral cortex, it is not surprising that individuals with NF1 have problems with cognitive functioning.

Another deviation often identified in individuals with NF1 is macrocephaly, as well as abnormalities in white and grey matter volumes. Steen et al. (2001) studied macrocephaly in relation to other brain abnormalities. They concluded that macrocephaly in young individuals with NF1 is a result of enlargement of brain tissue. They found enlargement of white matter volumes, also larger white matter volumes in the corpus callosum, and enlarged brainstems. Moore et al. (2000) investigated white and grey matter volumes in individuals with NF1. They found that the total brain volume, especially that of grey matter, was significantly greater in individuals with NF1 than in controls. This was more pronounced for younger participants. At the same time they found that the corpus callosum was significantly larger in the group with NF1. They surmised that these findings were related to macrocephaly and the cognitive profile of individuals with NF1, because they associated these findings with a delay in development of appropriate neuronal connections during brain development. Greenwood et al. (2005) reported significantly larger grey and white matter volumes in children with NF1 than in controls. The greatest differences were found in the cerebral white matter volume, mainly in the frontal lobes. Grey matter volume differences were mainly found in the parietal, occipital, and temporal regions. Whereas in controls increased grey matter volumes were related to IQ scores this was

not the case in children with NF1. The results of the several studies mentioned above regarding brain abnormalities reveal that abnormalities in the brains of individuals with NF1 are quite common. The studies investigating these abnormalities in relation to cognitive function illustrate that it is difficult to find a relation between specific brain abnormalities and cognitive functioning.

Above, cognitive findings and frequently occurring brain abnormalities were discussed in relation to NF1. Functional MRI studies have not yet been performed frequently in NF1. Working memory, which, as has been described before, has regularly been found to be impaired in NF1, has been associated with a relatively specific functional network of brain regions. Researchers have identified a cortical-subcortical-cerebellar network that is active in the 2-back condition, but less active or not at all in the 0-back condition of a commonly used working memory task, the N-back task (Schlösser, et al., 2003; Schlösser, Wagner, & Sauer, 2006). This network involves cortico-cortical connections comprising the parietal association cortex, ventrolateral prefrontal cortex, and the dorsolateral prefrontal cortex, as well as a cortico-cerebellar feedback loop comprising prefrontal cortex, the cerebellum, and thalamus.

In sum, NF1 is a disorder with a variable clinical expression, related to cognitive dysfunction, and involving many brain abnormalities. Impaired cognitive functioning is the problem most commonly reported in individuals with NF1. Working memory is proposed to be an important function, which is impaired in individuals with NF1. A working memory deficit could be possibly associated with or even underlying many other cognitive, social, or behavioral problems experienced by individuals with NF1. Individuals with NF1 commonly have brain abnormalities, and there are several indications that the brains of NF1 individuals develop somewhat differently than those of typically developing individuals. Nevertheless, the studies mentioned above have shown that it is difficult to relate cognitive deficits to specific brain abnormalities.

To date, only few fMRI studies have been conducted on individuals with NF1. Billingsley et al. (2003) investigated phonological processing during rhyme decisions by means of fMRI in

15 individuals with NF1 compared with 15 controls. Their research focused on the association between neuronal activity and reading disabilities. Their findings suggest that for the comparison of phonemic stimuli individuals with NF1 use inferior frontal cortices relative to posterior cortices differently than controls. The activation of the inferior frontal cortex was of a greater extent and degree than controls. The pattern of greater involvement of the inferior frontal cortices relative to temporal neocortical activity was predominantly found in the right hemisphere. In another fMRI study visual-spatial processing in 15 individuals with NF1, and 15 healthy controls was investigated (Billingsley et al., 2004). The authors hypothesized that neuronal activity in the occipital and parietal cortices would be less in the NF1 group. The results did indeed show less neuronal activity in anterior cortical regions and more activity in the middle temporal, parietal, and lateral occipital cortices during visual-spatial analysis. The authors suggest frontal cortical anomalies in individuals with NF1, this in turn may be a pathophysiological basis for cognitive deficits in these individuals. Finally, a fMRI study was performed to investigate visuospatial processing in 13 children with NF1 (Clements-Stephans, Rimrodt, Gaur, & Cutting, 2008). In line with their hypothesis they found that the children with NF1 tended to employ regions in the left hemisphere, while controls used regions in the right hemisphere. An unexpected finding was a decreased volume of activation in the primary visual cortex in individuals with NF1. They concluded that individuals with NF1 have difficulties with visuo-spatial processing due to an inefficient right hemisphere network. To the best of our knowledge, no fMRI study focusing on working memory in NF1 has been conducted yet.

The main aim of our study was to investigate whether or not the neuronal activity during performance on the N-back task differs between children with NF1 and controls. We expected the children with NF1 to show less activation in brain regions associated with the aforementioned working memory network, particularly in the more difficult 2-back condition. In the easier 0-back condition only maintenance is required, compared with the 2-back condition for which manipulation is required. It was expected that the children with NF1 and controls

would show more or less equal activation in the associated network of brain regions for the 0-back condition.

Our second aim was to investigate whether or not the performance on the working memory task outside the scanner would be different for children with NF1 than for controls. On the basis of previous research we expected children with NF1 to perform more poorly on the first condition of the working memory task than controls. In addition, when more cognitive control would be required, as is the case with the increase in working memory load, we expected the individuals with NF1 to perform even more poorly on the second condition of the task compared to controls.

Method

Participants

The sample consisted of 39 children and adolescents. Ages ranged between 8.2 and 19.1 *(*M_{age}= 13.12, *SD*=3.17). 21 children were individuals with NF1 (ages 8.2-18.8; M_{age} =12.54; *SD* =2.71; 7 female). From these 21 children, 13 children were included in the fMRI analysis (ages 9.6-18.8; M_{age} =12.95; *SD* =2.68; 6 female). These participants with NF1 were recruited through the Dutch Neurofibromatosis Association (Neurofibromatose Vereniging Nederland, NFVN). All children with NF1 met the diagnostic criteria of the National Institute of Health (1987). The remaining 18 children were controls (ages 9.2-19.1; M_{age} = 13.79; *SD* =3.59; 10 female). From these 18 children, 13 children were included in the fMRI analysis (ages 9.2-19.1; M_{age} = 12.95; *SD* =3.42; 6 female). Controls were siblings of the NF1 children or children recruited through elementary schools, secondary schools, acquaintances of the families with NF1 children or they were recruited for a parallel study investigating (social) cognition (*N*=6). The children with NF1 and controls who were included in the fMRI analysis were matched for age and gender. Exclusion criteria included a premature birth, a history of psychiatric illness (other than ADHD or an Autism Spectrum Disorder), endocrinological dysfunction, neurological illness (other than NF1), and use of psychotropic medication (other than stimulants to treat ADHD-symptomatology or sleep problems). The caretakers of the children completed the Child

Behavior Checklist (Achenbach, 1991) and a severity questionnaire specific for NF1 was developed to screen for the exclusion criteria. Moreover, contraindications for fMRI were taken into account, such as braces, a pacemaker, and metal objects in and around or on the body. The study was approved by the Leiden University Medical Center Institutional Ethics Review Board.

Procedure

Prior to the study, written informed consent was obtained from the participant and their caretaker to participate in the study. After receiving the written informed consent, an appointment was made to scan and a safety checklist was administered over the telephone (see Appendix 1 for the Dutch version of the safety checklist). The participant received a leaflet at home with information about the procedure before and in the scanner, and information about the study for the caretaker as well as for the participant. At the day of scanning the participant was welcomed in the central hall in the Leiden University Medical Center (LUMC). First, the participant was taken to a room with a MRI mock scanner. Here, a second informed consent, to give permission for the scan, was obtained. In addition, a second safety checklist was administered. The participant as well as their caretaker had the opportunity to ask questions and the tasks that were used in the actual scan session were practiced. In addition, the participant could acclimatize in the mock scanner and get familiar with the imaging procedures. The participant was accompanied to the scanner room where a last safety check took place. The children were checked for prohibited items such as earrings or a zipper on the pants. First, a survey scan and a reference scan were made for the radiologist of the LUMC. Second, a high resolution echo-planar imaging (EPI) scan was made and third an anatomical scan was made, which in this study were used for registration purposes. Fourth, a diffusion tensor imaging (DTI) scan was made to provide information about the magnitude and direction of molecular diffusion. Fifth, a social cognition task consisting of two runs with a break in between was performed in the scanner. Both runs consisted of eight blocks with eight trials in each block, and four versions were made for randomization purposes. Sixth, a working memory task was performed consisting of two runs with a break in between. Lastly, a resting state scan was made during

which the participant was presented a black screen for five minutes. The participants received the instruction to try to stay awake and to keep their eyes open (see Appendix 2 for a complete overview of the scan protocol). The total time spent in the LUMC was about two hours including approximately 45 minutes actual scan time. During the scan period the caretaker was asked to complete several questionnaires and an appointment was made to administer a number of tasks outside the scanner. A well-trained student assistant administered these tasks at school or at home in a silent room. Six tasks of the Wechsler Intelligence Scale for Children (WISC-III^{NL}; Wechsler, 1974, see Kaufman, Flanagan, Alfonso, & Mascolo, 2006) were performed, and three tasks of the Amsterdam Neuropsychological Tasks (ANT) battery (De Sonneville, 2005). Children received a gift and a voucher; the caretaker received a monetary compensation for travel costs.

Measurement instruments

N-back paradigm

To assess working memory in the scanner, the commonly used N-back paradigm (0-, 1-, and 2- back) was used (Schlösser, Wagner, & Sauer, 2006). In this task, a pseudorandom sequence of uppercase characters of the alphabet was presented on a screen. In the 0-back condition, the participant is required to press the yes-button (i.e., the index finger of the right hand) whenever the letter 'X' appeared on the screen. During the 1-back condition, the participant had to press the yes-button (i.e., the index finger of the right hand) when the letter they saw was the same letter as the letter before. In the 2-back condition, the participant had to press the yes-button (i.e., the index finger of the right hand) when the letter they saw was the same as two letters before. In each of the three conditions the child was asked to press the no-button (i.e., the index finger of the left hand) when the presented letter was not the same as respectively the X, the letter before, and two letters before. The task consisted of two runs with a break in between. The first run consisted of five blocks with twenty trials each and the second run consisted of four blocks with twenty trials in each block. The stimuli were presented for 1500 ms, and after every presented stimulus a fixation cross was shown varying from 500 till 700 ms. Every block lasted

for 42 seconds and in between the blocks an instruction was given on the screen for 15000 ms. Four different versions of the task were used for randomization purposes.

Memory Search 2D (MS2D)

To assess working memory outside the scanner, participants performed the MS2D computerized task of the ANT program. This task requires participants to remember target figures characterized by two specific features; color and shape (e.g. a red circle). In each trial four figures were presented on the screen: participants were required to press the yes-button (i.e., a response with the index finger of the preferred hand) when a target figure was visible on the screen and the no-button (i.e., a response with the index finger of the non-preferred hand) when none of the presented figures was a target figure. The task consisted of two conditions with 48 trials each. In the first condition participants had to remember one target figure and in the second condition they had to remember three target figures, see Figure 1. The working memory load and the cognitive control required to complete the task therefore increased from the first to the second condition.

(a) (b)

Figure 1. a) The target figure in the first condition. b) The three target figures in the second condition.

fMRI data acquisition

Scanning was performed with a standard whole-head coil on a 3-Tesla Philips Achieva MRI system at the LUMC. Visual stimuli were projected onto a screen that was viewed through a mirror at the head end of the magnet. Stimulus presentation and the timing of all stimuli were acquired using E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA). The children gave their answers pressing buttons attached to their legs. Head motion was restricted using a pillow and foam inserts that surrounded the head. A total of 239 T2*-weighted whole-brain EPIs were acquired (TR = 2.2 s; TE = 30 ms, flip angle = 80 degrees, 38 transverse slices, 2.75 x 2.75 x 2.75 mm). Before the functional runs, a high resolution EPI scan and a T1-weighted anatomical scan

were acquired for registration purposes (EPI scan: TR = 2.2 s; TE = 30 ms, flip angle = 80 degrees, 84 transverse slices, 1.96 x 1.96 x 2 mm; 3D T1-weigthed scan: TR = shortest; TE = 4.60 ms, flip angle = 8 degrees, 140 transverse slices, 1.16 x 1.20 x 0.875 mm, FOV = 224 x 168 x 177.33 mm). All anatomical scans were reviewed by a radiologist of the LUMC.

Data analysis

fMRI data analysis

Preprocessing. First, all raw data were examined for motion and other imaging artifacts. Next, the high resolution scan and the structural scan of each subject was brain extracted (i.e., non-brain matter removed) using BET (Smith, 2002). FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) version 5.98, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). The following pre-statistics processing was applied: motion correction using MCFLIRT (Jenkinson, Bannister, Brady, & Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 8.0 mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor; high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma = 60.0 s).

Neuronal activity. Next, within session analysis was performed on each subject's pre-processed data using GLM analysis in FEAT. The fMRI time series data were modeled as three separate independent variables (0-back, 1-back, and 2-back). The period of interest started with the presentation of the first stimuli and lasted until the last item disappeared; this was 42 seconds for each block. The models were convolved with a double gamma hemodynamic response function and its temporal derivative. The single subject analysis was performed with a voxel threshold (p= .01). One subject's fMRI data was registered onto that subject's brain extracted high-resolution image using a 3 degrees-of-freedom (DOF) linear fit. Then the brain extracted high resolution scan was registered onto the subject's brain extracted T1-weighted anatomical scans using a 6 DOF linear fit. Last, the brain extracted T1-weighted anatomical scan was registered onto standard space (the MNI 152 image) using a 12 DOF linear fit using FLIRT (Jenkinson et al., 2002; Jenkinson & Smith, 2001). For the second level analysis, between session

analyses were performed using a fixed-effect analysis. The two pre-processed runs of each subject were combined to estimate each subject's mean response. The third level analysis was the between subject analysis to estimate the group mean difference. The images, computed on a subject by subject basis, were submitted to group analysis. This analysis focused on three contrasts, respectively 2-back > 0-back, 2-back > 1-back, and 1-back > 0-back. Task related responses were considered significant if a cluster exceeded a stringent threshold of p=0.05 and $z \geq 2.3$. A mask was computed with the activated voxels of the NF1 group and the control group to be able to only investigate the networks in the brain that are activated during the task.

Neuropsychological data analysis

To investigate the task performance outside the scanner General Linear Model Repeated Measures analysis of variance was performed for the ANT task. Data were analyzed using Statistical Package for the Social Sciences for Windows (SPSS; version 19.0). For the MS2D ANT task the within-subject factor was working memory load (1 vs. 3 in part 1 and 2 respectively). The between-subject factor was group (NF1 vs. controls). The amount of correct responses was used as accuracy measure. The analysis was performed for the participants who where included in the fMRI analyses only, and for all the participants who performed this task. In the latter the analysis was performed with and without age as covariate.

Results

Neuronal activity results

In order to determine whether or not the neuronal activity in brain regions differed between children with NF1 and controls a group analysis was performed. The group analysis was performed for the three contrasts mentioned below. For every contrast, the group means were compared within the activated network of brain regions, instead of the activity in the entire brain. Next, the difference in neuronal activity within the activated network with unthresholded z-values was inspected.

2-back versus 0-back

First, we inspected the contrast in which the neuronal activity during the 2-back condition was compared with the neuronal activity during the 0-back condition. This contrast was of particular interest because the shift from maintenance to manipulation is larger than in the other two contrasts. In Figure 2, an overview is presented of the neuronal activity of activated clusters of voxels within the activated network (p = .05 and $z \geq 2.3$). The activity that is shown are the group mean for the NF1 children (blue) and the group mean for the controls (red).

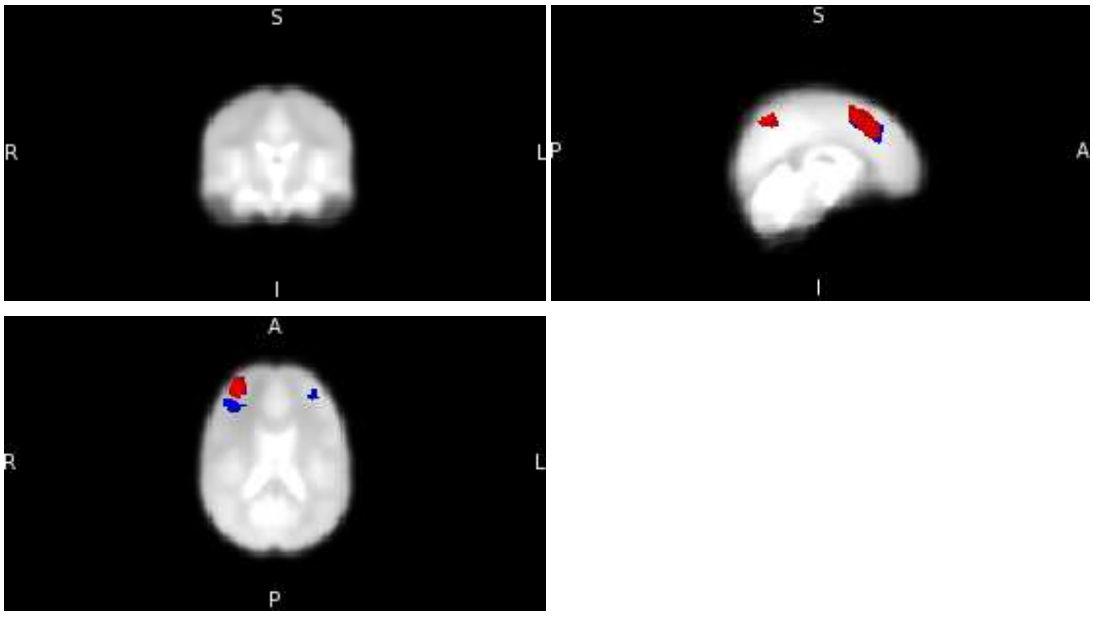

Figure 2. The neuronal activity of the NF1 children (blue) and controls (red) during working memory task performance (2-back versus 0-back).

The group mean comparison revealed a non-significant difference between the two groups. At the same time, a closer inspection of the differences in group means seems to shows a difference. Therefore, we inspected the unthresholded z values between two and five of the group means differences. In Figure 3, an overview is presented of the group mean differences of the activated clusters of voxels with a z-value between two and five.

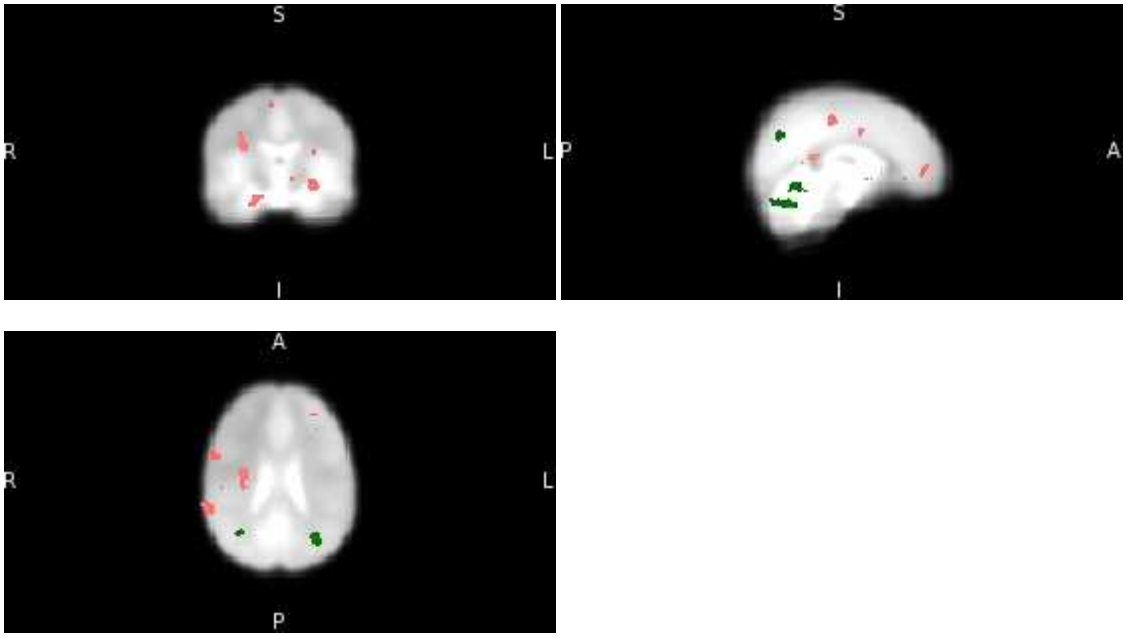

Figure 3. Group mean differences of activated clusters of voxels with an unthresholded *z* value between 2 and 5. NF1 group (pink) and controls (green).

These results indicate indeed a visible difference between the two group means. Therefore, the specific coordinates of highly activated clusters of voxels were investigated. In Table 1, an overview of a selection of the highest *z* values are presented for the two groups. The coordinates of the three coils in the MRI scanner (x, y, and z) are used to give an indication of the location of the brain region. In the NF1 group, activated voxels are mainly located in the anterior brain regions. In contrast, activated voxels in the controls are not only located in the anterior brain regions, but also in the posterior brain regions and cerebellum.

Table 1. *Overview of z values on different coordinates.*

	x	y	z	*z* value
NF1 group	62	79	38	3.85
	43	92	38	3.29
	53	62	31	3.22
	15	41	52	2.49
Controls	35	29	53	2.72
	60	29	57	2.81
	64	26	55	2.83
	34	30	52	2.83

2-back versus 1-back

Second, we inspected the contrast in which the neuronal activity during the 2-back condition was compared with the neuronal activity during the 1-back condition. In Figure 4 of Appendix 3, the group means differences are presented. The activity represents the clusters of activated voxels within the activated network (p = .05 and $z \geq 2.3$). The group means comparison of this contrast revealed a non-significant difference. In addition, a closer inspection of the unthresholded z values between two and five of the group mean difference resulted in Figure 5 of Appendix 3. The specific coordinates with the highest z values are presented in Table 2. The NF1 children mainly had activated clusters of voxels in the anterior brain regions, but the control children had activated clusters of voxels in both the anterior and posterior brain regions.

Table 2. *Overview of z values on different coordinates.*

	x	y	z	*z* value
NF1 group	31	60	46	3.16
	53	83	35	2.64
	52	61	32	3.44
	20	52	47	3.20
Controls	45	30	22	2.47
	65	25	55	3.48
	44	30	22	2.44
	22	30	55	2.80

1-back versus 0-back

Thirdly, we inspected the contrast in which the neuronal activity during the 1-back condition was compared with the neuronal activity during the 0-back condition. Both conditions particularly require the maintenance component of working memory. The analysis resulted in a non-significant difference between group means (p = .05 and $z \geq 2.3$). The unthresholded z values between two and five were inspected for group differences. The specific coordinates of highly activated clusters of voxels are presented in Table 3. Furthermore, in Figure 6 of Appendix 3 the results of the neuronal activity are presented. The children with NF1 had no activated clusters of voxels in the posterior brain regions, while the control group did. Both groups had activated clusters of voxels in the anterior brain regions, and left and right parietal lobe.

Table 3. *Overview of z values on different coordinates.*

	x	y	z	*z* value
NF1 group	46	62	26	2.38
	23	78	30	2.67
	51	54	58	2.65
	73	52	39	2.49
Controls	62	52	41	2.56
	71	38	41	2.55
	58	22	31	2.86
	40	68	46	2.50

Neuropsychological results

In order to determine whether or not the children with NF1 had more problems than controls with the working memory task outside the scanner, repeated measures GLM analyses were performed with the two conditions as within-subject factor and the two groups as between-subject factor. Children with NF1 had less correct responses in the more demanding second condition of the working memory task, see Table 4. This leads to an overall significant difference in accuracy of correct responses (F (1,19) =51.91, p =< .001, η_p^2 =.74). No significant group by condition interaction was found although it did approached significance (F (1,19) =2.87, p =.107, η_p^2 =.14).

Table 4. *Mean and standard deviations for the two conditions of the MS2D task for both groups (N=20)*

	Group	Mean	*SD*
MS2D condition 1	NF1 (*N*=13)	22.00	1.63
	Controls (*N*=7)	22.86	1.07
	Total (*N*=20)	22.30	1.49
MS2D condition 2	NF1 (*N*=13)	13.23	3.19
	Controls (*N*=7)	17.43	5.06
	Total (*N*=20)	14.70	4.33

In order to assess the accuracy of correct responses in a larger group, the participants who were not included in the fMRI analyses were added to the repeated measures GLM analysis. The children with NF1 had fewer correct responses compared with the controls in the first condition as well as in the more demanding second condition, see Table 5. This leads to an overall significant difference in accuracy of correct responses (F (1,29) =10.69, p = .003, η_p^2 =.26) and a significant group by condition interaction (F (1,29) =4.87, p = .035, η_p^2 =.14). However,

when age was added to the analysis as covariate the group by condition interaction turned out to be non-significant (F (1,28) =1.97, p = .171, η_p^2 =.06).

Table 5. *Mean and standard deviations for the two conditions of the MS2D task for both groups (N=33)*

	Group	Mean	*SD*
MS2D condition 1	NF1 (*N*=21)	22.19	1.53
	Controls (*N*=12)	22.08	0.90
	Total (*N*=33)	22.52	1.39
MS2D condition 2	NF1 (*N*=21)	13.76	4.12
	Controls (*N*=12)	18.25	5.14
	Total (*N*=33)	15.39	4.95

Discussion

The first aim of this study was to investigate whether or not the neuronal activity in brain regions differs between children with NF1 and controls during a working memory task. The commonly used N-back task was administered in the MRI scanner with three different conditions (0-back, 1-back, and 2-back). Of particular interest was the difference in neuronal activity between the 0-back condition and the 2-back condition. In the 0-back condition only maintenance is required, whereas in the 2-back condition manipulation is necessary. The latter condition is more demanding because of an increase in memory load, and as a result this task requires more cognitive control. Previous research has shown that a network of brain regions is active during working memory task performance, more specifically during the 2-back condition. This network consists of the parietal association cortex, the ventrolateral prefrontal cortex, the dorsolateral prefrontal cortex, as well as a cortico-cerebellar feedback loop comprising the prefrontal cortex, contralateral cerebellum, and thalamus (Schlösser, et al., 2003; Schlösser, Wagner, & Sauer, 2006). We expected the children with NF1 to show less activation than controls of the brain regions in the network associated with the 2-back condition, and similar neuronal activity in the 0-back condition.

Interestingly, within the network mentioned above we found differences between the two groups when neuronal activity was compared between the 2-back condition and 0-back

condition. Although the group means analyses within the activated network revealed non-significant differences, a closer inspection of the unthresholded *z* values yielded remarkable findings. Regarding the prefrontal cortex, the results showed that both groups had neuronal activity. However, the NF1 group had increased activity compared to the controls in the 2-back versus the 0-back condition. The neuronal activity related to the NF1 group was located throughout the whole prefrontal cortex; orbitofrontal, ventrolateral, and dorsolateral. In contrast, the neuronal activity related to the controls was situated in the ventrolateral and dorsolateral prefrontal cortex. In addition, the results showed neuronal activity for the controls in the parietal association cortex, and the cerebellum. In contrast, the NF1 group showed no increased neuronal activity in these two brain regions. The NF1 group also showed increased neuronal activity in the corpus callosum, which was absent in the controls. These results suggest a difference in brain regions involved between the NF1 group and controls, when the 2-back condition was compared with the 0-back condition.

Results regarding the difference in neuronal activity when the 2-back condition was compared with the 1-back condition were highly similar to those for the 2-back condition compared with the 0-back condition. Controls had increased neuronal activity in the parietal association cortex and cerebellum, whereas the NF1 group showed no increased activity in these brain regions. Regarding the prefrontal cortex, the NF1 group showed greater increases of neuronal activity than the controls throughout more or less the entire prefrontal cortex, whereas the controls had increased activity in the dorsolateral and ventrolateral prefrontal cortex. Comparison of the 1-back condition with the 0-back condition showed no difference in the mean neuronal activity of both groups. This is not surprising, taking into account that these two conditions both particularly required maintenance and the working memory load increase was minimal. Inspection of the group differences of the 1-back versus 0-back condition showed that the NF1 group had increased neuronal activity in the orbitoprefrontal cortex, whereas the results did not indicate increased activity in the parietal association cortex and cerebellum. In

contrast, the controls showed increased activity in the parietal association cortex, cerebellum, and prefrontal cortex.

These results suggest that in the controls different brain regions are active than in participants with NF1. The NF1 group showed increased activity throughout roughly the whole prefrontal cortex, whereas the increased activity of the controls was located in the dorsolateral and ventrolateral prefrontal cortices. This suggests that the brains of the controls are more differentiated or specialized. Where the NF1 children use almost the whole prefrontal cortex to perform the task, controls use some specific locations in their brains, and require less activation in those regions in order to perform the task. In the past, neuropsychological models often adopted a 'localisationist' approach, which attributed particular behavioral functions to specific brain regions. It was believed that the localization occurred before postnatal experience started playing a role. Differentiation begins prenatally; at the time of proliferation and migration, neurons move to predetermined destinations and then become components of particular cerebral regions (Anderson, Northam, Hendy, & Wrennall, 2001). An alternative view is that the cortex is initially undifferentiated with respect to function, but during the postnatal period gradually becomes differentiated in response to input of the thalamus (Anderson et al., 2001). With both approaches it is quite conceivable that the process of differentiation is disturbed by the dysfunction of the protein neurofibromin. The lack of neurofibromin or its dysfunction results in abnormal proliferation and differentiation. This might explain the difference in differentiation of brain regions between the children with NF1 and controls.

With regard to the brain differences in the prefrontal cortex there was another remarkable finding. Specifically, the cerebellum and parietal association cortex are active in the controls, as opposed to the activity in the NF1 group. This might suggest that there is a shift from posterior to anterior brain regions, for the controls and the NF1 group, respectively. The cerebellum has a role in motor control, attention, and a role in working memory performance. For instance, when a person memorizes a telephone number the cerebellum is active, whereas the parietal association cortex enables individuals to read, write, and solve mathematical problems (Carter

et al., 2011). In NF1 children these two brain regions showed no increased activity, but at the same time, the prefrontal cortex did show increased neuronal activity. The functions carried out by the prefrontal cortex are the so-called executive functions, of which working memory is a core component, although generally a network of brain regions is active during such control functions. Thus, in the NF1 group the anterior brain regions were more active, and in the controls the posterior brain regions were more active. The fact that the NF1 participants had a greater amount of activity in the prefrontal cortex, and less in the posterior brain regions might also because they compensate with the prefrontal cortex. It seems that they do not have differentiated or specialized brain regions and it might be that they compensate this with the involvement of more brain regions. The increase in activity in the prefrontal cortex and less in posterior regions was also found in the study of Billingsley and colleagues (2003). They hypothesized that this was caused by a mediator function of the prefrontal cortex. This shift could also be explained by the dysfunction or lack of neurofibromin. From research with a mouse model Shilyansky et al. (2010) concluded that the inhibitory networks in the prefrontal cortex, essential for working memory performance, are regulated by neurofibromin. The dysfunction of neurofibromin causes an increase in inhibition, which in turn ensures a decrease in information processing speed. This could explain the working memory impairments of individuals with NF1, as well as the increased prefrontal cortex activity in the individuals with NF1. Our hypotheses for the first aim were partly confirmed. Contrary to our expectation the NF1 children had more neuronal activity instead of less, but the brain regions involved in working memory performance did differ between the two groups.

The second aim of this study was to investigate whether or not performance on the working memory task outside the scanner was different for the NF1 children compared to controls. We expected the NF1 children to perform more poorly than the controls on the first condition of the task. In addition, because of the increase in working memory load, we expected the NF1 children to perform even more poorly on the second condition of the task than controls. The results revealed that, as expected, the NF1 children performed slightly poorer on the first

condition of the task, and obviously more poorly on the more demanding second condition of the task. This might be explained by the increase in cognitive control required in the second more demanding condition, and confirms a working memory deficit in NF1 individuals. The analysis was performed on two groups, one group consisting only of the participants that were included in the fMRI analyses, and the other group consisting of all the participants. In the latter group, the NF1 children performed slightly better than the controls at the first condition, but it should be mentioned that the NF1 group was almost twice as large as the group containing controls. For both groups the analyses revealed a main effect, which indicates that the controls had significantly more correct responses than the NF1 group. For the smaller group, no significant interaction between group and condition was found. This indicates that with the increase of the working memory load, the performance of the NF1 group did not deteriorate significantly more than the performance of the controls. In the larger group an interaction between group and condition was found, but this result disappeared when age was added to the analysis as covariate. This indicates that the decrease in correct responses for the NF1 group, as compared with the controls, might be related to age. This indication is confirmed by the study by Gathercole, Pickering, Ambridge and Wearing (2004), who investigated the different components of Baddeley and Hitch's information-processing model at different ages. They concluded that the different components are all present at the age of six years, and a linear increase in the capacity of each component was found from the age of four to early adolescence. Thus, this suggests that capacity increases linearly with age, and that the deterioration in correct responses may be related to age. These results are consistent with our hypotheses.

However, the results should be seen in the light of their limitations. Due to an error in the programming of the E-prime task, we were unable to evaluate the performance data of the N-back task in the scanner. As a result it was not possible to verify whether the participants performed well on the task, compare the data of the working memory tasks inside and outside the scanner, and relate task performance to neuronal activity. This is a serious limitation. Still, the task was practiced outside the scanner until experimenters were convinced participants

understood the task. In that light, the results could be interpreted assuming that differences in neuronal activity were observed despite similar performance levels for controls and children with NF1. Unfortunately, we can not be sure of this, particularly when considering that group differences outside the scanner on working memory tasks performance are often observed after practice sessions as well. The question however applies whether this information is extremely relevant to the current results, which particularly revealed the use of different brain regions to perform the task by controls and the NF1 group. This use of different brain regions may have resulted in poorer performance by NF1 children as it may have been less effective, or it may have been compensatory leading to similar performance. However, it has been shown that for optimal performance of the N-back task the network of brain regions has to be active that was observed for controls. If the NF1 children compensate with greater prefrontal activity, this is likely to result in reduced capacity for other activities requiring the prefrontal cortex. In addition, the fact that the analyses turned out to be not significant might be due to the small sample size, although a small sample size is not unusual in studies concerning participants with a genetic syndrome with similar incidences as NF1. The children and adolescents with NF1 were recruited through the Dutch Neurofibromatosis Association, which means that only members of this association were approached to participate. Consequently, a smaller part of the population was reached than would otherwise have been the case. In addition, not all participants were included in the fMRI analysis due to the matching for age and gender.

To date, our study is the first to have investigated neuronal activity during working memory task performance in children and adolescents with NF1. The results are promising, and may be important for understanding the relation between working memory and brain functioning, especially, because impaired cognitive functioning is the problem most commonly reported in NF1. Moreover, working memory, as a core cognitive function appears to be the most impaired aspect of cognitive control. Working memory may very well be associated with, or could even underlie, many other cognitive deficits, social, or behavioral problems. We hope this study has provided insights into the brain functioning of children and adolescents with NF1

compared to typically developing children and adolescents. These insights might aid to the development of treatment or intervention programs, because the result provide further evidence for a working memory deficit in NF1, as well as the neural basis for this working memory deficit. This evidence might be used as a guide to decide from which approach children, adolescents, and possibly adults would benefit most. Reinforcement of working memory abilities might contribute to better social, behavioural, and cognitive functioning of individuals with NF1. Imaginably, these results could also provide handles for medical researchers to develop medication. The role of neurofibromin in the development and working of the brains should be further investigated, and this could provide input for the development of medication or therapy. For instance, medicines may target the proper original function of neurofibromin, and gene therapy. This could address the basis of which cause the dysfunctioning in individuals with NF1. Future research should focus on differences in brain volumes and their possible relation with working memory performance. Perhaps, future researchers could investigate the link between working memory performance and neuronal activity. This would provide more insight in the specific brain regions related to working memory and any deficits related to this link. This mentioned link could be related to performance on social or cognitive tasks and neuronal activity. This would provide more insight in the working memory as underlying construct of social and cognitive problems.

In conclusion, we hope our study has shown that children and adolescents with NF1 have different neuronal activity than controls, and that different brain regions are active during working memory performance. The NF1 children had more neuronal activity in the prefrontal cortex than the controls. Controls used their parietal association cortex and cerebellum, whereas the NF1 group showed no increased activity in these brain regions. Furthermore, the NF1 children performed more poorly than controls on the working memory task outside the scanner. When more cognitive control was required the NF1 group performed even more poorly.

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Appendix 1

Dutch version of the MRI safety checklist

MRI Safety Checklist

Met behulp van Magnetic Resonance Imaging (MRI) is het mogelijk om duidelijke beelden van de hersenen te verzamelen. Hiervoor wordt gebruik gemaakt van een magnetisch veld. Dit magnetisch veld is, zowel op korte- als lange termijn, niet schadelijk voor de gezondheid. Het is echter niet voor iedereen mogelijk om mee te doen aan MRI onderzoek. Wij willen u daarom vragen onderstaande vragenlijst naar waarheid in te vullen en bij eventuele vragen of aanvullingen contact met ons op te nemen.

Heeft uw kind een pacemaker?	**JA**	**NEE**
Heeft uw kind een neurostimulator?	**JA**	**NEE**
Heeft uw kind vaatklippen?	**JA**	**NEE**
Is uw kind ooit geopereerd?`	**JA**	**NEE**
Indien uw kind ooit geopereerd is, zijn er objecten in het lichaam geplaatst of achtergebleven?	**JA**	**NEE**
Indien uw kind ooit geopereerd is, wat voor operatie betrof het en hoe lang is dit geleden?	**……..** **……..** **……..** **……..**	
Heeft uw kind een tatoeage?	**JA**	**NEE**
Heeft uw kind piercings?	**JA**	**NEE**
Heeft uw kind een gehoorapparaat?	**JA**	**NEE**
Heeft uw kind een insulinepomp?	**JA**	**NEE**
Is uw kind claustrofobisch?	**JA**	**NEE**
Is uw kind mogelijk zwanger?	**JA**	**NEE**
Heeft uw kind een spiraaltje laten plaatsen?	**JA**	**NEE**

Heeft uw kind permanente oogmake-up laten aanbrengen?	**JA**	**NEE**
Heeft uw kind wel eens met metaal gewerkt of gelast?	**JA**	**NEE**
Heeft uw kind een beugel?	**JA**	**NEE**
Heeft uw kind een bril? Zo ja, welke sterkte?	**……..**	

Overige opmerkingen/vragen:

Ik heb bovenstaande vragen naar waarheid ingevuld en de onderzoekers op de hoogte gesteld van alle metalen objecten die mogelijk in mijn lichaam aanwezig kunnen zijn. Ik ben op de hoogte gesteld van de risico's van het verstrekken van onjuiste of onvolledige informatie. Ik heb de gelegenheid gekregen vragen te stellen aan de onderzoekers en kan mijn toestemming, zonder opgaaf van reden, te allen tijde intrekken.

Naam onderzoeker:

Naam ouder:

Naam kind:

Plaats:

Datum:

Handtekening ouder Handtekening onderzoeker

Appendix 2

Complete overview of the scan protocol

- Survey scan
- Reference scan
- High resolution EPI scan
- PACS
- T1-weighted anatomical scan
- DTI scan
- Social information processing task part I
- Social information processing task part II
- Working memory task part I
- Working memory task part II
- Resting state scan

Appendix 3

2-back versus 1-back

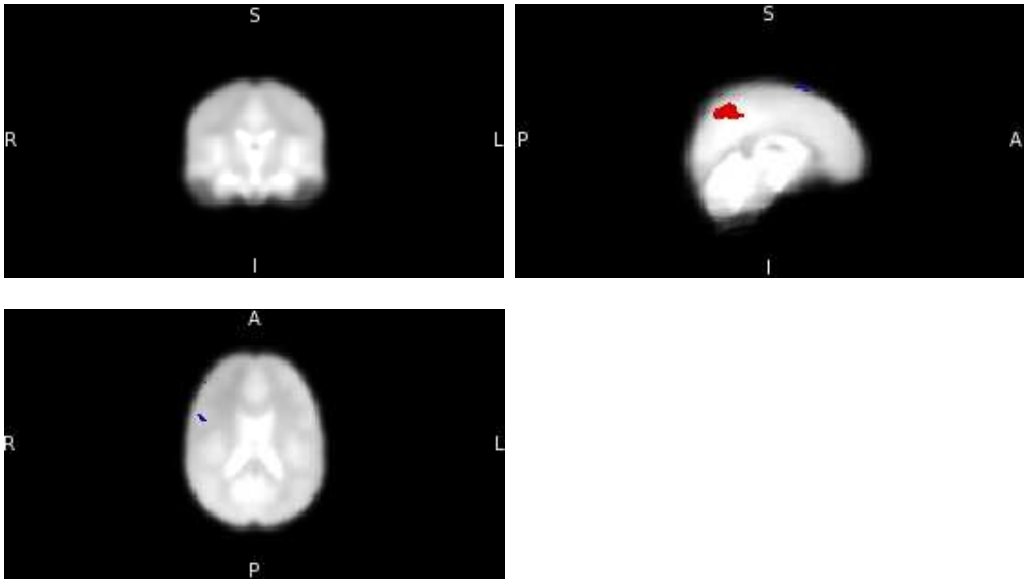

Figure 4. The neuronal activity of the group means within the activated network, the NF1 children (blue) and controls (red), during working memory task performance.

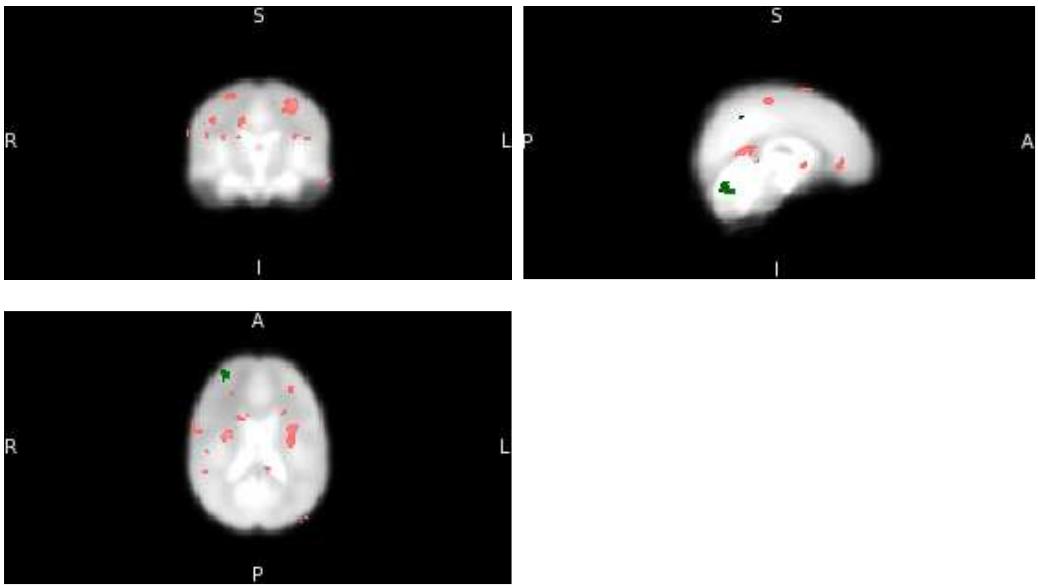

Figure 5. Group mean differences of activated clusters of voxels with an unthresholded *z* value between two and five. NF1 group (pink) and controls (green).

1-back versus 0-back

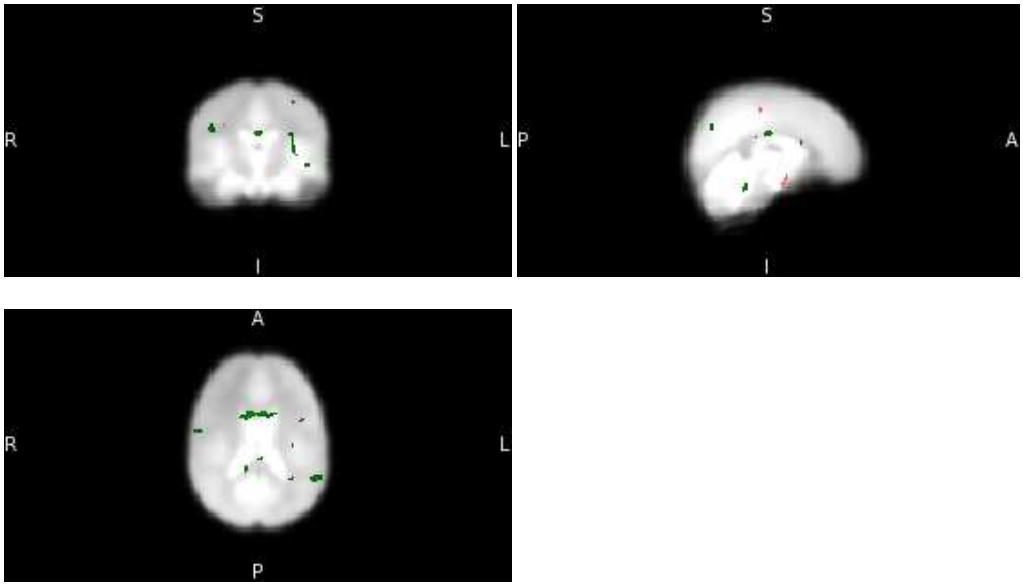

Figure 6. Group mean differences of activated clusters of voxels with an unthresholded *z* value between 2 and 5. NF1 group (pink) and controls (green).