Cerebellar Control of Saccades in Health and Disease

Cerebellaire Controle van Saccades bij Gezondheid en Ziekte

P.C.A. van Broekhoven

Cover: sagittal view of the cerebellum and stylized traces of saccadic and smooth pursuit eye movement data.

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Chapter 1

General introduction

Vision and eye movements

Vision is one of the senses that are used for gathering information about the surrounding environment. Patterns of light reflecting from the environment enter the eye and are projected onto the retina. The retina harbors photoreceptors, which transform the patterns of light into the electrical activity that neurons use to convey visual information. This information enables humans to observe and interact with the environment.

To gather detailed visual information about the properties of an object of interest, it must be projected on the fovea. The fovea is the part of the retina that has the highest density of photoreceptors and therefore provides the highest visual acuity. The projection of (moving) objects can be kept on the fovea using smooth pursuit eye movements, and new objects can be targeted by using saccadic eye movements.

Eye movements can be recorded and quantified with relative ease. Current technology enables us not only to record eye movements in healthy adults in a laboratory setting, but also in more challenging subjects such as children or patients, or to record concurrently with functional imaging in a MR-scanner. Eye movements can be studied at multiple levels, ranging from the quantification of reflexive behavior to the inference of cognitive strategies involved in voluntary eye movement control. With properly designed studies it is possible to assign a functional role to the different stages in the visuo-oculomotor pathways.

These characteristics propelled a large number of electrophysiological and behavioral studies on eye movements in non-human primates and humans. Functional imaging studies during the last twenty years have opened new ways for further investigations of the oculomotor system. The accumulating knowledge from these electrophysiological, behavioral and the more recent imaging studies has led to a detailed, but not complete, understanding of the neuronal pathways that subserve the oculomotor system. This also led to the appreciation that eye movements exhibit specific abnormalities when a confined part of the nervous system that is involved in these eye movements is affected by disease or trauma. Therefore, eye movement abnormalities have a clear clinical value and can contribute to the diagnostic process in a clinical setting.

This thesis focuses on the neuronal pathways and clinical use of saccadic and smooth pursuit eye movements.

Saccades and smooth pursuit

Eye movements can grossly be divided in two groups: compensatory eye movements and goal-directed eye movements. Compensatory eye movements stabilize the visual environment on the retina when the head moves relatively to the visual scene. Voluntary saccades and smooth pursuit are goal-directed eye movements that bring or keep an object on the retina.

Saccades

Saccades are fast 'jumps' of the eyes that shift the line of sight from one object in the visual scene to another. For example, when reading lines of text such as these, the eyes make a series of saccades from one word to another. In between two saccades the eyes are not moving for a short period, known as a fixation. During fixations the visual information can be processed in detail.

Saccades can be classified into two categories: reflexive and higher-order saccades. Reflexive saccades are saccades evoked by suddenly appearing targets. Higher-order saccades are made deliberately and involve more cognitive processing in order to determine when and where to move gaze. Higher-order saccades include voluntary, memory guided and delayed saccades.

All saccades are highly stereotyped eye-movements. There is a relatively fixed relation between the amplitude, duration and peak velocity called the main sequence (Bahill, 1975). The velocity of the eye can reach up to 500 degrees per second during a 30 degrees saccade that lasts 100 ms (Leigh and Zee, 1999). The velocity of the eye is so high that vision is impaired during the saccade. Saccades are ballistic movements, that is, a saccade is programmed before the saccade is executed. A correction while the saccade is in progress is not possible. Therefore, correction saccades must take place after the eye has landed near the target (Hopp and Fuchs, 2004). 'Near the target' is correct because in general saccades consistently tend to be a little too short. In other words, saccades tend to undershoot their target. This physiological undershoot is 'corrected' for by a small extra saccade or sliding movement, a glissade, until the target is foveated.

The saccadic neuronal circuitry of the saccadic system is simplified and schematized in figure 1a. Visual information from the retina is relayed to the primary visual cortex (V1). V1 processes visual information and sends the information to higher cortical areas (frontal and parietal eye fields, FEF and PEF respectively), which are responsible for planning and programming saccades (Moschovakis et al., 1996; Hopp and Fuchs, 2004). The FEF and PEF send their output to the superior colliculus (SC). The superior colliculus encodes the desired

eye displacement and sends that to the brainstem burst generator (BBG). The BBG in its turn generates and sends the appropriate motor command to the extraocular muscles. The superior colliculus also sends its output to the nucleus reticularis tegmentis pontis (NRTP), which projects to the cerebellum. The cerebellum is involved in maintaining saccadic accuracy. A more detailed description of the role of the cerebellum will be provided later.

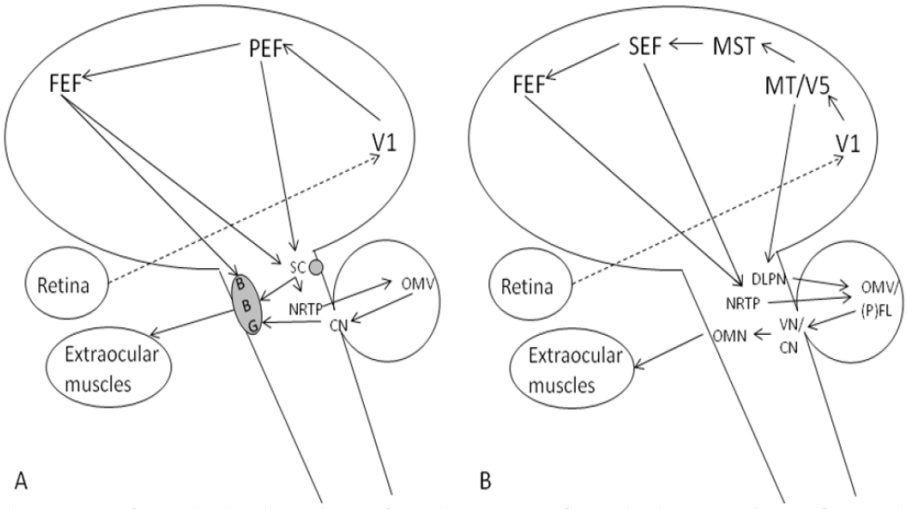

Figure 1A: schematic circuitry of saccades. **Figure 1B:** schematic circuitry of smooth pursuit.

Abbreviations: BBG = Brainstem Burst Generator, CN = Cerebellar Nuclei, DLPN = DorsoLateral Pontine Nuclei, FEF = Frontal Eye Field, NRTP = Nucleus Reticularis Tegmentis Pontis, OMV = Oculomotor Vermis, PEF = Parietal Eye Field, (P)FL = Flocculus/Parafloccullus, SC = Superior Colliculus, VN = Vestibular Nuclei.

Abnormal saccadic inaccuracy, besides the physiological undershoot, can be the result of changed functioning of extra-ocular muscles or due to altered neurological functioning (Buttner and Fuhry, 1995; Sweeney et al., 2004; Ramat et al., 2007; Adams et al., 2008). Physical growth, trauma or weakening of the extra-ocular muscles due to age can alter the force exerted on the eye by the extra-ocular muscles. Trauma or disease of the brain (including the cerebellum) changes the functioning of the neurological part of saccadic system. If possible, the saccadic system will change the amplitude and/or angle of the saccades over time to meet the new demands. This process of saccade modification is a form of motor learning known as saccade adaptation (Hopp and Fuchs, 2004).

Saccadic adaptation can also be induced in the laboratory. McLaughlin introduced the classical paradigm in 1967 (McLaughlin, 1967).

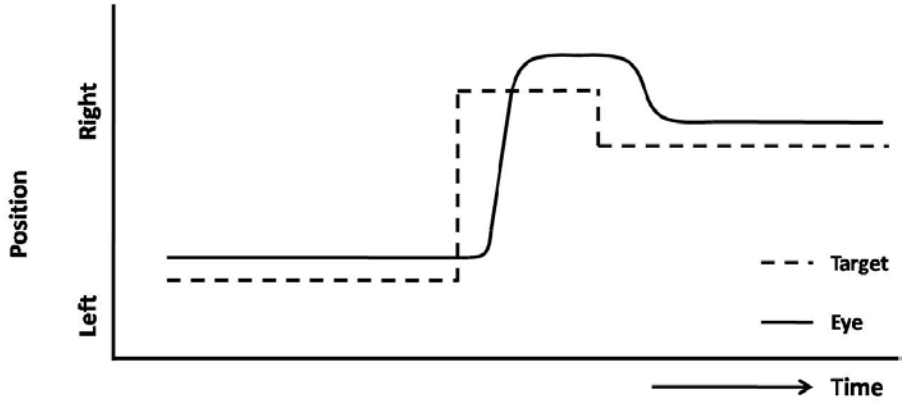

Figure 2. Saccade adaptation paradigm.

In the saccade adaptation paradigm (figure 2), a target (a colored dot against a black background) is presented on one side of a computer screen. This target disappears and a new target on the opposite side of the screen is presented. The subject makes a saccade to the new target. During the saccade, the target is shifted to a new position. Since vision is impaired during saccades (Bridgeman et al., 1975), these shifts remain unnoticed to the observer. After the saccade, the oculomotor system registers, unconsciously, an error between the saccadic end point and the target. A corrective eye movement is required to foveate the target. However, in the course of multiple trials, the amplitude of the saccades becomes adapted to the displaced target position. In other words, post-saccadic visual errors, induced by consistent intra-saccadic target steps, drive the gradual modification of subsequent saccadic amplitudes over a series of trials in order to retain saccadic accuracy (Wallman and Fuchs, 1998; Robinson et al., 2003; Hopp and Fuchs, 2004).

Smooth Pursuit

In contrast to saccades, which are used to direct the eyes to another object in the visual field, smooth pursuit is used to keep the point of gaze on an object that moves across the visual field. For example: smooth pursuit can be used to follow a sailing boat passing by on the water.

Smooth pursuit works at its best when the velocity of the moving object is low. Eyes can perfectly follow the target when it moves at speeds of up to 10 degrees per second (Schalen, 1980), but when the speed increases, accuracy drops rapidly (Schalen, 1980; Meyer et al., 1985). The maximum velocity is about 90 degrees

per second (Meyer et al., 1985). Smooth pursuit is not always accurate in tracking a target, especially when the target changes its speed or its trajectory, or is simply moving too fast. When the eyes lag behind the target, small saccadic eye movements can help to bring the fovea back on the target. These characteristic intrusions in the smooth pursuit movement are known as 'catch-up saccades'. In this way, smooth pursuit and saccades can work together to get an optimal result in tracking a moving target. Increasing numbers of catch-up saccades during smooth pursuit reflect increasing difficulty in tracking an object. Similar to the saccadic system, the smooth pursuit system is able to adapt to changes in demand as a result of physical change of the motor execution system or injuries (Kahlon and Lisberger, 1996; Takagi et al., 2000b).

A simplified scheme of the neuronal circuitry of smooth pursuit is given in figure 1b. Visual information from the retina is relayed to the primary visual cortex (V1). V1 processes the visual information and detects target movement. V1 sends the information to higher cortical areas. MT/V5 encodes target motion (Albright, 1993). MST also encodes motion of target, but additionally takes into account eye movements. This means that MST is probably concerned with moving targets against a textured background or stationary targets during self-movement (Leigh and Zee, 1999). Studies in humans and monkeys show that the frontal eye fields (FEF) are involved in initiation and maintenance of smooth pursuit (Rivaud et al., 1994; Petit et al., 1997; Tanaka and Lisberger, 2001; Rosano et al., 2002; Schraa-Tam et al., 2008). Electrophysiological studies in monkeys suggest that the supplementary eye fields (SEF) are involved in initiation and anticipation of movement direction of smooth pursuit (Missal and Heinen, 2001, 2004; de Hemptinne et al., 2008). In humans, results from a lesion study as well as a study using transcranial magnetic stimulation (TMS) yields similar results (Heide et al., 1996; Gagnon et al., 2006). Electrophysiological recordings in monkeys have revealed two parallel pathways projecting from cerebral areas via the brainstem to the cerebellum. The FEF and SEF project via the nucleus reticularis tegmentis pontis (NRTP) to the cerebellar oculomotor areas (Brodal, 1980a, b, 1982; Yamada and Noda, 1987; Shook et al., 1990; Suzuki et al., 1999; Giolli et al., 2001; Ono and Mustari, 2009). The other pathway stems from visual area MT/V5 and sends its projections via the dorsolateral pontine nuclei (DLPN) to the cerebellar oculomotor areas (Glickstein et al., 1985; Noda et al., 1990; Suzuki et al., 1990; Glickstein et al., 1994; Kralj-Hans et al., 2007). The cerebellar oculomotor areas on their turn project via the fastigial nucleus and vestibular nuclei to the oculomotor nuclei (Leigh and Zee, 1999).

Eye movements in a clinical setting

Eye movements can be of great value in determining underlying pathologies. In this section of the introduction a brief overview of several common eye movement abnormalities related to saccades, smooth pursuit and fixations will be provided. Many of these abnormalities can be readily observed in a clinical setting (see table 1).

A more quantitative description of the alleged eye movement abnormalities can be obtained by actually measuring the eye movements. Moreover, saccade adaptation cannot be tested without such equipment as one has to change the stimulation in response to the saccades. Complete control over the stimulation during testing can be obtained using a computer display. The patient can then be asked to look at relatively simple stimuli such as a dot that changes position now-and-then to evoke saccades or a dot that moves slowly in the field of view to evoke a smooth pursuit eye movement. Fixation behavior can be examined by having the patient look at a stationary dot for a specific period of time.

Functional lesions in specific brain areas can affect different types of eye movements simultaneously, as can be derived from the substantial overlap in the neuronal circuitry between saccades and smooth pursuit (table1 and figures 1a and 1b). Small focal lesions in these areas can lead to specific deficits in either type of eye movements. The next section of this introduction will discuss disorders of saccades, smooth pursuit and fixation.

Saccadic disorders

Innervation of eye muscles that is needed for fixations and saccadic eye movements can be described as a tonic innervation that keeps the eye in a certain position (fixation) and a burst of higher activity that is needed to make the muscles contract and drag the eye to a new position (saccade). The burst for movement is called the pulse and the difference in tonic innervation between two positions is called the step. Pulse-step mismatches will lead to saccades that end close to a target, undershoot or overshoot, with a subsequent sliding movement (glissade) towards the target. Losing the ability to keep the new firing frequency belonging to a step will result in a repetitive pattern of post-saccadic drift towards a neutral (center) position and a new saccade to foveate the target again. This pattern is called gaze-evoked nystagmus. Classification according to changes in the pulse-step pattern provides a framework for analyzing saccadic abnormalities. In normometric saccades, the pulse is step-signal is matched and adequate. When the step is adequate but the pulse is inadequate, the saccadic amplitude will be too

Table 1. Commonly encountered eye movement abnormalities and their site of origin in the brain.

Eye movement abnormalities	Observation	Localization
Saccade dysmetria	Multiple saccades are needed to look at a target; saccade is followed by correction saccade in opposite direction	Cerebellum Brainstem
Saccade slowing (*)	Saccade to a target are slow pursuit-like movements	Brainstem
Deficits in saccade motor learning (*)	Saccadic amplitudes cannot be modified in a saccade adaption paradigm	Cerebellum
Impaired saccade initiation	Decreased reaction time	Cerebrum Basal ganglia
Impaired smooth pursuit initiation	Delayed initiation Decreased initial velocity	Frontal eye fields Cerebellum
Deficits in smooth pursuit maintenance	Catch-up and back-up saccades	Frontal eye fields Brainstem Cerebellum
Deficits in fixation	Saccadic intrusions Nystagmus	Pons Cerebellum

Most of the eye movement abnormalities listed in table 1 can be observed directly, by a careful examination of the patient at the bedside. (*) Detection requires eye tracking equipment.

small or too big. Saccadic dysmetria, a pathological undershoot or overshoot, is often found in patients with cerebellar lesions (Selhorst et al., 1976b; Botzel et al., 1993). However, lesions in the brainstem can also result in saccadic dysmetria, similar as in cerebellar disease. In Wallenberg's syndrome, patients suffered a lateral medullary infarction. In these patients, saccades directed to the side of the lesion become hypermetric and saccades away from the side of the lesion become hypometric (Helmchen et al., 1994). It is thought that this saccadic dysmetria results from impaired signal transduction from the cerebellum via the fastigial nucleus to the brainstem (Helmchen et al., 1994). When the height of the pulse is too small, the saccade will undershoot the target and be followed by a drift of the eye, a glissade, towards the target. As noted above, this is seen in Wallenberg's syndrome patients, but also patients with Myasthenia Gravis can show hypometric saccades (Sollberger et al., 1986). An overshoot of the target with a subsequent glissade to reach the target is seen when the pulse is too high with a

correct step. This pathological overshoot is seen in cerebellar disease (Zee et al., 1976) and internuclear ophthalmoplegia (Baloh et al., 1978).

An adequate step, but a long pulse duration combined with a low height of the pulse renders slow saccades. Slow saccades point to a problem with the brainstem burst generator (BBG) (Scudder et al., 2002). For horizontal saccades, the burst arises in the parapontine reticular formation (PPRF). For vertical saccades this signal originates in the rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF). A lesion in these areas or loss of input from upstream brain areas can lead to decreased excitatory input to the oculomotor muscles and thus low velocity saccades. This is seen in progressive supranuclear palsy (Newman et al., 1970), Huntington's disease (Starr, 1967) olivopontocerebellar atrophy (Burk et al., 1997; Wessel et al., 1998) and Myasthenia Gravis (Yee et al., 1987).

Fast saccades are actually saccades that are too short. Due to the early interruption of the saccadic movement, the ratio between peak velocity and amplitude increases. This is seen in Myasthenia Gravis (Cogan et al., 1976; Khanna et al., 2007).

Changes in saccadic reaction time are the result of an initiation disturbance. Volitional saccades in basal ganglia disease can be characterized by increased latencies. For example, patients affected by Huntington's disease show longer latencies and more variability in reaction time in saccade generation (Avanzini et al., 1979; Peltsch et al., 2008). The effect of basal ganglia disease is probably by impaired signaling from the basal ganglia to the superior colliculus. The superior colliculus is known to play an important role in saccadic eye movements (Moschovakis et al., 1996). The increased saccadic latency is probably due to a disturbance in the pathway from the frontal eye fields via the basal ganglia to the superior colliculus, which results to increased inhibition of the superior colliculus (Peltsch et al., 2008).

Saccadic adaptation is the learning process in which saccadic amplitude is adjusted when saccades are consistently inadequate. No adaptation of saccades means that there is no correction of the pulse-step signal when saccades are not accurate. This loss of saccadic adaptation is seen in cerebellar disease (Waespe and Baumgartner, 1992; Straube et al., 2001).

Smooth pursuit disorders

Smooth pursuit stabilizes the image of a slow moving target of interest at the fovea. The focus in this part will be on deficits of horizontal smooth pursuit in humans. Three main components of smooth pursuit can be distinguished (Keller and Heinen, 1991). Firstly, retinal slip indicates the movement of the target of

interest. In general, a saccade is then needed to position the target near the fovea. Secondly, after the saccade, the eye starts to increase velocity to get up to speed with the target. The correct speed is reached when retinal slip is close to zero. Usually, when the eye is at the same speed as the target, a small second saccade is needed to put the target on the fovea. The eye is now tracking the target in position and motion. This part is called the initiation phase. The third part is sustained tracking of the target during which retinal slip is minimized. The ability to maintain tracking of a moving target is quantified as eye velocity divided by target velocity: gain. A gain of 1.0 is a perfect pursuit, gain smaller than 1.0 means that pursuit is too slow and a gain greater than one means that the pursuit is too fast. Impaired smooth pursuit, i.e. gain not equal to 1.0, results in 'catch-up saccades' or 'back-up saccades' that bring the target back on the fovea.

Smooth pursuit deficits can grossly be divided in impaired initiation and impaired sustained tracking. Deficits of initiation lead to a delay in pursuit onset or a low acceleration of the eye during the initial phase of pursuit. Human lesion studies revealed that the cerebellum participates in smooth pursuit initiation. Straube and colleagues report a decrease in eye velocity, most prominent in the first 20 ms of pursuit, in patients affected by unilateral cerebellar lesions (Straube et al., 1997). Moschner and colleagues present six patients affected by degenerative cerebellar lesions in which the onset of smooth pursuit was delayed (Moschner et al., 1999). Also the eye acceleration during the first 60 ms and peak velocity during sustained pursuit were decreased. Transcranial magnetic stimulation in healthy humans suggests that the frontal eye fields are involved in initiation of contraversive pursuit (Drew and van Donkelaar, 2007).

Impaired sustained smooth pursuit can be classified as being unidirectional (i.e. ipsiversive or contraversive) or bidirectional. Low gain smooth pursuit is the predominant sort of deficit. Few reports show data of high gain pursuit. Ipsiversive impaired smooth pursuit designates disorders of pursuit towards the side of the brain with a lesion. This can result from lesions in many parts of the smooth pursuit neuronal circuitry. In humans, impaired ipsiversive smooth pursuit is reported in lesions in the frontal cortex (Morrow and Sharpe, 1995; Heide et al., 1996), parieto-occipital lobe (Bogousslavsky and Regli, 1986), pons (Gaymard et al., 1993; Ahn et al., 2007) and the cerebellum (Zee et al., 1976; Baloh et al., 1978; Moschner et al., 1999). Pure contraversive impaired smooth pursuit (disorders of pursuit away from the side of the brain with a lesion) is reported in Wallenberg's syndrome (Helmchen et al., 1994). In other reports of contraversive impaired smooth pursuit, ipsiversive smooth pursuit was affected as well (Morrow and Sharpe, 1990; Lekwuwa and Barnes, 1996; Barton and Sharpe, 1998). Barton

and Sharpe (1998) described 17 patients with several smooth pursuit deficits, among which 3 patients showed increased contraversive pursuit gain after lesions in the posterior internal capsule. Cerebellar lesions can lead to a general decrease in eye velocity during smooth pursuit (Moschner et al., 1999).

Fixation disorders

Disorders of gaze fixation result in blurred vision and difficulties with reading. Two major types of eye involuntary eye movements that interfere with gaze fixation are pathological nystagmus and saccadic intrusions (Leigh and Zee, 1999). Examination of fixation can be complicated by the occurrence of square-wave jerks. These small saccadic eye movements also occur in healthy subjects (Leigh and Zee, 1999) and are thus not always a sign of pathology. These saccadic intrusions become pathological when they interfere with fixation of a target of interest on the fovea. Macrosaccadic oscillations are large horizontal saccades that generally occur when a patient attempts to shift visual fixation but they can also occur during attempted fixation. These ocular oscillations are seen in genetic cerebellar ataxias (Swartz et al., 2003), with lesions affecting the cerebellar vermis, the cerebellar nuclei and in multiple sclerosis (Selhorst et al., 1976a). Rarely, macrosaccadic oscillations are seen with pontine lesions (Averbuch-Heller et al., 1996; Kim et al., 2007). The neuronal substrate of macrosaccadic oscillations is not clear yet. The cerebellum (Selhorst et al., 1976a; Swartz et al., 2003) and the omnipause neurons in the pons (Averbuch-Heller et al., 1996; Kim et al., 2007) are suggested as sites of origin. Other saccadic oscillations as ocular flutter (horizontal saccades) or opsoclonus (omnidirectional) are seen in diseases affecting the brainstem and/or the cerebellum (Leigh and Zee, 1999), though the precise mechanism behind these saccadic intrusions has yet to be elucidated (Tilikete and Pelisson, 2008).

Nystagmus consists of a repetitive slow drift (slow phase) of the eye during attempted fixation. The deviation from the fixation position is then corrected by a small saccade-like movement (quick phase) to bring the eye back to the initial position. Nystagmus is named after the quick phase: the quick phase is to the left in left nystagmus, the quick phase is directed downward in downbeat nystagmus. A complete description of the different types of nystagmus and their neuronal substrate is beyond the scope of this introduction, so a few easy to recognize types of nystagmus are presented. A major distinction made when examining nystagmus is between gaze evoked nystagmus and the occurrence of nystagmus with the eye in central position. Gaze-evoked nystagmus is the most common form of nystagmus seen in a clinical setting. This form nystagmus appears when

fixation in an eccentric position, usually lateral or upward, is attempted (Leigh and Zee, 1999). A possible explanation why gaze-evoked nystagmus is often seen in clinical practice is because it is a common side effect of medications (Leigh and Zee, 1999). Disease processes that result in gaze-evoked nystagmus are located in the brainstem and cerebellum (Buttner and Buttner-Ennever, 2006). Key structure in this is the neural integrator (Moschovakis, 1997). The neural integrator generates an eye position signal (a 'step' as in saccade generation, described above) that leads to tonic innervation of the extraocular muscles, which holds the eye in eccentric gaze. When the eye position signal is inadequate, the eyes will drift to the central position (slow phase). This drift will be corrected by a saccade. The eye will be brought back to the eccentric position by a quick phase (figure 3).

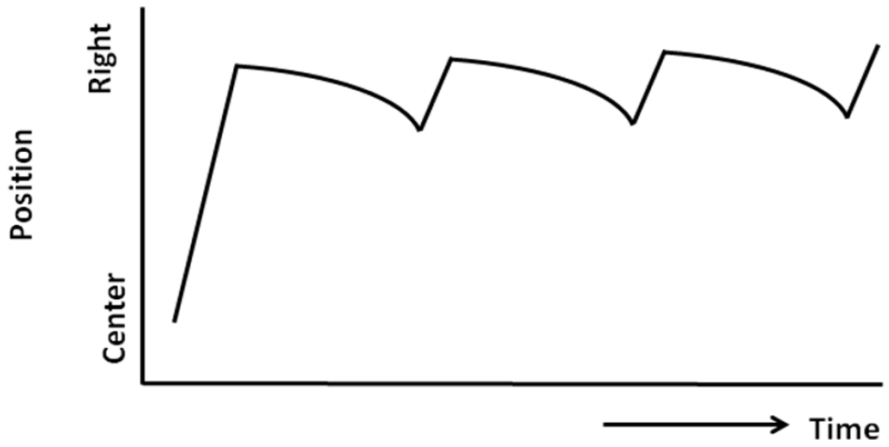

Figure 3. Gaze evoked nystagmus. The eyes move from a center position to the right and start to drift back to the center position (slow phase). A correction saccade brings the eyes back to the eccentric position.

Two types of vertical nystagmus occurring when the eyes are in central position are downbeat nystagmus and upbeat nystagmus (Leigh and Zee, 1999). Downbeat nystagmus occurs in around 40% of the cases without a known underlying pathology (Wagner et al., 2008). Most of the cases in which the aetiology is known occur with cerebellar degeneration and Chiari malformations (Halmagyi et al., 1983; Wagner et al., 2008). A less common but important cause is medical treatment with lithium carbonate (Williams et al., 1988; Halmagyi et al., 1989). Downbeat nystagmus is often accompanied by other oculomotor abnormalities as impaired smooth pursuit. Although in more than half of the cases

of downbeat nystagmus an underlying disease could be assessed, the exact pathophysiology is yet unclear (Marti et al., 2008; Wagner et al., 2008). Upbeat nystagmus occurs with lesions at the pontomedullary (that is the level of the vestibular nuclei) or pontomesencephalic junction (Fisher et al., 1983; Pierrot-Deseilligny and Milea, 2005; Tilikete and Pelisson, 2008; Lee et al., 2009). The pathophysiology of upbeat nystagmus is even less well understood than of downbeat nystagmus (Halmagyi and Leigh, 2004). As noted above, the pathophysiology of both upbeat and downbeat nystagmus has yet to be elucitdated. Since no reports on vertical nystagmus as a result of cerebral lesions are published, the cerebellum and brainstem are likely to be the site of origin. A current theory about the mechanisms behind vertical nystagmus holds that asymmetries in the cerebello-brainstem network that normally stabilizes vertical gaze may lead to these forms of nystagmus (Dieterich, 2007).

The cerebellum

The saccadic system and the smooth pursuit system can learn, i.e. adapt to changing demands. Although it is still not entirely clear which part(s) of the brain is (are) responsible, the cerebellum is likely to play an important role. Experimental lesions of the monkey cerebellum interfere with execution of saccades, smooth pursuit and the adaptation of both eye movements (Takagi et al., 1998; Takagi et al., 2000b; Straube et al., 2001; Rambold et al., 2002). Furthermore, it has a role in other forms of motor learning as the blink reflex and vestibulo-ocular reflex (Rambold et al., 2002; Maschke et al., 2003; Blazquez et al., 2004; Ramat et al., 2007).

The cerebellum is a remarkable part of the brain, located in the caudaldorsal part of the skull. Although the cerebellar volume is only about one tenth of the whole brain, (figure 4) it contains more neurons than the two cerebral hemispheres together (Andersen et al., 1992). Macroscopically, in the coronal plane, the human cerebellum can be divided into three parts: a longitudinal wormlike midline structure, the vermis, and two lateral hemispheres. In the transverse plane, the cerebellum is divided into the flocculonodular lobe and the corpus cerebelli by the fissura postrolateralis. The corpus cerebelli then is divided into a lobus anterior and a lobus posterior by the fissura prima. The lobus anterior and the lobus posterior are further divided into several lobuli, which contain the characteristic folia seen on sagittal views. Functionally, the cerebellum can be divided in three separate regions. Phylogenetically, the oldest part is the vestibulocerebellum, which comprises the flocculonodular lobe. The

vestibulocerebellum receives input from the vestibular system and visual information. Its main concern is balance and eye movements. Output is projects directly to the vestibular nuclei. The second part is the spinocerebellum, which comprises the vermis and the adjacent intermediate parts of the hemispheres. The vermis is predominantly concerned with the proximal parts of the body and eye movements. The intermediate parts of the hemispheres are involved in the control of limbs. Although together named the spinocerebellum, these areas project via different nuclei. The vermis projects to the cortex and brainstem via the fastigial nucleus. The intermediate parts of the hemispheres project via the interposed nucleus to the rubrospinal and corticospinal tracts. Thirdly, the cerebrocerebellum comprises the lateral hemispheres. It receives input from the cerebral cortex and is predominantly concerned with complex motor behavior. Output is projected to the motor, premotor and prefrontal cortices via the dentate nucleus.

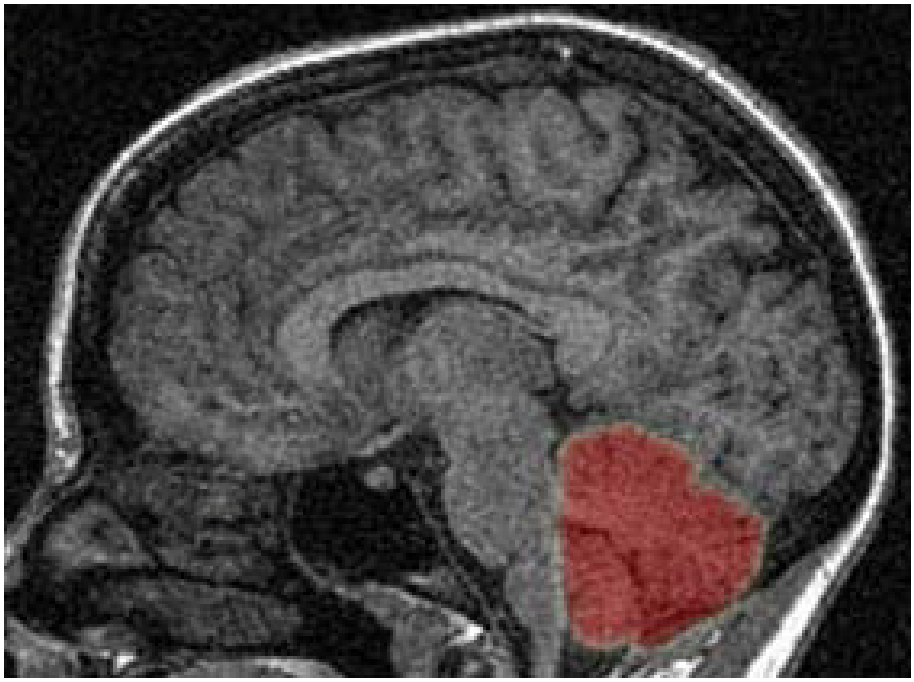

Figure 4. Sagittal view of the brain. The cerebellum highlighted in red.

The architecture of the cerebellar cortex is strikingly regular and uniform throughout. This architecture reflects the flow of information. The principle cell in the cerebellar cortex and the sole provider of output from the cerebellum is the

Purkinje cell. A vast amount of information converges on these cells. The Purkinje cell has an elaborate dendritic tree on which it can receive input from up to 200.000 parallel fibers (Fox and Barnard, 1957). These parallel fibers convey information from nuclei in the spinal cord and brainstem to the Purkinje cell. This information stems from the periphery and the cerebral cortex. In addition, each Purkinje cells receives input from the inferior olive by means of one climbing fiber, which forms multiple synapses on one the Purkinje cell. In contrast: one climbing fiber contacts up to ten Purkinje cells. The climbing fiber conveys somatosensory, visual and cerebral cortical information. The cerebellum interacts with the cerebral cortex by means of closed corticocerebellar loops. In these loops, the cerebral cortex projects via nuclei in the pons to the cerebellar cortex. The Purkinje cells in the cerebellar cortex project back to the cerebral cortex via the thalamus (Ramnani, 2006).

The architecture and connections to other parts of the brain enable the cerebellum to evaluate and integrate sensory perception, coordination and motor behavior. It uses feedback on body position to fine-tune motor movements and learning of motor skills. Additionally, it is claimed to be involved in cognitive-affective regulation (Schmahmann, 1991; Baillieux et al., 2008; Hoppenbrouwers et al., 2008).

Adaptation: neuronal basis

The cerebellum is capable of making small changes in its circuitry. This change is called plasticity and takes place at synapse-level (the synapse is the place where two neurons 'connect'). By means of this plasticity, the strength of synapses of parallel fibers on Purkinje cells is altered when adjustment of the cerebellar output is needed (Ito, 2000). The strength of the synapse can be adjusted in the sense that it becomes stronger (potentiation) or weaker (depression).

The cerebellum and eye movements

The role of the cerebellum role in saccades and smooth pursuit is firmly established by abundant research on non-human primates and humans. Different approaches, such as electrophysiology, microstimulation and lesion studies in non-human primates (Kase et al., 1980; Noda and Fujikado, 1987a; Ohtsuka and Noda, 1995; Krauzlis and Miles, 1998; Takagi et al., 1998; Barash et al., 1999), lesion studies in human patients (Botzel et al., 1993; Straube et al., 2001) and functional imaging studies (using PET & fMRI) (Anderson et al., 1994; Desmurget et al., 2000; Dieterich et al., 2000; Hayakawa et al., 2002) have

generated a vast amount of data on this topic. This data has shown that with respect to saccades and smooth pursuit, the cerebellum is involved in maintaining accuracy and adaptation of these eye movements (Stone and Lisberger, 1986; Robinson et al., 2002; Catz and Thier, 2007).

The cerebellum is of different importance for saccades and smooth pursuit, though. The flocculus-paraflocculus complex and the dorsal vermis both play a role in smooth pursuit (Ilg and Thier, 2008; Schraa-Tam et al., 2008), though the flocculus appears to be particularly important for smooth pursuit eye movements in combination with the vestibular system (Nagao, 1992; Rambold et al., 2002). The flocculus-paraflocculus complex seems mostly to be concerned with both initiation and maintenance ongoing smooth pursuit, as lesions of this area produces deficits of initiation as well as maintaining smooth pursuit (Rambold et al., 2002). The dorsal vermis appears to encode the sum of gaze velocity and retinal image velocity, which is important for maintaining saccadic accuracy. Stimulation of the dorsal vermis during smooth pursuit results in acceleration of the eyes during ongoing smooth pursuit (Krauzlis and Miles, 1998). Additionally, lesions of the posterior vermis impair the smooth pursuit short-term adaptation (Takagi et al., 2000a). Total cerebellectomy even completely abolished smooth pursuit. This is in contrast to the role of the cerebellum in saccades. Lesions of the dorsal vermis impair saccadic accuracy (Barash et al., 1999) and lesions to the flocculus/paraflocculus complex produce post-saccadic drift (Optican et al., 1986). But even after total cerebellectomy, saccades can be made, though they are less accurate and show post-saccadic drift (Optican and Robinson, 1980; Optican et al., 1986).

Electrophysiological and microstimulation studies in the cerebellar oculomotor vermis (lobuli VI-VII) show vermal involvement in saccade generation (Kase et al., 1980; Noda and Fujikado, 1987b; Ohtsuka and Noda, 1995; Krauzlis and Miles, 1998). Additionally, functional imaging studies in humans using Positron Emission Tomography (PET) and functional magnetic resonance imaging (fMRI) (Anderson et al., 1994; Desmurget et al., 2000; Dieterich et al., 2000; Hayakawa et al., 2002) reported cerebellar activation during saccades. The cerebellum, more specifically the oculomotor vermis, is also involved in maintaining the accuracy of saccades. Lesions of the oculomotor vermis impaired the initiation, accuracy and dynamics of saccades in monkeys (Takagi et al., 1998; Barash et al., 1999). Human patients with cerebellar degeneration, cerebellar infarcts or congenital malformations also showed an increase in saccadic variability (Botzel et al., 1993; Straube et al., 2001).

With respect to saccadic adaptation, electrophysiological recordings in monkeys showed that neurons in vermis VI and VII are active in a saccade adaptation task (Catz et al., 2005; Soetedjo and Fuchs, 2006). Microstimulation of the vermis VI and VII in monkeys changes the amplitude of saccadic eye movements (Keller et al., 1983). Lesions of the oculomotor vermis impair the gradual modification of saccadic amplitudes in a saccade adaptation task (Takagi et al., 1998; Barash et al., 1999). Similarly, patients with cerebellar lesions (Straube et al., 2001), cerebellar atrophy (Waespe and Baumgartner, 1992) or paraneoplastic cerebellar ataxia (Coesmans et al., 2003) show impairments in or complete lack of saccadic adaptation capacities. A functional imaging study using PET showed cerebellar activations during saccadic adaptation (Desmurget et al., 1998; Desmurget et al., 2000; Coesmans et al., 2003).

Cerebellar disease

Infarcts, degeneration (inherited or acquired) and tumors can compromise cerebellar functioning and produce profound effects on motor behavior. This is characterized by ataxia (loss of accuracy and timing of movements), hypotonia (a general low muscle tone in the body) and intention tremors (trembling while a voluntary movement is executed).

Eye movements exhibit specific abnormalities when the cerebellum is affected. Saccades become dysmetric (mostly hypermetric) and more variable (Zee et al., 1976; Buttner et al., 1994), which means that they land next to the target. Subsequently, more corrective saccades must be made to foveate the target. Impairment of eye velocity during smooth pursuit renders it insufficient in tracking a moving target. This results in 'catch up saccades' during smooth pursuit to keep the target on the fovea. Furthermore, cerebellar lesions result in impaired adaptation of saccades (Waespe and Baumgartner, 1992; Takagi et al., 1998; Barash et al., 1999; Straube et al., 2001; Coesmans et al., 2003) and smooth pursuit (Takagi et al., 2000a; Ono and Mustari, 2007).

Video-oculography

Video-oculography is a non-invasive method for the recording of eye movements. The basic setup of this type of eye tracking consists of a video camera that captures images of the eye. The eye is illuminated with infrared light and an image of the eye is acquired by an infrared video camera. Dedicated image analysis software calculates the position of the center of the eye pupil in each image.

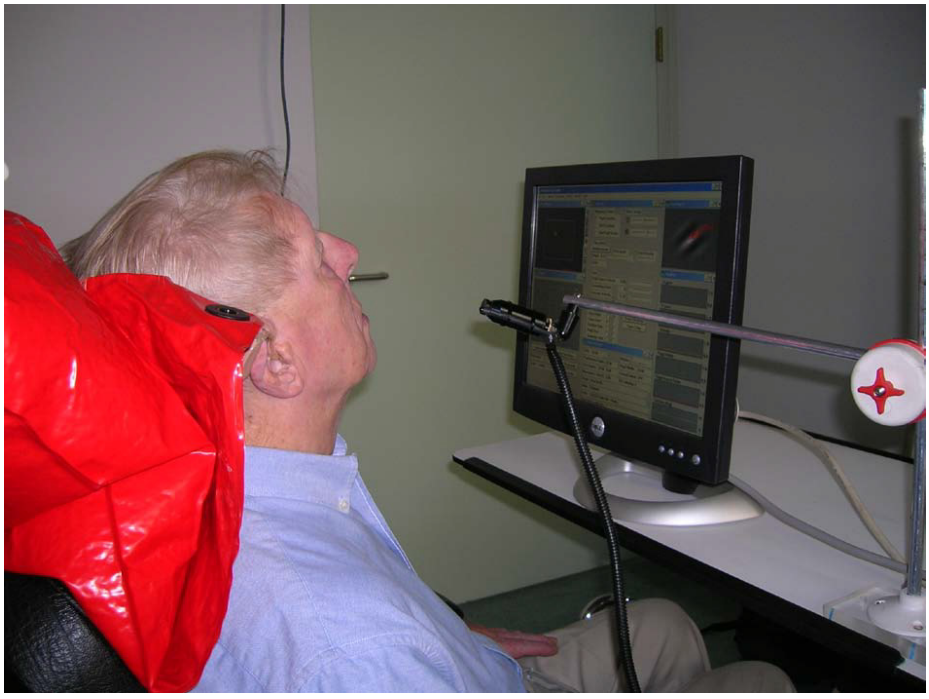

Figure 5. VOG-setup.

In a typical setup, the relative position between the camera and the head of the subject is fixed, by either attaching the camera to the head, or, as in our setups by fixing both camera and head to the environment. The latter can be achieved by using a vacuum cushion or some other type of head restraint.

Therefore, the position of the pupil relative to the camera changes when the eye rotates. This pupil position can be translated into a gaze direction. This is done via a calibration routine. In this routine, the subject is asked to look at specific points in the visual environment and the pupil position within the camera is marked. In other words, known gaze directions are mapped to measured pupil positions. The inverse mapping (pupil position to gaze direction) is applied to subsequent recordings.

With video-oculography gaze direction over time can be measured when subjects are viewing stimuli, and eye movement behavior can be analyzed with relative ease. For instance, the direction and duration of fixations towards pictures can be quantified, or the smooth rotations of the eye in response to a moving OKR stimulus or a single object during smooth pursuit can be inspected for intermittent saccades.

In the experiments presented in this thesis we used two different video-oculographic setups. Both setups required specific adjustments. The setup that was used during magnetic resonance imaging (MRI) needed to be compact, non-magnetic and inert to the electromagnetic environment of the MR scanner. In addition, the eye tracker and the presentation device for the stimuli needed to fit into the rather small space within the MR head coil and scanner bore. In our experiments we used a MR compatible eye tracker (Real Eye RE-4601 Imaging System. Avotec Inc., Stuart, Florida, USA) and visual stimulation device (Silent Vision SV-7021 Fiber Optic Visual System; Avotec Inc., Stuart, Florida, USA).The integrated display goggles and eye tracking device allowed for recording eye movements during visual stimulation within the MR environment.

The second setup was used in a clinical setting. We recorded eye movements in bedridden patients, and therefore the whole setup needed to be mobile (figure 5). We used a hospital cart with a flip-top table on which the setup could be positioned. The computer monitor, on which the stimuli were presented, could be positioned in front of the patient. The camera of the eye tracker (ViewPoint EyeTracker & QuickClamp System, Arrington Research, USA) was attached to a positioning arm so that it could be optimally positioned for the eye movement recording. Furthermore, as the patients would be seated in bed, the position of the camera relative to the head needed to be fixed. Head movements were constrained by a vacuum collar around the neck. This fixated the head in the desired position and supported the head as well.

Functional MRI

Magnetic Resonance Imaging (MRI) is an imaging method that uses the distribution and electromagnetic properties of hydrogen atoms to generate detailed images of the human body tissues. In a clinical setting it is commonly used to differentiate between normal and pathological tissue.

Functional MRI (fMRI) is a way to visualize activity in the brain. Most fMRI experiments consist of a baseline (rest) condition and one or more active conditions. In a typical fMRI experiment, a series of MR images are acquired while a subject executes a specific task. This task consists of two or more conditions. If the neuronal activity of a certain brain area increases during the active condition, blood flow and oxygen consumption in that area will change accordingly. These physiological changes are reflected in the strength of the MR signal that is being measured, the so-called blood oxygenation level dependent (BOLD) response.

Statistical comparisons of the signal measured in the resting condition and the signal measured in an active condition can yield functional images, showing the differences of activation in specific brain areas. Identification of the specific area is done by subsequent overlay of the functional images on the anatomical images of the subject. For example, a subject performs a simple task in which he makes repetitive movements with his fingers (active condition) and is holding still (baseline condition). Comparison of the brain activations might show that the precentral gyrus of the cerebrum (also known as the primary motor cortex) is indeed involved in execution of body movements (Yousry et al., 1997).

fMRI has several advantages and disadvantages when compared to other imaging methods. Similar to electroencephalography (EEG), fMRI is not invasive. This is opposed to Positron Emission Tomography (PET), which requires a radioactive labeled tracer drug to be injected in the patient in order to be able to detect activation. fMRI provides a spatial resolution in the order of millimeters, which is superior over PET or EEG. This allows for a more precisely localized activation. However, the temporal resolution of fMRI is rather poor, being in the order of about 2-3 seconds, dependent on the equipment. This means that fast changing activation is very hard to detect with fMRI.

In two experimental chapters described in this these we aimed at investigating the involvement of the cerebellum in the control of saccadic eye movements. therefore, the cerebellum constitutes approximately only 10 percent of the whole brain volume. Specific BOLD responses arising from this area might be quite hard to distinguish as signal changes during different conditions might arise from areas that are relatively close to each other, and could potentially mask each other. However, previous fMRI research has shown promising results for the use of fMRI in cerebellar research (Dieterich et al., 2000; Hayakawa et al., 2002; Bense et al., 2006). Improvement of functional MRI techniques in order to achieve more detailed results in cerebellar research will help to obtain a better understanding of cerebellar functioning. By performing pilot studies during which we adjusted the scan parameters, we gathered the necessary empirical data to increase the sensitivity for cerebellar activation.

Paraneoplastic Neurological Syndromes

Paraneoplastic neurological syndromes (PNS) are 'remote effects' of cancer. By definition, these neurological effects are not due to infiltration of the tumor or its metastases into surrounding tissue, nor are they due to infection, ischemia or tumor treatment (Darnell and Posner, 2003).

It is currently thought that most, if not all paraneoplastic neurological syndromes are autoimmune mediated diseases. The tumor expresses a protein that is normally exclusively expressed in the nervous system. Due to the ectopic expression of this protein, the body orchestrates an immune attack on that antigen in the tumor. Unfortunately, the immune system does not differentiate between the tumor tissue and healthy neurons, so neurons that express similar antigens are under attack as well.

The symptoms and signs of a paraneoplastic neurological syndrome precede the diagnosis of a tumor in 70% of the cases (Dalmau et al., 1992; Graus et al., 2001; Sillevis Smitt et al., 2002). The delay in tumor diagnosis can take up to years. In some cases, a tumor is even never identified. Tumor growth might be slowed as a result of the immune attack (Darnell and DeAngelis, 1993; Rauer and Andreou, 2002), which makes diagnosis challenging. In some cases, the tumor may have gone into spontaneous regression (Darnell and DeAngelis, 1993).

Virtually every part of the nervous system can be affected in paraneoplastic neurological syndromes. Only single cell-types can be targeted, such as Purkinje cells in anti-Yo associated paraneoplastic cerebellar degeneration, but multifocal involvement with simultaneous deterioration of sensory, motor and/or cognitive function as in some anti-Hu PNS patients is also possible.

Hu-PNS

Patients with paraneoplastic syndromes affecting the central nervous system have antibodies in their serum and CSF. These antibodies are thought to play a role in the disease process. One of the relatively common and well characterized antibodies is the Hu-antibody. This antibody reacts with a neuronal RNA/DNA binding protein called HuD (Szabo et al., 1991). Hu-antibody associated PNS (Hu-PNS) is most often associated with small cell lung cancer (SCLC) (Dalmau et al., 1992; Lucchinetti et al., 1998). In general, PNS affect probably only 0.01 percent of all patients with cancer (Darnell and Posner, 2003). Patients affected by Hu-PNS present with a wide variety of symptoms, depending on the location and extend of neuronal loss. Sensory neuronopathy, autonomic neuropathy, encephalomyelitis, limbic encephalitis, subacute cerebellar degeneration or a combination of these syndromes are possible presentations of Hu-PNS (Graus et al., 2001; Sillevis Smitt et al., 2002).

The prognosis for Hu-PNS patients is poor. Even when they are treated for the underlying tumor and receive concomitant immunomodulatory therapy (Keime-Guibert et al., 1999; Keime-Guibert et al., 2000; Graus et al., 2001; Sillevis Smitt et al., 2002), only 5-7% of the patients show neurological improvement. In

most patients, the neurological deterioration reaches a plateau and leaves the patient severely disabled.

Pathogenesis of Hu-PNS

The destruction of healthy neurons in Hu-PNS is thought to be mediated by antibodies and/or cytotoxic T-cells (Benyahia et al., 1999; Albert et al., 2000; Darnell and Posner, 2003). Pathological examination of affected areas of the nervous system in Hu-PNS shows loss of neurons with localized inflammatory cell infiltrates (Voltz et al., 1998; Bernal et al., 2002; Plonquet et al., 2002). Furthermore, increased numbers of B and T cell subsets in the CSF of Hu-PNS patients add to the evidence for the autoimmune pathogenesis of Hu-PNS (de Graaf et al., 2008). However, a pathogenic role for Hu-antibodies (Graus et al., 1991; Sillevis Smitt et al., 1995) or a direct pathogenic role for cell mediated immunity is not yet established (de Beukelaar et al., 2007).

Diagnosis and motor function evaluation

Diagnosis of a paraneoplastic syndrome is by large the process of exclusion of other possibilities. The presenting symptoms of the patient and signs of inflammation in CSF then can suggest a paraneoplastic neurological syndrome, but the definite diagnosis of PNS is made by detection of antibodies in serum or CSF (Graus et al., 2004).

In cerebellar and other neurological disorders, motor disturbances often are important clinical features. In Hu-PNS patients affected by subacute cerebellar degeneration, obvious symptoms in this respect are deficits in balance and gait. Less obvious to the observer, but not less severe are the specific oculomotor abnormalities, such as dysmetric saccades and non-smooth pursuit (see above). Motor disturbances can thus play an important role in evaluation of Hu-PNS patients. Mild motor disturbances, however, can be quite difficult to assess properly. Since PNS-patients affected by cerebellar ataxia may also show oculomotor disturbances (Leigh and Zee, 1999), video-oculography (VOG) and the quantitative analysis of the eye movements, may provide a useful tool in the assessment of (cerebellum-mediated) motor dysfunction.

Current Treatment options

Current treatment regimes aim for diagnosis of the underlying malignancy, as the only factor that might positively influence the outcome of PNS is eradication of the underlying tumor (Keime-Guibert et al., 1999). Trials with immunomodulatory therapies as plasmapheresis (Graus et al., 1992), intravenous

immunolgobulins (Uchuya et al., 1996) or immunomodulatory drugs (Keime-Guibert et al., 2000; Shams'ili et al., 2006) did not produce consistent satisfactory treatment results, although in individual cases a positive result was achieved (Graus et al., 2001; Sillevis Smitt et al., 2002; Oh et al., 2005).

Overview of this thesis

This thesis aims to provide more insight in the neurophysiological aspects of saccadic error detection, differences between voluntary and reflexive oculomotor control of saccades and oculomotor control of smooth pursuit. The practical use of knowledge from research in oculomotor control will be put to the test by trying to assess cerebellar involvement in paraneoplastic neurological patients.

The first part of this thesis, chapter 2, 3 and 4, aims to provide a more detailed understanding of the neurophysiological substrate of saccades and smooth pursuit. Two aspects of saccadic eye movements will be considered. First, visual error detection is a critical aspect of saccadic adaptation. Although theoretically the cerebellum is a good candidate, it is yet not clear which part of the brain is responsible for this error detection. In chapter 2 we will attempt to clarify this issue by means of a visual stimulation task while performing functional MRI of the whole brain and additionally do the same experiment with a focus on the cerebellum. Second, in chapter 3 we will try to provide more insight in possible differences in recruitment of neuronal pathways between voluntary and reflexive saccades.

Neurophysiological studies in monkeys suggest that smooth pursuit and fixation suppression of optokinetic nystagmus are two behavioral phenomena that may be generated by overlapping but distinct pathways. In chapter 4 we will, by direct comparison, try to assess differences in the neuronal circuitry of smooth pursuit and fixation suppression of optokinetic nystagmus. Additionally, this research in healthy human subjects might reveal activity in areas that were previously not considered to be involved in these types of oculomotor behavior.

In the second part of this thesis, eye movement abnormalities and their use in a clinical setting will be evaluated. Chapter 5 will try to value of quantification of saccadic eye movements and smooth pursuit as part of the assessment of neurological damage in patients with paraneoplastic neurological disorders. In chapter 6 will we look at the effect of Pregnyl on anti-Hu antibody associated paraneoplastic neurological syndromes and simultaneously follow neurological disease progression by evaluation of eye movement abnormalities.

Chapter 2

Cerebellar Contributions to the Processing of Saccadic Errors

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Abstract

Saccades are fast eye movements that direct the point of regard to a target in the visual field. Repeated post-saccadic visual errors can induce modifications of the amplitude of these saccades, a process known as saccadic adaptation. Two experiments using the same paradigm were performed to study the involvement of the cerebrum and the cerebellum in the processing of saccadic errors using functional magnetic resonance imaging and in-scanner eye movement recordings. In the first active condition, saccadic adaptation was prevented using a condition in which the saccadic target was shifted to a variable position during the saccade towards it. This condition induced random saccadic errors as opposed to the second active condition in which the saccadic target was not shifted. In the baseline condition, subjects looked at a stationary dot. Both active conditions compared with baseline evoked activation in the expected saccade-related regions using a stringent statistical threshold (the frontal and parietal eye fields, primary visual area, MT/ V5, and the precuneus (V6) in the cerebrum; vermis VI–VII; and lobule VI in the cerebellum, known as the oculomotor vermis). In the direct comparison between the two active conditions, significantly more cerebellar activation (vermis VIII, lobules VIII-X, left lobule VIIb) was observed with random saccadic errors (using a more relaxed statistical threshold). These results suggest a possible role for areas outside the oculomotor vermis of the cerebellum in the processing of saccadic errors. Future studies of these areas with, e.g., electrophysiological recordings, may reveal the nature of the error signals that drive the amplitude modification of saccadic eye movements.

Introduction

Saccades are fast and accurate eye movements that are encoded within several distinct brain regions, such as the frontal, supplementary, and parietal eye fields (FEF, SEF, and PEF) (Moschovakis et al., 1996; Hopp and Fuchs, 2004). The cerebellum is involved in maintaining saccadic accuracy (Leigh and Zee, 1999; Ramat et al., 2007), and saccade-related activation in the human cerebellum has been shown previously using positron emission tomography (PET) (Anderson et al., 1994; Desmurget et al., 1998) and functional magnetic resonance imaging (fMRI) (Dieterich et al., 2000; Hayakawa et al., 2002).

Saccadic accuracy can be artificially reduced in the laboratory using a so-called saccade adaptation paradigm (McLaughlin, 1967) in which the onset of a saccade triggers an intrasaccadic shift of the target to a new position. Post-saccadic visual errors, induced by consistent intra-saccadic target steps, drive the gradual modification of subsequent saccadic amplitudes over a series of trials in order to retain saccadic accuracy, i.e., to minimize saccadic errors (Wallman and Fuchs, 1998; Robinson et al., 2003; Hopp and Fuchs, 2004)

In a classical saccadic adaptation paradigm, systematic saccadic errors induce a gradual change of saccadic amplitudes in order to minimize the saccadic error (Wallman and Fuchs, 1998; Robinson et al., 2003). In this paradigm, the processing of saccadic errors and the actual modification of saccadic amplitudes are confounded because changes in saccadic amplitude are invariably accompanied by changes in saccadic errors. In order to separate the processing of saccadic errors from the actual modification of saccadic amplitudes, Desmurget et al. (Desmurget et al., 2000) implemented a paradigm in which the intra-saccadic target step was variable. They showed that variable intrasaccadic target steps did induce saccadic errors and corrective eye movements, but this so-called random paradigm did not lead to an overall gradual modification of saccadic amplitudes over a series of trials.

Behavioral studies showed that such a single but random saccadic error has an effect on the amplitude of the subsequent saccade (Frens, 2004; Srimal et al., 2008). This suggests that the brain is quite efficient in processing saccadic errors, for which the cerebellum is a likely candidate (Hopp and Fuchs, 2004). However, in the only study comparing variable intra-saccadic target steps to a condition without intra-saccadic target steps, using PET imaging, no cerebral or cerebellar activation was observed (Desmurget et al., 2000). It is possible that the neuronal

activity related to the processing of increased saccadic errors is simply too small to detect with PET.

In the present study, the activation in the cerebrum and the cerebellum related to the processing of saccadic errors was investigated using fMRI, which provides a better spatial and temporal resolution than PET (Le Bihan et al., 1995). In order to induce saccadic errors without sustained saccadic amplitude changes, we used variable forward and backward intrasaccadic target steps, similar to the random paradigm of Desmurget et al. (Desmurget et al., 2000).

In two separate experiments, we first looked at activation in the cerebral cortex, and in the second experiment, we focused on activation in the cerebellum so that we could obtain more detailed information about this structure using the same behavioral paradigm. We hypothesized that specific cerebellar regions are involved in the processing of saccadic errors.

Materials and methods

Subjects

Written informed consent was obtained from each participant prior to this study, which was approved by the Institutional Review Board. Subjects could participate in either one or both of the two experiments (*whole brain* and *cerebellum*) that were performed. Seventeen subjects (10 male, 7 female; on average 27 years of age, range 19 to 60 years) participated in the whole brain experiment, and 23 subjects (15 male, 8 female; on average 28 years of age, range 19 to 60 years) participated in the cerebellum experiment. Six of these subjects participated in both experiments. None of the subjects had any known neurological or visual deficits other than minor refractive anomalies. None of the subjects wore spectacle correction during the experiments, as minor refractive anomalies could be adjusted for by the goggle system that was used for displaying the stimuli. All subjects reported good visual acuity during the experiment.

Data acquisition

Data were acquired on a 1.5T MRI scanner (Signa CV/I; General Electric, Milwaukee, USA) using a dedicated 8-channel head coil. An anatomical image covering the whole brain was acquired using a 3D high-resolution inversion recovery FSPGR T1 weighted sequence (repetition time (TR)/echo time (TE)/inversion time (TI) 9.99/2/400 ms; flip angle 20 degrees, 320x224 matrix with a rectangular field-of-view of 22cm, 1.2 mm slice thickness with no gap; ASSET factor 2; acquisition time 5 minutes).

In both the whole brain and the cerebellum experiment, functional imaging was performed with single-shot gradient-echo echo-planar imaging (EPI) sequences in transverse orientation that is sensitive to blood oxygenation level dependent (BOLD) contrast. For the whole brain experiment, the imaging volume covered the whole brain (TR/TE 4500/50 ms, 64 x6 4 matrix with a field-of-view of 22 cm, 2.5 mm slice thickness, 48 contiguous slices, voxel size of 2. 5x 3.4 x 3.4 mm^3; 10:03 minutes acquisition time, including 18 seconds of dummy scans that were discarded). For the cerebellum experiment, the imaging volume only covered the whole cerebellum with higher spatial and temporal resolution (TR/TE 3000/30 ms, 96x96 matrix with a rectangular field-of-view of 24 cm, 2.5 mm slice thickness, 18 contiguous slices, voxel size of 2.5 x 2.5 x 2.5 mm^3; 10:00 minutes acquisition time, including 12 seconds of dummy scans that were discarded).

Eye-tracking

Eye movements (monocular, left eye) were registered continuously with the Real Eye RE-4601 Imaging System (Avotec Inc., Stuart, Florida, USA) with a 60 Hz sampling rate. Online monitoring of eye movements and recording was done with the iViewX Eye Tracking System (SensoMotoric Instruments, Teltow, Germany). The system was calibrated before each scan session with the built-in 3-by-3 point calibration routine. Online eye position was send through a dedicated ethernet connection to the stimulation computer, where it was used for on-line updating of the visual display.

Experimental Design

Both experiments consisted of a blocked design in which the baseline condition and one of two active conditions ("*no-step*" and "*random-step*") were presented in alternation. The sequence of conditions started and ended with the baseline condition. The order of the two active conditions was switched halfway the experiment (e.g., baseline – no-step – baseline – random-step … baseline – no-step – baseline – random-step – (*switch*) – baseline – random-step – baseline – no-step … baseline – random-step – baseline – no-step – baseline).

In the whole brain experiment, each of the two active conditions was presented six times and the baseline condition was presented 13 times. An active condition lasted for 31.5 s, during which seven volumes were acquired, and baseline conditions lasted randomly either for 13.5 or 18 s, during which three or four volumes were acquired, respectively. In total, 42 volumes per active condition and 46 volumes of the baseline condition were acquired.

In the cerebellum experiment, each of the two active conditions was presented eight times and the baseline condition was presented 17 times. An active condition lasted for 24 s, during which eight imaging volumes were acquired, and the baseline condition lasted for 12 s, during which four imaging volumes were acquired. In total, 64 volumes per active condition and 68 volumes of the baseline condition were acquired.

In the cerebellum experiment, each active condition lasted for 24 seconds during which time 8 imaging volumes were acquired. The baseline-condition lasted for 12 seconds during which 4 imaging volumes were acquired. Each of the two active conditions was presented 8 times and the baseline condition was presented 17 times. In total, 64 volumes per active condition and 68 volumes of the baseline condition were acquired.

Stimulus paradigm

The experiments were performed in darkness. The visual stimuli were binocularly presented by means of a goggle-based system (Silent Vision SV-7021 Fiber Optic Visual System; Avotec Inc.). The optical components were mounted on top of the head coil. Screen resolution was 1,024×768 pixels and the refresh rate 60 Hz. The visual stimulus was a single yellow dot (0.9° in diameter) presented against a black background. Overall luminance of the whole display was 0.43 cd/m^2. Subjects were instructed to continuously look at that dot. In the baseline condition, the yellow dot was positioned in the center of the screen for the duration of the block.

Both active conditions consisted of a series of several trials per block, during which the dot jumped repeatedly between the left and right side of the screen. At the beginning of each trial, the dot was shown at 9.0° on the left. After a random interval of 1 to 2 s, the dot disappeared from the left side and then flashed once at 9.0° on the right for a period of 100 ms. The flashed dot evoked a saccadic eye movement towards it.

The onset of this primary saccade was estimated online using a position threshold, halfway between the initial position on the left and the target on the right. The saccade triggered the dot to reappear after about 100 ms after saccade detection on the right side of the screen, on a position dependent of the specific active condition (no-step or random-step). After 1 to 2 s, the dot on the right disappeared and the next trial was initiated by the appearance of the dot on the left side. This procedure was repeated for the duration of the active block (31.5 s

in the whole brain experiment or 24 s in the cerebellum experiment), yielding about eight to ten trials per block.

The only difference between the two active conditions was the position where the dot reappeared when triggered by the saccade to the right. In the no-step condition, the position where the dot reappeared was the same as the flashed position, i.e., 9.0° on the right. In the random-step condition, the position where the dot reappeared varied randomly between one out of seven possible positions (5.1°, 6.4°, 7.7°, 9.0°, 10.3°, 11.6°, and 12.9° on the right).

In contrast to existing literature on saccade adaptation where sustained targets are commonly used (Hopp and Fuchs, 2004), the initial saccade to the right was evoked by a flashed target. Usually the intra-saccadic target step and the concurrent removal of the initial saccadic target are triggered by the onset or the peak velocity of the saccade. This point in time can be estimated very reliably in setups with higher eye movement recording frequencies. However, the recording frequency of the in-scanner eye tracking device is limited to 60 Hz, and therefore, this moment of saccadic onset or maximum eye velocity could easily have been missed. In the present stimulation protocol, the dot had already disappeared at the onset of the saccade, preventing subjects from perceiving the offset as well as the actual displacement of the target after the saccade. In a pilot experiment outside the scanner, we performed a saccadic adaptation experiment using flashed targets and a consistent intra-saccadic target step and observed that subjects did normally adapt the amplitudes of their saccades toward these flashed targets. None of the subjects in the present study reported that they saw differences between the two active conditions.

Analysis

Eye movements

Eye movement recordings were analyzed off-line using Matlab (version 6.5, The MathWorks, Inc., Natick, MA, USA). Data points in which the vertical eye position deviated from zero by more than 2° were marked as missing data and discarded from analysis. These data include both blinks and tracking failures. Horizontal eye position data were subsequently smoothed using a Savitsky–Golay polynomial filter.

Saccades were marked automatically using a velocity criterion of 50° per second. Saccadic onsets were defined as the moments of minimum eye velocity in the 50-ms periods before eye velocity exceeded this criterion. Likewise, saccadic offsets were defined as the moments of minimum eye velocity in the 50-ms periods

after eye velocity dropped below the criterion. By definition, the automatically detected saccades needed to have a minimum amplitude of 3° and a minimum duration criterion of 40 ms. Subsequently, the eye movement recordings were checked manually to ensure proper automatic detection of the large primary saccades and to manually include small correction saccades.

For each subject and each condition, the numbers of all primary and corrective saccades were counted. In the active conditions, saccades that moved the eye back to the fixation point on each trial were ignored; in the baseline condition, all (spontaneous) saccades were included. The average amplitude gain of the primary saccades toward the flashed target on the right was calculated for the two active conditions. The gain was defined as the ratio between the amplitude of the primary saccade and the distance to the target (18°). A gain of 1 indicates that a saccade lands perfectly on target. Paired t tests were used to assess significant differences in number of saccades and in primary saccade amplitude gains between the conditions in each of the experiments.

Functional imaging data

The functional imaging data were analyzed using statistical parametric mapping software (SPM 2, distributed by the Wellcome Department of Cognitive Neurology, University College London, UK) implemented in MATLAB (version 6.5, Mathworks, Sherborn, MA, USA). For both studies, motion correction and co-registration were performed according to the methodology provided in SPM2. Brain volumes were normalized to the standard space defined by the Montreal Neurological Institute (MNI) template. The normalized data had a resolution of $2\times2\times2$ mm^3 and were spatially smoothed with a three-dimensional isotropic Gaussian kernel, with a full width half-maximum of 8 mm in the whole brain experiment and 6 mm in the cerebellum experiment.

Statistical parametric maps were calculated for each subject. Movement parameters resulting from the realignment preprocessing were included as regressors of no interest to further reduce motion artifacts. The model was estimated with a high pass filter with a cutoff period of 128 s. For each subject and for each experiment, a t-contrast map was calculated for each of the two active conditions between the active condition and the baseline condition (active > baseline).

The individual t-contrast maps were used for second level random effect group analysis. One sample t tests were performed to assess main effects ([$active_{no\text{-}step}$ > baseline] and [$active_{random\text{-}step}$ > baseline]) in both experiments separately. To investigate the differences in activation between the two active conditions

corrected for baseline activation (direct comparisons), we used a paired t test ([active no-step > baseline] versus [active$_{random\text{-}step}$ > baseline] and vice versa). All tests were thresholded at $p<0.05$ with false discovery rate (FDR) correction for multiple comparisons and at a minimum cluster size of 10 voxels. When no voxels were found to survive the threshold using FDR correction, a more lenient correction for multiple comparisons at cluster level was used.

Anatomic labeling of the observed areas of activation in SPM was done using the macroscopic anatomic parcellation procedure of the MNI MRI single-subject brain (Tzourio-Mazoyer et al., 2002). Reporting of activation is focused on the brain areas that are involved in eye movements, namely, the FEF, SEF, PEF, primary visual area, MT/V5, V6, and the cerebellum.

Results

Eye Movements

Qualitative inspection showed that the eye movement data were insufficient in two subjects in the whole brain experiment and in five subjects in the cerebellum experiment. This included tracking failures, problems in keeping fixation during the baseline condition, and a lack of saccadic eye movements in the active conditions. These 7 subjects were discarded from further analysis, leaving 15 subjects in the whole brain experiment and 18 subjects in the cerebellum experiment. The eye movement behavior of these subjects consisted of stable fixations in the baseline condition and saccadic eye movements toward the targets in the active conditions (see figure 1).

Number of saccades and saccadic amplitudes

In both experiments (whole brain and cerebellum), the saccadic eye movement behavior was similar in the two active conditions (no-step and random-step) with respect to the number of (primary plus correction) saccades and saccadic gains (Table 1). Subjects made more saccades per block in either active condition than in the baseline condition ($p<0.001$). The number of saccades was slightly but not significantly increased in the random-step condition of the cerebellum experiment when compared to the no-step condition ($p=0.05$). In all conditions, the primary saccades towards the flashed target on the right were slightly hypometric with a gain below 1 (Table 1). No differences in these gains were observed between the random-step and no-step conditions in both experiments ($p>0.8$). In the no-step condition, these hypometric primary saccades were followed by a small rightward correction saccade, whereas in the random-step condition, the direction and

amplitude of the correction saccade following the primary saccades depended on the position of the target after the intra-saccadic step.

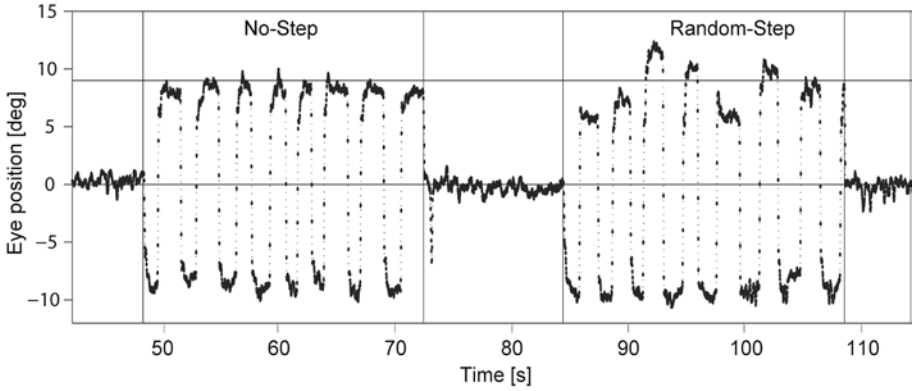

Figure 1. Eye Movements. An example of the eye movement behavior of one subject in the cerebellum study. Shown are a no-step condition (between 48s and 72s) and a random-step condition (between 84s and 108s) interspersed with the baseline condition (fixation) during which no saccades were made. In the no-step condition, the initial saccade and the post-saccadic correction movement brings the eye to the fixed target position of 9 degrees to the right. In the random-step condition, the final target position is variable, explaining the varying fixation positions after the primary saccade and the corrective movements if needed.

Functional imaging data

The results of the random effects group analyses in both experiments are shown in table 2 for the no-step condition and in table 3 for the random-step condition.

No-Step condition

Comparison of the no-step condition with the baseline condition in the whole brain experiment revealed bilateral activation in the precentral gyrus (FEF) and in the superior parietal gyrus and unilateral activation in the right inferior parietal gyrus (PEF). Bilateral activation was also found in the middle temporal gyrus (MT/V5) and the precuneus (V6). No significant activation was found in the supplementary motor area (SEF).

Comparison of the no-step condition with the baseline condition in the cerebellum experiment revealed activation in the vermis VI and VII and bilateral activation in lobule VI of the cerebellar hemispheres.

Random-step condition

Comparison of the random-step condition with the baseline condition in the whole brain experiment revealed bilateral activation in the precentral gyrus (FEF), in the superior parietal gyrus, and unilateral in the right inferior parietal gyrus (PEF). Bilateral activation was also found in the middle temporal gyrus (MT/V5) and the precuneus (V6). No significant activation was found in the supplementary motor area (SEF).

Comparison of the random-step condition with the baseline condition in the cerebellum experiment revealed activation in the vermis VI and VII and bilateral activation in lobule VI of the cerebellar hemispheres.

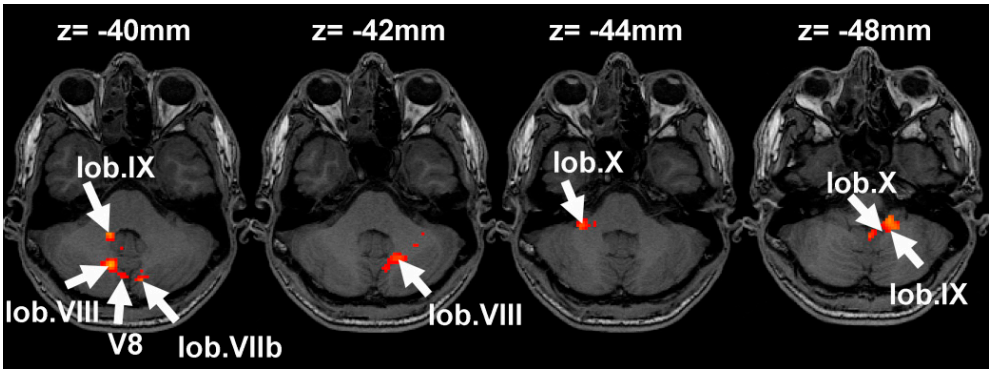

Figure 2. Direct Comparison. Four axial slices showing areas of activation of the direct comparison ([random-step > baseline] > [no-step > baseline]) in the cerebellum experiment (lob. = lobule; V8 = vermis VIII). All areas were thresholded at $p < 0.05$ corrected for multiple comparisons at cluster level and a minimum cluster size of 10 voxels.

Direct comparison

Direct comparison of the two active conditions (no-step vs. random-step and vice versa) yielded no significant activation in either experiment using FDR correction at voxel level. The correction for multiple comparisons was therefore relaxed to correction at cluster level.

The comparison of the no-step condition with the random-step condition ([$\text{active}_{\text{no-step}}$ > baseline] > [$\text{active}_{\text{random-step}}$ > baseline]) revealed activation in the right middle temporal gyrus (MT/V5). For the cerebellum experiment, this comparison revealed no significant activation.

The comparison of the random-step condition with the no-step condition ([$\text{active}_{\text{random-step}}$ > baseline] > [$\text{active}_{\text{no-step}}$ > baseline]) revealed activation in the left middle temporal gyrus (MT/V5). For the cerebellum experiment, the analysis yielded activation in vermis VIII and bilateral activation in the lobules VIII, IX,

and X, and unilateral activation in the left lobule VIIb of the cerebellar hemispheres (Table 4 and Fig. 2).

Discussion

The principal aim of this study was to evaluate the involvement of the cerebrum and the cerebellum in the processing of saccadic errors. We used a modification of the classical saccade adaptation paradigm in which the random intra-saccadic target steps do lead to an increase in saccadic error. Although these increased errors might induce transient changes in saccade amplitudes (Frens, 2004; Srimal et al., 2008), the random distribution of the saccadic errors prevents the sustained adaptation of saccades, as would have been observed in a classical saccade adaptation paradigm with a fixed intra-saccadic target step.

We performed two fMRI experiments, one on the whole brain and one in which we focused on the cerebellum, using different scan parameters to increase the sensitivity for cerebellar activation.

Our main observation was an increase in cerebellar activity with an increase in saccadic errors. This activity was located in vermis VIII, in the hemispheric lobules VIII, IX, and X bilaterally, and in the left lobule VIIb. In a previous study with a similar experimental paradigm using PET, this cerebellar activation was not observed (Desmurget et al., 1998; Desmurget et al., 2000), which is possibly due to the lower resolution achieved with PET imaging. When we looked at the main effects of each condition separately, activation related to saccadic eye movements was observed in the expected cerebral and cerebellar regions.

Cerebral Activation

In the whole brain experiment, cerebral activation was

located in known visual areas [primary visual area (PVA/V1), MT/V5 and the precuneus (V6)] as well as in eye movement related areas [frontal (FEF) and parietal (PEF) eye fields] in both conditions (saccades without induced errors and saccades with random saccadic errors). PVA/V1, MT/V5, and V6 process visual information, which is used to guide saccadic eye movements. The FEF and PEF are involved in the planning and generation of saccadic eye movements (Moschovakis et al., 1996; Hopp and Fuchs, 2004). Activation in these areas is consistent with the results from previous functional imaging studies on saccadic eye movements (Anderson et al., 1994; Mort et al., 2003; Matsuda et al., 2004).

The direct comparisons of the conditions without target steps and with random intra-saccadic target steps in the whole brain experiment only revealed

activation in MT/V5, which is an area specifically involved in the processing of real and apparent visual motion (Born and Bradley, 2005). Although subjects did not see the target step consciously, it is possible that the use of a flashed target and the reappearance of the target after the saccade may have induced differences in low-level processing of apparent motion between the two conditions. Apparent motion can induce fMRI activation of MT/V5 (Goebel et al., 1998), although it does not explain the observed laterality of the activation, dependent on the direction of the comparison, in the present study.

Cerebellar activation

In the cerebellum experiment, activation was found in the vermis VI and VII (known as the oculomotor vermis) and in the cerebellar hemispheric lobule VI bilaterally in both conditions (no target step and random intra-saccadic target step). These findings are consistent with previous imaging studies in humans on saccade eye movements using fMRI (Dejardin et al., 1998; Dieterich et al., 2000; Hayakawa et al., 2002; Nitschke et al., 2004).

Several human and non-human primate studies suggest that the oculomotor vermis is critically involved in the accurate performance of saccadic eye movements. Electrophysiological recordings in vermis VI and VII of the cerebellum yield neuronal activity during saccade generation (Kase et al., 1980; Ohtsuka and Noda, 1995), and microstimulation in these areas evokes saccadic eye movements (Noda and Fujikado, 1987b; Krauzlis and Miles, 1998). Lesions in vermal areas V–VIII, which include the oculomotor vermis, impaired the initiation, accuracy, and dynamics of saccades in monkeys (Takagi et al., 1998; Barash et al., 1999). Similarly, studies in human patients with cerebellar degeneration, cerebellar infarcts, or congenital malformations showed an increase in saccadic variability (Botzel et al., 1993; Straube et al., 2001).

With respect to saccadic adaptation, electrophysiological recordings in monkeys showed that neurons in vermis VI and VII are active in a saccade adaptation task (Catz et al., 2005; Soetedjo and Fuchs, 2006). Microstimulation of the vermis VI and VII in monkeys changes the amplitude of saccadic eye movements (Keller et al., 1983). Finally, lesions of vermis V–VIII impair the gradual modification of saccadic amplitudes in a saccade adaptation task (Takagi et al., 1998; Barash et al., 1999). Similarly, patients with cerebellar lesions (Straube et al., 2001), cerebellar atrophy (Waespe and Baumgartner, 1992), or paraneoplastic cerebellar ataxia (Coesmans et al., 2003) showed impairments in or complete lack of saccadic adaptation capacities. In a functional imaging study

using PET, cerebellar involvement during saccadic adaptation was confirmed (Desmurget et al., 1998; Desmurget et al., 2000).

The direct comparisons of the two saccadic conditions in our cerebellum experiment revealed an increase in activation in vermis VIII and in the hemispheric lobules VIII, IX, and X bilaterally and in left lobule VIIb during the condition with random intra-saccadic target steps compared to the condition without target steps. It could be argued that this increase in activation may, in part, be related to a difference in motor activity due to differences in the number, direction, and size of saccades between the no target step and the random intra-saccadic target step condition. Recently, it has been suggested that corrective saccades may indeed induce activation in several brain structures (Haller et al., 2008). However, we did not observe any difference in the number of primary and correction saccades between the two conditions in either experiment. Furthermore, despite the obvious differences in the corrective saccade metrics, their amplitudes are still small in both conditions. Neurophysiological recordings suggest that saccade size and direction of small saccades do not have an effect on the neuronal activity of cerebellar Purkinje cells (Kase et al., 1980). Therefore, we postulate that the increase in activation is related to the increase in saccadic errors, which should be confirmed using advanced electrophysiological paradigms in animals.

To the best of our knowledge, the involvement of these areas has not been reported previously in the literature on electrophysiological and microstimulation studies in monkeys related to saccadic eye movements, except for lobule VIIb, nor was activation observed using PET imaging (Desmurget et al., 2000). The lack of activity in the saccade-related regions of the cerebellum might relate to the results of electrophysiological studies in monkeys, which suggests that the complex spike activity of cerebellar Purkinje cells does not signal saccadic errors but changed saccadic amplitudes (Catz et al., 2005), although another study suggested that spiking activity might be related to the direction of errors (Soetedjo and Fuchs, 2006). However, the relationship between Purkinje cell activity and the BOLD response remains to be elucidated.

Lobule VIIb of the cerebellum is known to be involved in the generation of saccades (Leigh and Zee, 1999). Furthermore, left-and rightward saccades are under the control of the ipsilateral cerebellar hemisphere. Although the total number of saccades was not different between the two conditions, it can be argued that the increase in activity of the left lobule VIIb in the random-step condition is related to a relative increase in leftward saccades. This is not unlikely given the physiological undershoot of normal saccadic behavior. Saccades tend to fall short

of the target so that in general, correction saccades will be directed to the right in the no-step condition. In the random-step condition, the target is sometimes shifted backwards. Correction saccades in the random-step condition can thus also be directed to the left as well as to the right.

Vermis VIII (pyramis) is not part of the oculomotor vermis, but has been reported to be involved in the performance of hand movements (Nitschke et al., 2005). A previous fMRI study on saccades and hand movements suggested that this area may be related to the execution of a sequence of saccades (Nitschke et al., 2004).

Lobules VIII, IX, and X have been reported to be involved in eye movements other than saccades, namely, in smooth pursuit and in the adaptation of the vestibulo-ocular reflex (VOR) (Rambold et al., 2002; Thier and Ilg, 2005). These areas receive mossy fiber input from the dorsolateral region of the pontine nuclei, relaying information from cerebral visual areas (Glickstein et al., 1994). They also receive input from the inferior olivary nuclei (Brodal and Brodal, 1985; Noda and Mikami, 1986).

The nature of olivary input to the cerebellum in oculomotor learning processes has been a subject of extensive research and is generally thought to serve as an error signal (Ito, 2000; Winkelman and Frens, 2006).

Similar to saccades, the accuracy of smooth pursuit and VOR eye movements depends on the processing of visual errors (Raymond and Lisberger, 1996). The nature of the error signals, however, may vary. The visual error after a discrete saccadic eye movement is static and consists of the perceived distance between the fovea and a stationary target. This error is then used to execute a corrective eye movement. In contrast, the visual error in smooth pursuit and the vestibulo-ocular reflex is regarded as dynamic. For instance, the visual error during smooth pursuit is the perceived distance of the fovea to a moving target in the visual field. Adequate smooth pursuit therefore requires a dynamic adjustment of eye velocity and proper anticipation of the future position of the target to keep it projected on the fovea (Barnes and Asselman, 1991).

Activity in the cerebellar lobules VIII–X related to saccadic errors may therefore represent the visual error signals used for the maintenance of saccadic accuracy (Wallman and Fuchs, 1998). Future studies using electrophysiological recordings of these areas might provide more insight into the nature of the cerebellar activity in the processing of static and dynamic visual errors.

For the assessment of fMRI activation in the direct comparison of the two active conditions, we used a more lenient multiple comparison correction, as at the most stringent correction for multiple comparisons, no voxels were seen to

survive the threshold for significance. This lack of statistical power may be due to two things: first, in the direct comparison, only small differences in activation are likely to be measured, as the two conditions are very similar. Second, it has become clear from neurophysiological studies that the activation related to the processing of visual errors and saccade adaptation depends on small electrophysiological changes and not all cells in an involved area are committed to the same task. For example, subgroups of cells may be dedicated to a specific direction of movement. Furthermore, the changes in activity are not always dependent on increase or decrease of neuronal firing rate, but can also depend on changes in the timing of neuronal firing (Catz et al., 2005; Takeichi et al., 2005; Soetedjo and Fuchs, 2006). These factors increase variation and noise, thus reducing statistical power. It should be noted, on the other hand, that the more lenient multiple comparisons correction did not reveal any significant activation in the whole brain experiment. Neurophysiological recording and stimulation studies will be needed to corroborate the present findings, which are the first to suggest activity in areas outside the oculomotor vermis in relation to errors in saccadic motor performance. In a follow-up study, the size of the visual error can be changed randomly on each trial, which allows for a parametric analysis of the time course of the activation in the various cerebellar arias using an event-related fMRI design.

If areas outside the oculomotor areas indeed participate in saccadic error processing, it is conceivable that these areas are also involved in the modification of saccadic amplitudes, such as in the normal saccadic adaptation paradigm. In the classical saccade adaptation paradigm, subjects are presented with consistent errors rather than the random errors such as those used in the present study. In a functional MRI study using a classical saccade adaptation paradigm, it may, however, be too difficult to detect the contribution of these areas to saccadic error processing for various reasons. First, consistent saccadic errors induce sustained changes in saccadic amplitudes during the course of the experiment (adaptation). These motor changes may lead to confounding changes in motor signals in the cerebrum and cerebellum. Second, the saccadic adaptation process during the course of the experiment will reduce the magnitude of the saccadic errors, which, in turn, results in a concurrent reduction of the brain activation related to saccadic errors. Finally, subjects vary greatly with respect to the speed at which they adapt their saccadic amplitudes over a series of trials: some subjects need a few trials of post-saccadic errors, whereas others need tens of trials or do not adapt systematically at all. The present paradigm of using random step saccadic errors minimizes these confounding factors associated with consistent errors.

Conclusion

Using fMRI, we found an increase in cerebellar activity, but not in cerebral activity, related to random saccadic errors. These results suggest a possible role for areas outside the oculomotor vermis in the processing of saccadic errors. Future studies with, e.g., electrophysiological recordings in monkeys, may be directed specifically towards these areas to corroborate our findings and to investigate the precise nature of the error signals that drive the modification of saccadic eye movements.

Tables

Table 1. Eye Movements

Experiment	Parameter	Baseline	No-step	Random-step
Whole brain	N saccades / block	1.0 ± 0.2	24.5 ± 4.6	25.9 ± 3.6
	Saccadic Gain		0.93 ± 0.11	0.94 ± 0.12
Cerebellum	N saccades / block	1.0 ± 0.3	17.9 ± 2.5	19.5 ± 2.3
	Saccadic Gain		0.89 ± 0.13	0.89 ± 0.12

The number of saccades per block (N saccades / block) and the gain of the primary saccades (saccadic gain) for each of the three conditions (baseline, no-step and random-step) for each of the experiments (whole brain and cerebellum) across subjects (mean ± standard deviation). Note that in the baseline condition no primary saccades were made and that the block durations of the two active conditions were different between the two experiments (31.5 seconds in the whole brain experiment and 24 seconds in the cerebellum experiment).

Table 2. Areas of activation (no-step > baseline)

Cluster size	T-value	MNI coordinates			Anatomical area	Side	% (*)	Functional area
		x	y	z				
Whole brain experiment								
5734	8.92	-14	-76	-2	Lingual Gyrus	L	14.4	PVA/V1
					Lingual Gyrus	R	11.8	PVA/V1
					Calcarine Gyrus	R	11.1	PVA/V1
					Calcarine Gyrus	L	10.8	PVA/V1
					Middle Temporal Gyrus	L	8.2	MT/V5
					Cuneus	L	6.5	PVA/V1
					Middle Occipital Gyrus	L	5.6	PVA/V1
					Fusiform Gyrus	R	3.4	
					Supramarginal Gyrus	L	3.1	
					Cuneus	R	2.6	PVA/V1
					Angular Gyrus	L	2.2	
					Superior Temporal Gyrus	L	1.9	

					Inferior Occipital Gyrus	L	1.5	PVA/V1
					Superior Occipital Gyrus	L	1.1	PVA/V1
45	6.98	-42	36	-14	Inferior Frontal Gyrus	L	100.0	
380	6.59	-48	-10	42	Postcentral Gyrus	L	60.5	
					Precentral Gyrus	L	37.4	FEF
294	6.31	52	-6	38	Precentral Gyrus	R	59.9	FEF
					Postcentral Gyrus	R	38.8	
133	5.84	20	4	4	Globus Pallidus	R	45.1	
					Putamen	R	39.9	
386	5.63	42	-64	10	Middle Temporal Gyrus	R	74.6	MT/V5
					Middle Occipital Gyrus	R	8.8	
104	5.63	58	-50	38	Inferior Parietal Gyrus	R	48.1	
					Angular Gyrus	R	36.5	
					Supramarginal Gyrus	R	11.5	
177	5.59	32	-64	56	Superior Parietal Gyrus	R	78.5	PEF
					Inferior Parietal Gyrus	R	10.7	PEF
					Angular Gyrus	R	7.3	
75	5.33	-20	16	4	Putamen	L	82.7	
					Caudate	L	5.3	
229	5.3	-26	-6	8	Putamen	L	52.0	
					Globus Pallidus	L	28.4	
					Thalamus	L	2.2	
134	5.12	50	40	-14	Inferior Orbital Frontal Gyrus	R	82.8	
					Middle Frontal Gyrus	R	17.2	
243	5.02	62	-42	10	Superior Temporal Gyrus	R	60.9	
					Middle Temporal Gyrus	R	38.3	
26	5.00	40	-10	48	Precentral Gyrus	R	100.0	FEF
19	4.4	18	-44	0	Lingual Gyrus	R	68.4	
					Precuneus	R	31.6	V6
49	4.36	-34	-58	60	Superior Parietal Gyrus	L	98.0	PEF
					Precuneus	L	2.0	V6
19	4.29	12	-16	0	Thalamus	R	42.1	
23	4.26	-24	-74	24	Superior Occipital Gyrus	L	69.6	PVA/V1
					Middle Occipital Gyrus	L	30.4	PVA/V1
14	4.01	22	-84	52	Superior Parietal Gyrus	R	57.1	

17	4.01	46	-36	34	Supramarginal Gyrus	R	76.5	
14	3.87	-56	-8	-28	Inferior Temporal Gyrus	L	64.3	
					Middle Temporal Gyrus	L	35.7	
11	3.64	-50	4	-2	Superior Temporal Gyrus	L	45.5	
					Rolandic Operculum	L	36.4	
					Temporal Pole	L	9.1	
					Inferior Opercular Frontal Gyrus	L	9.1	
10	3.58	32	-80	10	Middle Occipital Gyrus	R	60.0	
Cerebellum experiment								
88	6.33	8	-72	-14	Cerebellum VI	R	81.82	Oculomotor
					Vermis VII		14.77	
					Vermis VI		3.41	
36	6.09	-30	-66	-24	Cerebellum VI	L	100	Oculomotor
70	5.48	40	-60	-26	Cerebellum VI	R	100	Oculomotor
32	5.17	-8	-76	-12	Cerebellum VI	L	100	Oculomotor

All areas were thresholded at $p < 0.05$ with FDR correction for multiple comparisons. (L: left hemisphere, R: right hemisphere; FEF: frontal eye fields, PEF: parietal eye fields, MT/V5: motion-sensitive area, V6: precuneus and PVA/V1: primary visual areas/V1). (*) The unassigned areas for each cluster are not listed in the table.

Table 3. Areas of activation (random-step > baseline)

Cluster size	T-value	MNI coordinates			Anatomical area	Side	% (*)	Functional area
		x	y	z				
Whole brain experiment								
8677	11.91	10	-78	-12	Lingual Gyrus	L	12.9	PVA/V1
					Lingual Gyrus	R	12.08	PVA/V1
					Calcarine Gyrus	L	8.98	PVA/V1
					Calcarine Gyrus	R	8.38	PVA/V1
					Middle Occipital Gyrus	L	6.49	
					Middle Temporal Gyrus	R	4.64	MT/V5
					Fusiform Gyrus	R	4.53	
					Cuneus	L	3.81	PVA/V1
					Fusiform Gyrus	L	3.68	
					Superior Occipital Gyrus	L	3.24	PVA/V1
					Cuneus	R	3.16	PVA/V1

					Middle Temporal Gyrus	L	3.02	MT/V5
					Inferior Occipital Gyrus	L	2.96	PVA/V1
					Superior Occipital Gyrus	R	2.37	PVA/V1
					Middle Occipital Gyrus	R	1.99	PVA/V1
					Supramarginal Gyrus	L	1.29	
410	7.30	-20	-64	60	Superior Parietal Gyrus	L	70.24	PEF
					Precuneus	L	23.17	V6
181	7.20	68	-36	14	Superior Temporal Gyrus	R	85.08	
					Middle Temporal Gyrus	R	11.05	
					Supramarginal Gyrus	R	2.21	
534	6.15	28	-62	54	Superior Parietal Gyrus	R	69.48	PEF
					Inferior Parietal Gyrus	R	11.24	PEF
					Angular Gyrus	R	7.49	
					Precuneus	R	2.81	V6
248	5.59	-48	-2	40	Precentral Gyrus	L	63.71	FEF
					Postcentral Gyrus	L	35.89	
61	5.07	26	6	6	Putamen	R	88.52	
					Globus Pallidus	R	3.28	
					Insula	R	3.28	
27	4.96	56	-54	42	Inferior Parietal Gyrus	R	62.96	
					Supramarginal Gyrus	R	33.33	
					Angular Gyrus	R	3.7	
32	4.70	-64	-34	0	Middle Temporal Gyrus	L	100	
179	4.68	60	-6	46	Precentral Gyrus	R	92.74	FEF
					Inferior Opercular Frontal Gyrus	R	6.7	
20	4.51	-30	-4	42	Precentral Gyrus	L	40	
21	4.35	48	-42	30	Supramarginal Gyrus	R	95.24	
13	4.31	20	-28	-4	Hippocampus	R	38.46	
					Thalamus	R	7.69	
10	4.12	36	-6	-14	Hippocampus	R	20	
45	3.88	50	12	2	Inferior Opercular Frontal Gyrus	R	55.56	
					Inferior Triangular Frontal Gyrus	R	42.22	
					Inferior Orbital Frontal Gyrus	R	2.22	
19	3.88	-14	4	12	Caudate	L	52.63	
					Putamen	L	10.53	

20	3.81	-60	-32	-10	Middle Temporal Gyrus	L	100	
20	3.74	50	2	16	Rolandic Operculum	R	70	
					Precentral Gyrus	R	15	
					Inferior Opercular Frontal Gyrus	R	10	
18	3.55	-24	-2	6	Putamen	L	72.22	
					Globus Pallidus	L	27.78	
Cerebellum experiment								
136	6.69	8	-74	-14	Cerebellum VI	R	77.21	Oculomotor
					Vermis VII		16.18	
78	5.84	-34	-64	-20	Cerebellum VI	L	100	Oculomotor
66	5.78	-8	-76	-12	Cerebellum VI	L	100	Oculomotor
100	5.19	36	-64	-22	Cerebellum VI	R	100	Oculomotor

All areas were thresholded at $p < 0.05$ with FDR correction for multiple comparisons. (L: left hemisphere, R: right hemisphere; FEF: frontal eye fields, PEF: parietal eye fields, MT/V5: motion-sensitive area, V6: precuneus and PVA/V1: primary visual areas/V1). (*) The unassigned areas for each cluster are not listed in the table.

Table 4. Areas of activation ([random-step > baseline] > [no-step > baseline]) in the cerebellum experiment

Cluster size	**T-value**	**MNI coordinates**			**Anatomical area**	**Side**	**% (*)**	**Functional area**
		x	**y**	**z**				
1099	5.08	12	-44	-40	Cerebellum IX	R	13.92	Oculomotor
		12	-66	-40	Cerebellum VIII	R	11.19	Oculomotor
		-16	-38	-48	Cerebellum IX	L	9.19	Oculomotor
		-18	-60	-42	Cerebellum VIII	L	8.28	Oculomotor
		28	-38	-44	Cerebellum X	R	4.73	Oculomotor
		-10	-76	-40	Cerebellum VIIb	L	2.46	Oculomotor
		-16	-36	-48	Cerebellum X	L	1.91	Oculomotor
		4	-74	-40	Vermis VIII		1.18	

All areas were thresholded at $p < 0.05$ corrected for multiple comparisons at cluster level and a minimum cluster size of 10 voxels. (L: left hemisphere, R: right hemisphere). (*) The unassigned areas for each cluster are not listed in the table.

Chapter 3

Cortical and cerebellar activation induced by reflexive and voluntary saccades

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Abstract

Reflexive saccades are driven by visual stimulation whereas voluntary saccades require volitional control. Behavioral and lesional studies suggest that there are two separate mechanisms involved in the generation of these two types of saccades. This study investigated differences in cerebral and cerebellar activation between reflexive and self-paced voluntary saccadic eye movements using functional magnetic resonance imaging. In two experiments (whole brain and cerebellum) using the same paradigm, differences in brain activations induced by reflexive and self-paced voluntary saccades were assessed. Direct comparison of the activation patterns showed that the frontal eye fields, parietal eye field, the motion-sensitive area (MT/ V5), the precuneus (V6), and the angular and the cingulate gyri were more activated in reflexive saccades than in voluntary saccades. No significant difference in activation was found in the cerebellum. Our results suggest that the alleged separate mechanisms for saccadic control of reflexive and self-paced voluntary are mainly observed in cerebral rather than cerebellar areas.

Introduction

Saccades are fast rotatory eye movements that serve to move the eyes as quickly as possible, so that an object of interest is projected onto the fovea where it can be visually processed in detail (Schall, 1995; Hayakawa et al., 2002; Schiller and Tehovnik, 2005; Amlot and Walker, 2006). Saccades can be classified into two broad categories: reflexive and higher-order saccades. Reflexive saccades are saccades toward suddenly appearing targets and are described as reflexive or targeting saccades. Higher-order saccades have a more volitional nature and include voluntary, memory-guided and delayed saccades. Voluntary saccades are made with a cognitive judgment in order to determine when and where to move gaze (Straube and Deubel, 1995; Leigh and Zee, 1999; Walker et al., 2000).

The neurophysiological circuit that drives saccadic eye movements includes several distinct regions of the brain (Leigh and Zee, 1999). The mesencephalic and the pontine reticular formations of the brainstem encode the motor signals that drive the eye muscles. The superior colliculus encodes the direction and amplitude of the saccadic eye movements (Leigh and Zee, 1999). Several cortical brain areas, such as the frontal eye fields (FEF), the supplementary eye fields (SEF), the parietal eye fields (PEF), and the motion sensitive area (MT/V5), are also known to be involved in saccadic generation. Furthermore, the cerebellum is involved in maintaining saccadic accuracy (Leigh and Zee, 1999). Specific areas of the human cerebellum, such as lobules VI and VII and the fastigial nucleus, have been implicated in maintaining saccadic accuracy (Zee et al., 1976; Straube and Deubel, 1995). Patients with cerebellar lesions often show inaccurate saccades (known as saccadic dysmetria) that do not resolve over time (Straube and Deubel, 1995).

Several functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) imaging studies have shown activation in the human cerebellum during saccadic eye movements (Desmurget et al., 1998; Dieterich et al., 2000; Hayakawa et al., 2002; Nitschke et al., 2004). For instance, Hayakawa et al. observed activity in the posterior vermis and the bilateral hemispheres of the cerebellum when subjects made saccades between two stationary targets (Hayakawa et al., 2002).

It has been suggested that there are two separate and largely independent mechanisms involved in the generation of reflexive saccades and voluntary saccades (Deubel, 1995). Mort et al. aimed to compare the cortical activation patterns induced by these two types of saccades with fMRI (Mort et al., 2003). In their paradigm, reflexive saccades were evoked by flashing a peripheral spot to

the left or the right of the fixation spot. Voluntary saccades were evoked by means of an arrow cue as an indicator to the observer to change their point of gaze to a dot pointed at by the arrow. Their results suggested that FEF and PEF were more activated during voluntary saccades and that the angular gyrus and the precuneus were more activated during reflexive saccades (Mort et al., 2003). Furthermore, behavioral studies showed that modification of the amplitudes of reflexive saccades in a saccade adaptation paradigm (McLaughlin, 1967) does not influence the amplitudes of voluntary saccades, and vice versa (Erkelens and Hulleman, 1993; Gaveau et al., 2005; Alahyane et al., 2007). The cerebellum is an important brain structure in saccadic gain control (Ron and Robinson, 1973; Straube and Deubel, 1995; Desmurget et al., 1998; Barash et al., 1999; Straube et al., 2001). Desmurget et al. (2000) demonstrated that the oculomotor vermis of the cerebellum is activated in a saccade adaptation paradigm. The results of the behavioral studies suggest that the cerebellum might also be involved in a different way in maintaining the accuracy of reflexive and voluntary saccades.

In the present study, fMRI was used to investigate putative differences in cerebral and cerebellar activation patterns between reflexive and self-paced voluntary saccadic eye movements. We performed two experiments using the same experimental paradigm. The first experiment aimed to assess cerebral activations of saccadic eye movements and to compare the results with data from existing literature. The second experiment focused on the cerebellum, specifically obtaining more detailed activation within this brain structure. We hypothesized that the differences between reflexive and self-paced voluntary saccadic eye movements might be reflected by differences in cerebral as well as in cerebellar activation patterns.

Materials and methods

Subjects

Written informed consent was obtained from each participant prior to the study, which was approved by the Institutional Review Board. Subjects could participate in either one or both of the two experiments that were performed: whole brain and cerebellum. A total of 26 healthy volunteers (13 men, 13 women; average age 26.7 years, range 22–37 years) participated in the whole brain experiment, and 26 healthy volunteers (15 men, 11 women; average age

26.7 years, range 22–37 years) participated in the cerebellum experiment. Ten of these subjects participated in both experiments. None of the subjects had any known neurological or visual defects other than minor refractive anomalies. None

of the subjects wore spectacle correction during the experiments, as minor refractive anomalies could be adjusted for by the goggle system that was used to display the stimuli. All subjects reported good visual acuity during the experiment.

Data acquisition

For each subject the images were acquired on a 1.5T MRI scanner (Signa CV/I; General Electric, Milwaukee, USA) using a dedicated 8-channel head coil. For the anatomical image, a 3D high-resolution inversion recovery FSPGR T1-weighted sequence covering the entire brain was acquired (repetition time (TR)/echo time (TE)/inversion time (TI)/9.99/ 2/400 ms, flip angle 20°, 320 × 224 matrix with a rectangular field-of-view of 22 cm, 1.2 mm slice thickness with no gap; parallel imaging factor of 2). Acquisition time was 5 min.

Functional imaging

For functional imaging, a single-shot gradient-echo echo-planar imaging (EPI) sequence in transverse orientation was used in each study that is sensitive to blood oxygenation level dependent (BOLD) contrast. For the whole brain experiment, the imaging volume covered the entire brain (TR/TE 4,500/50 ms, 64 × 64 matrix with a rectangular field-of-view of 22 cm, 2.5 mm slice thickness, 48 contiguous slices; voxel size of 2.5 × 3.5 × 3.5 mm^3). Acquisition time was 10:03 min per scanning session (including 18 s of dummy scans that were discarded). For the cerebellum experiment, the imaging volume only covered the whole cerebellum with higher spatial and temporal resolution (TR/TE 3,000/50 ms, 96 × 96 matrix with a rectangular field-of-view of 24 cm, 2.5 mm slice thickness, 18 contiguous slices; voxel size of 2.5 × 2.5 × 2.5 mm^3). Acquisition time was 10:00 min per scanning session (including 12 s of dummy scans that were discarded).

Eye tracking

Eye movements (monocular, left eye) were registered continuously with the Real Eye RE-4601 Imaging System (Avotec Inc., Stuart, FA, USA) with a 60 Hz sampling rate. Online monitoring of eye movements and recording was done with the iViewX Eye Tracking System (SensoMotoric Instruments, Teltow, Germany). The system was calibrated before each scan session with the built-in 3-by-3 point calibration routine.

Stimulus paradigm

The experiments were performed in near darkness. The visual stimuli were binocularly presented by means of a goggle-based system (Silent Vision SV-7021 Fiber Optic Visual System; Avotec Inc.). The optical components were mounted on top of the head coil. Screen resolution was 1,024 × 768 pixels and the refresh rate was 60 Hz.

The visual stimulation was exactly the same for both experiments (whole brain and cerebellum), and consisted of three different visual displays, corresponding to three experimental conditions. In all three displays, three horizontally aligned dots (0.9° of visual angle in diameter) were presented on a dark background. The horizontal separation between the dots was 9°, and the central dot was centered in the subject's visual field-of-view. The overall luminance was 0.43 cd/m^2.

In the fixation condition (baseline), the central dot was yellow, and the two peripheral dots were gray. Subjects were instructed to look at the yellow dot continuously. In the first active condition (reflexive saccades), the central dot and one of the peripheral dots were gray, and the other one was yellow. The two peripheral dots were intermittently yellow with a random interval between 1 and 2 s. In this condition saccade pace was therefore imposed by the other peripheral dot turning yellow.

In the second active condition (voluntary saccades), the central dot was gray, and both peripheral dots were yellow. Subjects were instructed to change their point of gaze between the two dots about every second, thus performing self-paced voluntary saccadic eye movements.

Task design

An experiment consisted of a block design in which the baseline condition [fixation (F)] and one of the two active conditions [reflexive (R) saccades and voluntary (V) saccades] were presented in alternation. The sequence of conditions started and ended with the baseline condition. The order of the two active conditions was switched halfway through the experiment [F–R–F–V…F–R–F–V–F– (switch)–V–F–R…F– V–F–R-F].

In the whole brain experiment, an active condition lasted for 31.5 s during which seven volumes were acquired. The baseline (fixation) condition lasted either 13.5 s (six times) or 18 s (seven times) during which three or four volumes, respectively, were acquired. Each of the two active conditions was presented six times and the baseline condition was presented 13 times in total. In the cerebellum experiment, each active condition lasted for 24 s during which time eight volumes were acquired. The baseline condition lasted for 12 s during which

four volumes were acquired. Each of the two active conditions was presented eight times and the baseline condition was presented 17 times.

Analysis

Eye movements

The eye movement recordings were analyzed offline. Saccadic eye movements were extracted semi-automatically using an eye velocity criterion of 30°/s and checked manually. The total number of saccades was counted, and the average number of saccades per second was calculated for each subject, for each of the three conditions and for each of the two experiments. Paired *t* tests were used to assess differences between the conditions for significance.

Subjects were excluded from the analyses if they showed inadequate eye movement behavior, such as not looking at the yellow dot continuously or making more than one saccade per second in the voluntary condition. Subjects, in whom eye tracking failed due to technical problems, were also excluded.

Functional imaging data

The functional imaging data were analyzed using statistical parametric mapping software (SPM 2, distributed by the Wellcome Department of Cognitive Neurology, University College London, UK) implemented in MATLAB (Version 6.5, Mathworks, Sherborn, MA, USA). For both studies, motion correction and co-registration were done according to the methodology provided by SPM2 (Tzourio-Mazoyer et al., 2002). The time-series of images were realigned using a least square approach and a six parameter spatial transformation. The central image in the time-series was the reference to which all subsequent images were realigned (Friston et al., 1995). Motion parameters were checked for each subject to ascertain that no excessive motion (>3 mm translation or >1.5° rotation) has occurred. None of the scan sessions had to be discarded due to excessive motion.

Brain volumes were normalized to the standard space defined by the Montreal Neurological Institute (MNI) template. The normalized data had a resolution of 2 × 2 × 2mm^3 and were spatially smoothed with a 3D isotropic Gaussian kernel, with a full width half maximum of 8 mm for the whole brain experiment and 6 mm for the cerebellum experiment.

Statistical parametric maps were calculated for each subject. Movement parameters resulting from the realignment pre-processing were included as regressors of no interest to further reduce motion artifacts. The model was estimated with a high-pass filter with a cut-off period of 128 s. For each subject

and for each experiment, t-contrast maps were calculated between each of the two active condition and the baseline condition [(active$_{reflexive}$ > baseline) and (active$_{voluntary}$ > baseline)] and between the two active conditions.

The individual t-contrast maps were used for second level random effects (group) analysis. One sample *t* tests were performed for each of the conditions and each of the experiments separately: (active$_{reflexive}$ > baseline) and (active$_{voluntary}$ > baseline). To investigate the differences in brain activation between the reflexive and the voluntary saccade conditions directly we used an analysis of covariance (ANCOVA) in which we compared active$_{reflexive}$ versus active$_{voluntary}$ and vice versa. In order to ensure that the differences in brain activation between these two active conditions are not caused by differences in the number of saccades made during the active condition, for each subject we counted the numbers of saccades made in the active conditions of the behavioral experiments ($N_{reflexive}$ and $N_{voluntary}$). From these numbers we obtained the ratio [($N_{reflexive}$ - $N_{voluntary}$)/($N_{reflexive}$ + $N_{voluntary}$)] for each active condition, which was entered as a regressor of no interest. All tests were thresholded at $P < 0.05$ with false discovery rate (FDR) correction for multiple comparisons and at a minimum cluster size of 10 voxels. Reporting of activation is focused on the brain areas that are involved in saccadic eye movements, namely the FEF, SEF, PEF, MT/V5, precuneus (V6), cingular and angular gyri, PVA/V1 and the cerebellum. In the whole brain experiment we focused on cerebral activations, whereas the cerebellum study allowed for a more detailed assessment of cerebellar activation.

Results

Eye movements

Inspection of the eye movement behavioral data showed that eye tracking failed in four subjects and that 12 subjects did not perform properly during the experiments (seven subjects in the whole brain experiment, and five subjects in the cerebellum experiment): four subjects made too many saccades in the voluntary conditions and eight subjects did not look at the yellow dot continuously. These 16 subjects were excluded from further analyses leaving 18 subjects for each of the two experiments; nine of these subjects participated in both experiments.

For the whole brain experiment, the average (±SD) number of saccades per second was 0.11 ± 0.04 for the fixation condition, 0.71 ± 0.05 for the reflexive condition and 0.81 ± 0.15 for the voluntary condition. For the cerebellum experiment, the average (±SD) number of saccades per second was 0.09 ± 0.05 for

the fixation condition, 0.70 ± 0.07 for the reflexive condition and 0.72 ± 0.09 for the voluntary condition.

As expected, the number of saccades per second was significantly higher in each of the active conditions than in the baseline condition for both the whole brain experiment and the cerebellum experiment. For the whole brain experiment, the number of saccades per second made in the voluntary condition was higher than in the reflexive condition ($P = 0.012$). In the cerebellum experiment subjects made an equal number of saccades in both active conditions ($P = 0.55$). In both experiments, the average number of saccades per second made in the reflexive condition matched the number of target onsets (0.7 per second). None of the subjects made saccades toward the target before it turned yellow in the reflexive condition.

fMRI activation

The results of the random effects group analysis for the whole brain experiment and for the cerebellum experiment are shown in Tables 1 and 2. In the cerebellum experiment no significant activation was found at a threshold of $P < 0.05$ with FDR correction. Therefore, we used a more lenient statistical threshold of $P < 0.05$ corrected for multiple comparisons at cluster level and with a minimum cluster size of 10 voxels.

Reflexive saccades

Analysis of the reflexive saccade condition for the whole brain experiment revealed bilateral activation in the precentral gyrus (frontal eye fields, FEF), in the superior parietal gyrus (parietal eye fields, PEF) and in the middle temporal gyrus (MT/V5) (Fig. 1). Unilateral activation was found in the left angular gyrus. The cerebellum experiment revealed bilateral activation in lobule VI and unilaterally in crus I on the left (Fig. 2). Activity was also found in vermis VI and VII.

Voluntary saccades

Analysis of the voluntary saccade condition for the whole brain experiment revealed bilateral activation in the precentral gyrus (FEF) and unilateral activation in the left inferior parietal gyrus (PEF) (Fig. 3). The cerebellum experiment revealed only unilateral activation in the right lobule VI (Fig. 4). Activity was also found in vermis VI and VII.

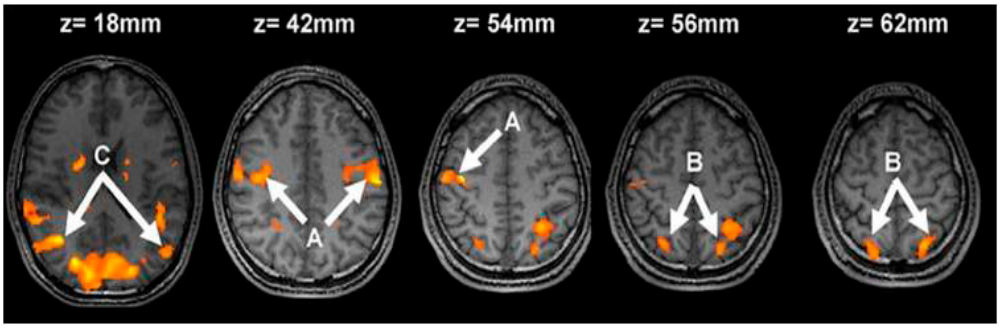

Figure 1. Reflexive saccade eye movement: activation clusters in the whole brain study for reflexive saccades versus fixation. All areas were thresholded at $P < 0.05$ with FDR correction for multiple comparisons and with a minimum cluster size of 10 voxels. (Labels: *A* FEF, *B* PEF *C* MT/V5)

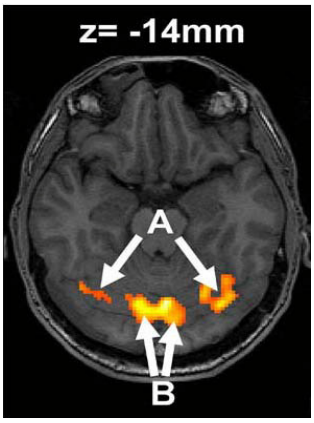

Figure 2. Reflexive saccade eye movement: activation clusters in the cerebellum study. All areas were thresholded at $P < 0.05$ with correction for multiple comparisons at cluster level and with a minimum cluster size of 10 voxels. (Labels: *A* cerebellum lobule VI, *B* vermis VI and VII)

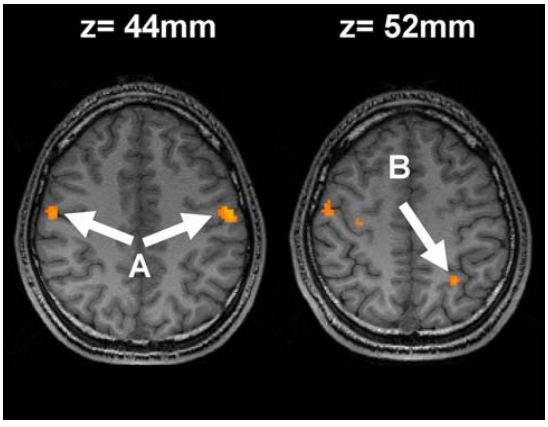

Figure 3. Voluntary saccade eye movement: activation clusters in the whole brain study for voluntary saccades versus fixation. All areas were thresholded at $P < 0.05$ with false discovery rate correction for multiple comparisons and with a minimum cluster size of 10 voxels. (Labels: *A* FEF, *B* PEF)

Direct comparison between reflexive and voluntary saccades

Results of the direct comparison between the two active (saccade) conditions are given in Table 3, and visualized in Fig. 5. When the reflexive saccade condition was compared with the voluntary saccade condition ($active_{reflexive} > active_{voluntary}$) for the whole brain experiment, the analysis yielded bilateral activation in the precentral gyrus (FEF), in the inferior and superior parietal gyrus (PEF), in the middle temporal gyrus (MT/V5), the precuneus (V6), and the angular and the anterior cingulate gyrus, and unilateral right activation in the posterior cingulate gyrus.

When the reflexive saccade condition was compared with the voluntary saccade condition ($active_{reflexive} > active_{voluntary}$) in the cerebellum experiment, the analysis yielded unilateral activation in the left lobule VI. This area of activation, however, was part of the large activation cluster in the fusiform gyrus, similar to that observed in the whole brain experiment. The actual part of this larger cluster being located in the left cerebellar lobule VI was less than 10 voxels.

For both the whole brain and the cerebellum experiment, no significant activation was found when the voluntary saccade condition was compared with the reflexive saccade condition.

A summary of the results is presented in Table 4.

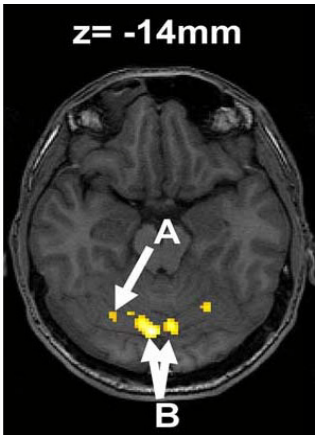

Figure 4. Voluntary saccades eye movement: activation clusters in the cerebellum study. All areas were thresholded at $P < 0.05$ with correction for multiple comparisons at cluster level and with a minimum cluster size of 10 voxels. (Labels: *A* cerebellum lobule VI, *B* vermis VI and VII)

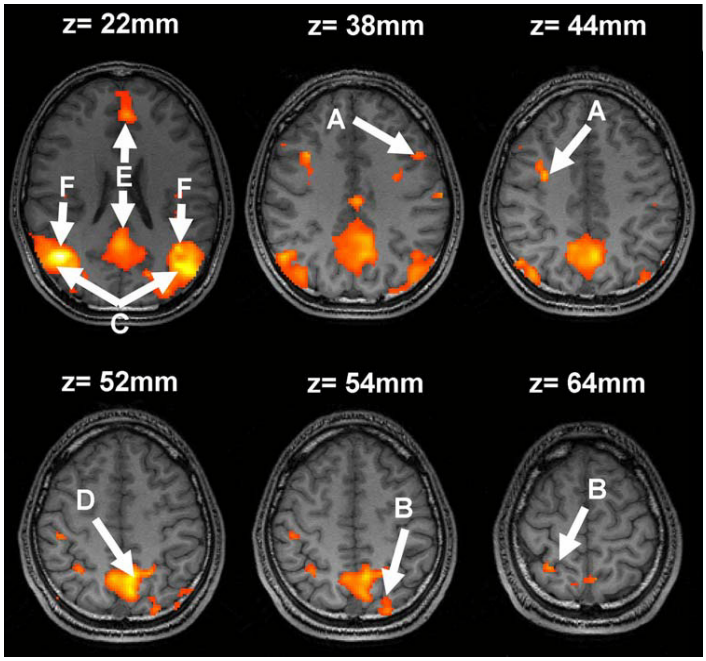

Figure 5. Activation clusters for reflexive versus voluntary saccade eye movement in the whole brain study. All areas were thresholded at $P < 0.05$ with false discovery rate correction for multiple comparisons and with a minimum cluster size of 10 voxels. (Labels: *A* FEF, *B* PEF, *C* MT/V5, *D* precuneus, V6, *E* cingulate gyrus, *F* angular gyrus)

Discussion

This study investigated differences in brain activation patterns between reflexive and voluntary saccadic eye movements. These two types of saccades were compared in two different experiments in which we looked for cerebral activation and for specific activation in the cerebellum. Numerous functional imaging studies have investigated brain activation related to reflexive or voluntary saccades separately. PET studies have shown activation during reflexive saccades in FEF (Anderson et al., 1994; Sweeney et al., 1996), PEF (Anderson et al., 1994), cerebellum, striate cortex and posterior temporal cortex (Sweeney et al., 1996). fMRI studies have also shown activation in FEF (Petit et al., 1997; Luna et al., 1998; Muri et al., 1998; Berman et al., 1999; Nobre et al., 2000), PEF (Luna et al., 1998; Muri et al., 1998; Berman et al., 1999; Nobre et al., 2000), and the cerebellum (Nobre et al., 2000), as well as in SEF (Luna et al., 1998; Berman et al., 1999), the precuneus (Berman et al., 1999), the cingulate gyrus (Berman et al., 1999; Nobre et al., 2000), MT/V5, PVA/V1 and the midbrain. In general, subjects in these studies were asked to execute saccadic eye movement towards suddenly appearing peripheral targets. In our study, we also found that reflexive saccades yielded activation in the FEF, PEF, MT/V5 and in

the angular gyrus, as well as in the cerebellum, more specifically in the cerebellar lobule VI, crus I and in the vermis VI and VII.

For voluntary saccades, PET studies have shown activation in FEF (Fox et al., 1985; Petit et al., 1996; Law et al., 1998), SEF (Fox et al., 1985; Petit et al., 1996; Law et al., 1998), PEF (Petit et al., 1996), PVA/V1 (Fox et al., 1985), the anterior cingulate cortex (Paus et al., 1993), the precuneus (Petit et al., 1996), the midbrain and the cerebellar vermis (Petit et al., 1996; Law et al., 1998). fMRI studies have also shown activation in FEF (Darby et al., 1996; Corbetta et al., 1998), SEF (Darby et al., 1996), PEF, PVA/V1, and the posterior vermis of the cerebellum (Corbetta et al., 1998), as well as in V4. In general, subjects in these studies were asked to execute self-paced voluntary horizontal saccades. In our study, self-paced voluntary saccades yielded activation in the FEF and PEF only, while activation was also found in the cerebellar lobules VI and in the vermis VI and VII.

Taken together, the previous imaging studies suggest a considerable overlap in brain activation during both types of saccadic eye movements. However, direct comparison studies of the brain activation patterns induced by reflexive and voluntary saccades are scarce. So far, only one fMRI study has investigated differences in activation patterns between both types of saccades (Mort et al., 2003). In the latter study, voluntary saccades were evoked by the sudden onset of a central cue indicating the direction of the saccade that was to be made. Using more lenient statistical thresholds than used in our study, the authors reported that, relative to voluntary saccades, the precuneus and the angular gyri were more strongly activated during reflexive saccades and that, relative to reflexive saccades, FEF and PEF were more strongly activated during voluntary saccades (Mort et al., 2003). In our study it was found that, relative to self-paced voluntary saccades, the MT/V5, the precuneus, and angular and cingulate gyri were more strongly activated during reflexive saccades. Relative to reflexive saccades, we found no area of interest which was more activated during self-paced voluntary saccades. In the cerebellum we found no significant differences in activation patterns between reflexive and voluntary saccadic eye movements.

Frontal, supplemental and parietal eye fields (FEF, SEF, PEF)

Studies on FEF and SEF lesions in human patients and nonhuman primates suggest that both areas are either not dominantly involved in the generation of reflexive saccades or that damage can be compensated for on a behavioral level by other areas, such as the brainstem (Schiller et al., 1980; van der Steen et al., 1986; Lee and Tehovnik, 1995; Sommer and Tehovnik, 1997; Schiller and Chou, 1998; Dias and Segraves, 1999). However, lesions of the PEF in non-human primates

(Lynch and McLaren, 1989) and humans (Pierrot-Deseilligny et al., 1991) considerably delay the onset of reflexive saccades, suggesting that the PEF might be more essential than the FEF or SEF for the adequate performance of reflexive saccades (Gaymard et al., 1993).

On the contrary, the contribution of FEF and PEF to voluntary saccades has not been extensively evaluated by lesion studies. Until now, the most studied form of voluntary saccades is the memory-guided saccades (Pierrot-Deseilligny et al., 1991; Rivaud et al., 1994; Sommer and Tehovnik, 1997). These studies suggest that unilateral FEF lesions in humans slow down the onset of contralesional memory-guided saccades. Similarly, comparable data on the contribution of PEF to voluntary saccades are scarce as well.

We found that PEF was more activated in reflexive than voluntary saccades, which is in accordance with the lesion studies (Lynch and McLaren, 1989; Pierrot-Deseilligny et al., 1991; Gaymard et al., 1993). The PEF is thought to be involved in saccades in terms of visuospatial localization, attention and integration (Pierrot-Deseilligny et al., 1991; Muri, 2006). These cognitive processes are likely to be involved in a reflexive saccade condition. At each target onset, the brain needs to attend to it, localize the peripheral target and encode the appropriate spatial directions for the saccadic eye movement towards it (i.e. integration) (Matsuda et al., 2004). In the voluntary condition these processes may have a less prominent role, which might relate to the increased activation in PEF in the reflexive saccade condition.

We also found that the FEF was more activated during the generation of reflexive rather than voluntary saccades, which seems to be at odds with the physiological and functional studies mentioned above. However, it has been proposed that the FEF, which is related to the preparatory stage of saccadic responses, sends out intention and readiness signals to the superior colliculus (Connolly et al., 2002). Since in our paradigm subjects were aware that they were about to make reflexive saccades, the activation in the FEF could reflect such intention and readiness signals.

The increased activation in the FEF and PEF during the generation of reflexive compared to voluntary saccades is in contrast to an earlier study which found that FEF and PEF were more activated during voluntary saccades (Mort et al., 2003). In the latter study eye movement was not recorded during scanning and the number of saccades may not have matched the number of stimuli. In the present study, we recorded the in-scanner behavior and found that the number of saccades in the different active conditions was different. However, adding the number of saccades as a regressor in the fMRI analysis did not change the differ-

ences in the activation between reflexive and voluntary saccades. We did not observe any significant activation in the SEF during saccadic eye movements. Activation in the SEF related to reflexive or voluntary saccade eye movements has been reported by some (Fox et al., 1985; Darby et al., 1996; Muri et al., 1996; Petit et al., 1996; Law et al., 1998; Luna et al., 1998; Berman et al., 1999; Nobre et al., 2000), but not by others (Anderson et al., 1994; Mort et al., 2003).

Motion-sensitive area (MT/V5)

Neurons in the MT area of monkeys are specifically responsive to visual motion, selectively for both direction and speed, and have receptive field sizes of up to 25° in visual angle (Zeki, 1974; Baker et al., 1981; Van Essen et al., 1981; Maunsell and Van Essen, 1983; Felleman and Kaas, 1984; Churchland and Lisberger, 2001). Functional imaging studies in humans have shown that the human homologue, area MT/V5, is also highly responsive to visual motion stimuli (Zeki et al., 1991; Watson et al., 1993; Tootell et al., 1995). We found that MT/V5 was bilaterally more activated in the reflexive than in the voluntary saccade condition, although no real visual motion was present in either condition. This activation could be explained by a phenomenon known as apparent motion (Wertheimer, 1912). When two stimuli at two different locations are turned on and off in alternation (as was the case in our reflexive condition), subjects often perceive this as one single stimulus moving between two locations, rather than two stimuli flashing in alternation at the two locations. This percept is absent when the two stimuli are presented simultaneously, as in our voluntary saccade condition. Such a stimulus, in which the apparent motion phenomenon occurs, evokes activation in the human MT/V5 region (Goebel et al., 1998) and in area MT of monkeys (Mikami, 1991; Albright, 1993), just like a true visual motion stimulus.

Precuneus, cingulate gyrus and angular gyrus

The present study demonstrated that the precuneus, the posterior and anterior cingulate and the angular gyri showed more activation during reflexive and voluntary saccades.

Studies in non-human primates suggest that the precuneus belongs to part of the neural network specialized for the processing of spatially-guided behavior (Selemon and Goldman-Rakic, 1988). Functional imaging studies have shown that the precuneus is involved in reflexive saccadic eye movements (Berman et al., 1999) and is associated with shifts of spatial attention (Cavanna and Trimble, 2006). The anterior cingulate gyrus is involved in target detection (Posner and

Petersen, 1990) and is activated during self-paced saccades (Paus et al., 1993; Petit et al., 1996; Sweeney et al., 1996) and during reflexive saccades (Berman et al., 1999; Mort et al., 2003). Neurons in the posterior cingulate cortex of primates fire instantly to assign the spatial coordinates after a saccade in which the eye position signals are provided and to permit monitoring of either eye or self motion (Olson et al., 1996). Functional studies have shown that the posterior cingulate cortex is involved in confirming the new target position during reflexive saccades (Mort et al., 2003). Lesion studies show that the main area facilitating the triggering of reflexive visually-guided saccades, but not the voluntary saccades (Cavanna and Trimble, 2006), is located in the posterior parietal cortex, in or near the superior part of the angular gyrus (Pierrot-Deseilligny, 1991; Pierrot-Deseilligny et al., 1991).

Cerebellar activation

The cerebellum plays an important role in the control rather than in the generation of saccadic eye movements. The vermis (VI and VII) are involved in controlling the accuracy and timing of saccades (Noda et al., 1990; Voogd and Barmack, 2006).

Microstimulation of vermis VI and VII in the alert monkey induce and influence saccadic eye movements (Ron and Robinson, 1973). The Purkinje cells of vermis VI and VII project to the caudal part of the fastigial nucleus, which projects to the vestibular nuclei and saccade-related brainstem nuclei (Noda et al., 1990). Electrophysiological experiments and clinical studies suggest that the vermis VI and VII are involved in the direction-selective control of saccade metrics and in saccadic adaptation (Kase et al., 1980; Suzuki and Keller, 1988; Fuchs et al., 1993). Disrupting the posterior vermis, especially area VI, VII and paravermis, in humans using transcranial magnetic stimulation also suggest that these areas are related to the execution of visually-guided saccades ((Hashimoto and Ohtsuka, 1995). Lesioning the oculomotor vermis in monkeys leads to a clear shortening of saccades (saccadic hypometria), an increase in saccadic amplitude variability and loss of adaptive capability of saccadic amplitudes (Takagi et al., 1998; Barash et al., 1999). Although hypometria dissolves within a year, saccadic amplitudes remain highly variable (Barash et al., 1999). Saccadic behavior of patients with lesions of the vermis VI and VII suggest that there is a dissociation between the extent of saccadic variability and the lack of adaptive capability (Straube et al., 2001). Indeed, an increased variability in saccadic accuracy does not generally lead to diminished saccadic adaptation (van der Geest et al., 2006). Lesions of the vermis VI and VII and the posterior hemispheres do not only affect

saccadic accuracy, but may also delay the covert orientation of visuospatial attention (Townsend et al., 1999).

Several fMRI studies have reported activation of the cerebellum during saccadic eye movements. The cerebellar hemispheres were bilaterally activated during both voluntary (Dieterich et al., 2000) and reflexive saccades (Hayakawa et al., 2002). Activation in vermis VI and VII was observed in reflexive saccades (Hayakawa et al., 2002; Nitschke et al., 2004). Dieterich et al. proposed that vermis IX and lobules IV and V, as well as a small portion of vermis VIII, might be involved in oculomotor performance and the activation of the cerebellar hemispheres could possibly reflect visuospatial attention processes (Dieterich et al., 2000). Nitschke et al. suggested that the vermis VI and VII of the cerebellum play a predominant role in the control of visually-triggered saccadic eye movements, and are involved in processing visuospatial working memory and attention (Nitschke et al., 2004).

In accordance with these previous studies, we also found activation in cerebellar lobule VI and vermis VI and VII, for both types of saccades. However, when we compared the two types of saccades directly, no significant difference in activation was found. So, although the cerebellum is thought to be critically involved in saccade amplitude modifications in humans (Desmurget et al., 1998; Straube et al., 2001), the lack of transfer between the adaptation of reflexive and voluntary saccades as observed in behavioral studies (Erkelens and Hulleman, 1993; Gaveau et al., 2005; Alahyane et al., 2007) is not reflected by differences in cerebellar activation between the two types of saccades. There are two possible, and not mutually exclusive, explanations for the lack of differential activation. It is possible that the modification of amplitudes of the two types of saccades is processed by different sets of neurons within the same cerebellar regions. Alternatively or additionally, the lack of transfer between voluntary and reflexive saccade adaptation arises on a cerebral level.

In the present experiments, voluntary saccades were self-paced saccades made between two targets. The present paradigm was chosen to mimic the saccadic adaptation experiments in which voluntary and reflexive saccades were dissociated with respect to their amplitude modifications. However, compared to other voluntary eye movement tasks (such as antisaccades and memory-guided saccades), the present task does not engage cognitive processes of inhibitory control and working memory which would recruit frontal areas. Hence, the lack of findings in these regions is not entirely unexpected. Moreover, subjects are able to actively suppress a reflexive saccade using cognitive processes of inhibition. It can be argued that the presently observed differences in circuitry between

voluntary and reflexive saccades are mainly related to a self-paced mechanism which is, in our view, still volitional in nature.

Conclusion

The execution of reflexive saccades induced stronger activation in several cerebral areas, but not in the cerebellum, than the execution of self-paced voluntary saccades. This could indicate that functional difference in maintaining the accuracy of the two types of saccades is mediated on a cerebral level, or that it involves overlapping cerebellar regions with possible functional differences.

Tables

Table 1. Areas of activation (reflexive saccades > fixation)

Cluster size	T-value	MNI coordinates			Anatomical area	Side	% (*)	Functional area
		x	y	z				
Whole brain study								
14,877	7.78	46	-74	-2	Middle temporal gyrus	R	4.89	MT/V5
					Middle temporal gyrus	L	3.6	MT/V5
					Lingual and calcarine gyri	L	31.33	PVA/V1
					Middle, inferior and superior occipital gyri, cuneus			
					Lingual and calcarine gyri	R	26.56	PVA/V1
					Middle, inferior and superior occipital gyri, cuneus			
					Fusiform gyrus	R	4.38	
					Fusiform gyrus	L	2.65	
					Angular gyrus	L	1.18	Angular gyrus
					Superior temporal gyrus	L	1.01	
1,124	6,022	40	-10	54	Precentral gyrus	R	51.6	FEF
					Postcentral gyrus	R	30.78	
					Middle frontal gyrus	R	3.2	
454	5.78	-60	-14	42	Postcentral gyrus	L	68.72	
					Precentral gyrus	L	26.87	FEF
394	4.64	-24	-62	62	Superior and inferior parietal gyrus	L	91.11	PEF
					Postcentral gyrus	L	2.54	
99	4.51	24	-48	40	Superior and inferior parietal gyrus	R		PEF
146	4.28	26	-62	56	Superior parietal gyrus	R		PEF
Cerebellum study								
409	7.02	-4	-76	-14	Cerebellum VI	L	38.39	Oculomotor area
					Cerebellum VI	R	20.29	
					Vermis VI		18.09	
					Vermis VII		4.16	
					Cerebellum crus 1	L	2.44	

All areas were thresholded at $P < 0.05$ with FDR (whole brain study) or at cluster level ($P < 0.05$) corrected for multiple comparisons (cerebellum study) and with a minimum cluster size of 10 voxels.

L left hemisphere, *R* right hemisphere, *FEF* frontal eye fields, *PEF* parietal eye fields, *MT/V5* motion-sensitive area (MT/V5), *PVA/V1* primary visual areas (V1).

(*) The unassigned areas for each cluster are not listed in the table.

Table 2. Areas of activation (voluntary saccades > fixation)

Cluster size	T-value	MNI coordinates			Anatomical area	Side	% (*)	Functional area
		x	y	z				
Whole brain study								
323	8.35	-62	2	20	Postcentral gyrus	L	44.89	FEF
					Precentral gyrus	L	19.81	
					Rolandic opercular gyrus	L	16.72	
					Inferior frontal opercular gyrus	L	14.24	
2,848	7.62	-16	-78	6	Lingual and calcarine gyri Middle, inferior and superior occipital gyri, cuneus	R	40.81	PVA/V1
					Lingual and calcarine gyri, Middle, inferior and superior occipital gyri, cuneus	L	49.31	PVA/V1
					Fusiform gyrus	R	1.19	
31	5.1	-34	-74	-6	Inferior and middle occipital gyri	L	61.92	PVA/V1
36	4.94	28	-84	24	Middle and superior occipital gyri	R	100	PVA/V1
14	4.37	-28	-50	54	Inferior parietal gyrus	L	100	PEF
137	4.32	56	-8	44	Precentral gyrus	R	57.66	FEF
					Postcentral gyrus	R	33.58	
					Middle frontal gyrus	R	8.76	
					Middle frontal gyrus	R	8.76	
Cerebellum study								
48	4.65	6	-76	-20	Cerebellum VI	R	70.83	Oculomotor area
					Vermis VI		20.83	
					Vermis VII		8.33	

All areas were thresholded at $P < 0.05$ with FDR (whole brain study) or at cluster level ($P < 0.05$) corrected for multiple comparisons (cerebellum study) and with a minimum cluster size of 10 voxels.

L left hemisphere, *R* right hemisphere, *FEF* frontal eye fields, *PEF* parietal eye fields, *MT/V5* motion-sensitive area (MT/V5), PVA/V1 primary visual areas (V1).

(*) The unassigned areas for each cluster are not listed in the table.

Table 3. Areas of activation (reflexive saccades > voluntary saccades)

Cluster size	T-value	MNI coordinates			Anatomical area	Side	% (*)	Functional area
		x	y	z				
Whole brain study								
20,961	9.11	46	-60	22	Middle temporal gyrus	R	10.92	MT/V5
					Middle temporal gyrus	L	9.78	MT/V5
					Precuneus	L	8.18	V6
					Inferior and middle occipital gyri	L	7.47	PVA/V1
					Precuneus	R	5.83	V6
					Angular gyrus	R	4.33	Angular gyrus
					Fusiform gyrus	L	3.6	
					Angular gyrus	L	3.58	Angular gyrus
					Inferior and middle occipital gyri	R	4.49	PVA/V1
					Fusiform gyrus	R	2.73	
					Inferior and superior temporal gyrus, hippocampal gyrus	R	7.07	
					Inferior temporal gyrus, parahippocampal gyrus	L	5.15	
					Posterior cingulate gyrus	R	1.54	Posterior cingulate gyrus
					Middle cingulate gyrus	L	1.3	
					Lingual gyrus	R	1.04	
139	4.96	30	-46	64	Superior and inferior parietal gyrus	R	71.95	PEF
					Post central gyrus	R	28.06	
277	4.76	32	0	44	Middle frontal gyrus	R	28.16	FEF
					Precentral gyrus	R	25.99	
					Inferior opercular frontal gyrus	R	19.86	
918	4.5	-2	44	20	Superior middle frontal gyrus	L	40.41	
					Superior middle frontal gyrus	R	14.81	
					Anterior cingulate gyrus	L	12.53	Anterior cingulate gyrus
					Middle orbital frontal gyrus	R	10.24	
					Middle orbital frontal gyrus	L	10.02	
					Anterior cingulate gyrus	R	7.19	Anterior cingulate gyrus
25	3.94	-52	-62	52	Angular gyrus	L	16	Angular gyrus
					Inferior parietal gyrus	L	16	
77	3.73	-24	-78	54	Superior and inferior parietal gyrus	L	63.63	PEF
26	3.6	2	-36	60	Precuneus	L	46.15	V6
					Paracentral lobule	R	30.77	
					Paracentral lobule	L	3.85	
					Precuneus	R	3.85	V6
15	3.56	-6	28	14	Anterior cingulate gyrus	L	33.33	Anterior cingulate gyrus
14	3.24	-46	16	38	Middle frontal gyrus	L	92.86	FEF
					Precentral gyrus	L	7.14	
Cerebellum study								
103	7.07	-44	-44	-22	Cerebellum VI	L	6.8	Oculomotor area

All areas were thresholded at $P < 0.05$ with FDR (whole brain study) or at cluster level ($P < 0.05$) corrected for multiple comparisons (cerebellum study) and with a minimum cluster size of 10 voxels. L left hemisphere, R right hemisphere, FEF frontal eye fields, PEF parietal eye fields, MT/V5 motion sensitive area (MT/V5), V6 Precuneus, PVA/ V1 primary visual areas (V1).

(*) The unassigned areas for each cluster are not listed in the table.

Table 4. A summary of areas of activation in the functional areas of interest for the whole brain and the cerebellum experiments.

Areas	Reflexive saccade	Voluntary saccade	Reflexive > voluntary	Voluntary > reflexive
Whole brain study				
FEF	B	B	B	-
SEF	-	-	-	-
PEF	B	L	B	-
MT/V5	B	-	B	-
Precuneus/V6	-	-	B	-
Angular gyrus	L	-	B	-
Cingulate gyrus	-	-	B	-
Cerebellum study				
Lobule VI	B	R	-	-
Crus I	L	-	-	-
Vermis VI	+	+	-	-
Vermis VII	+	+	-	-

All areas were thresholded at $P < 0.05$ with FDR (whole brain study) or at cluster level ($P < 0.05$) corrected for multiple comparisons (cerebellum study) and with a minimum cluster size of 10 voxels.

B bilateral, *L* left hemisphere, *R* right hemisphere, *FEF* frontal eye fields, *SEF* supplementary eye fields, *PEF* parietal eye fields, *MT/V5* motion-sensitive area (MT/V5), *PVA/V1* primary visual areas (V1).

+ with significant activation, - no significant activation.

Chapter 4

An fMRI study on smooth pursuit and fixation suppression of the optokinetic reflex using similar visual stimulation

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Abstract

This study compares brain activation patterns evoked by smooth pursuit and by fixation suppression of the optokinetic reflex (OKR) using similar retinal stimulation. Functional magnetic resonance imaging (fMRI) was performed during smooth pursuit stimulation in which a moving target was presented on a stationary pattern of stripes, and during fixation suppression of OKR in which a stationary target was presented on a moving pattern of stripes. All subjects could effectively ignore the background pattern and were able to keep the target continuously on the fovea with few saccades, in both experiments. Smooth pursuit evoked activation in the frontal eye fields (FEF), the supplementary eye fields (SEF), the parietal eye fields (PEF), the motion-sensitive area (MT/V5), and in lobules and vermis VI of the cerebellum (oculomotor areas). Fixation suppression of OKR induced activation in the FEF, PEF, and MT/V5. The direct comparison analysis revealed more activation in the right lobule VI of the cerebellum and in the right lingual and calcarine gyri during smooth pursuit than during fixation suppression of OKR. Using similar retinal stimulation, our results show that smooth pursuit and fixation suppression of the OKR appear to activate largely overlapping pathways. The increased activity in the oculomotor areas of the cerebellum during smooth pursuit is probably due to the presence of an active eye movement component.

Introduction

Both smooth pursuit and fixation are oculomotor behaviors dedicated to keep the image of an object projected onto the fovea so that the object can be visually processed in great detail (Carpenter, 1977; Kandel ER, 2000). Smooth pursuit is commonly evoked by a small moving target whereas fixation is used to foveate a stationary target. For these oculomotor behaviors to be effective, the motion of the visual background relative to the target has to be ignored. A moving background could evoke an optokinetic reflex (OKR) and leads to an eye movement pattern known as optokinetic nystagmus (OKN). The suppression of a moving background during fixation on a stationary target is referred to as fixation suppression of the OKR.

Neurophysiological studies in monkeys suggest that smooth pursuit and fixation suppression of OKR are two behavioral phenomena that may be generated by overlapping but distinct pathways. Microstimulation of specific regions in the motion-sensitive temporal areas (MT/V5) (Komatsu and Wurtz, 1988), the oculomotor vermis of the cerebellum (Krauzlis and Miles, 1998) or the dorsolateral pontine nuclei (May et al., 1985), can cause changes in pursuit eye velocity during ongoing pursuit, but is ineffective during fixation on a stationary target. Electrophysiological studies suggest that certain parietal lobe neurons discharge during fixations but not during smooth pursuit (Lynch and McLaren, 1989).

However, the patterns of brain activity observed in humans during functional imaging studies with positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) induced by smooth pursuit of a single target (Berman et al., 1999; Petit and Haxby, 1999; O'Driscoll et al., 2000; Tanabe et al., 2002; Konen et al., 2005) are similar to those observed during fixation suppression of OKR (Bucher et al., 1997; Dieterich et al., 1998; Bense et al., 2006). Both smooth pursuit and fixation suppression of OKR have been found to elicit activation in the frontal (FEF), supplementary (SEF) and parietal eye fields (PEF), as well as in various visual areas such as the primary visual area (PVA/V1) and the motion- sensitive area MT/V5.

In the above studies, comparison between patterns of brain activity during smooth pursuit and fixation suppression of OKR is, however, hampered by differences in the visual stimulation used. Smooth pursuit eye movements were studied using a single target on a homogeneous (dark) featureless background and this condition was contrasted with fixation on a stationary single target ((Berman

et al., 1999; Petit and Haxby, 1999; O'Driscoll et al., 2000; Tanabe et al., 2002; Konen et al., 2005). Fixation suppression of OKR, on the other hand, was studied using a single fixation target presented on a moving pattern, and this condition was compared with OKR stimulation using the same moving pattern without a fixation target (Bucher et al., 1997; Dieterich et al., 1998; Bense et al., 2006). Thus, the retinal stimulation is different between these studies on either smooth pursuit or fixation suppression of OKR.

The present study compares brain activation evoked by smooth pursuit and fixation suppression of OKR while using a similar retinal stimulation. For smooth pursuit, the stimulus was a single target moving against a stationary background consisting of a pattern of stripes, whereas for fixation suppression of OKR, the stimulus was a stationary single target with a moving background of stripes. Thus, in both cases the same visual background, which is moving relative to the smooth pursuit or to the fixation target, needs to be suppressed. We hypothesize that both smooth pursuit eye movement and fixation suppression of OKR will activate largely overlapping pathways, in line with previous studies. However, direct comparison of smooth pursuit and fixation suppression of OKR might yield differences related to the type of oculomotor behavior that is generated while the retinal stimulation is kept the same.

Methods

Subjects

Written informed consent was obtained from each participant prior to the study, which was approved by the Institutional Review Board. Twenty-two healthy volunteers (12 men, 10 women; average age of 27 years, range 22–43 years) participated in the study. None of the subjects had any known neurological or visual defects other than minor refractive anomalies. No one wore spectacle correction and all reported good visual acuity during the experiment.

Data acquisition

For each subject the images were acquired on a 1.5T MRI scanner (Signa CV/I; General Electric, Milwaukee, USA) using a dedicated 8-channel head coil. For the anatomical image, a 3D high resolution inversion recovery FSPGR T1 weighted sequence covering the whole brain including the cerebellum was acquired (repetition time (TR)/echo time (TE)/inversion time (TI) 9.99/2/400 ms, flip angle 20 degrees, 320 × 224 matrix with a rectangular field-of-view of 22

cm, 1.2 mm slice thickness with no gap; ASSET factor 2). Acquisition time was 5 min.

For functional imaging, a single-shot gradient-echo echo-planar imaging (EPI) sequence in transverse orientation was used, that is sensitive to blood oxygenation level dependent (BOLD) contrast. The imaging volume covered the entire brain including the whole cerebellum (TR/TE 2500/40 ms, flip angle 60 degrees, 96 × 96 matrix with a field-of-view of 26 cm, 5.5 mm slice thickness and 1 mm gap, 19 slices; voxel size of 5.5 × 2.7 × 2.7 mm^3). Acquisition time was 5 min 40 s per experiment (including 20 s of dummy scans that were discarded from further analysis).

Stimulus paradigm

Each subject participated in two experiments. The experiments were performed in near darkness. The visual stimuli were binocularly presented by means of a goggle-based system (Silent Vision SV-7021 Fiber Optic Visual System; Avotec Inc., Stuart, Florida, USA). The optical components were mounted on top of the head coil. Screen resolution was 1024 × 768 pixels and the refresh rate was 60 Hz. In both experiments the visual stimulation consisted of one single red dot (1.0 degrees of visual angle in diameter) presented on a background pattern of black and white stripes (luminance contrast of about 1). Each stripe had a width of 0.9 degrees. The overall luminance of the whole stimulus was 13.9 cd/m^2. Subjects were instructed to look at the dot continuously in all conditions.

In the first experiment (smooth pursuit), the dot was either positioned in the centre of the field-of-view and was not moving (baseline condition) or it was moving towards the left and right sinusoidally (motion condition). The moving dot in the motion condition stopped exactly at the center of the field-of-view during the change of condition to avoid resetting saccades. In this experiment the background pattern was always stationary. In the second experiment (OKR suppression), the background pattern was either stationary (baseline), or it was moving towards the right and left sinusoidally (motion condition). In this second experiment the dot was always stationary at the center of the field-of-view. The frequency (0.15 Hz) and the sinusoidal amplitude (10 degrees of visual angle) was the same for the moving dot in the smooth pursuit experiment and for the striped pattern in the OKR suppression experiment. So, the retinal stimulation was the same in the two experiments, if the dot was kept onto the fovea.

Both experiments consisted of a block design with two conditions (baseline and motion). In each experiment, the baseline and the motion condition were

presented in alternation. The baseline and the motion condition were presented eight times. Each condition lasted 20 s during which time eight volumes were acquired. The order of the experiments was pseudo-randomly performed across subjects. Eye movements (monocular, left eye) were registered continuously with the Real Eye RE-4601 Imaging System (Avotec Inc., Stuart, Florida, USA) with a 60 Hz sampling rate during each scanning session. Recording and online monitoring of eye movement behavior was done with the iViewX Eye Tracking System (SensoMotoric Instruments, Teltow, Germany). The system was calibrated before each scan session with the built-in 3-by-3 point calibration routine.

Analysis

Behavioral data

The eye movement recordings were analyzed offline. Eye velocity was calculated using a second-order polynome filter. Saccadic eye movements were extracted semi-automatically using an eye velocity criterion of 30 degrees/second and checked manually. The total number of saccades and blinks were counted. For each subject, the average number of saccades and blinks per block, and the gain (i.e., the ratio of eye velocity and target velocity; a gain of one indicates the eyes are moving as fast as the stimulus) were calculated for each of the two conditions (baseline and motion) in each of the two experiments (smooth pursuit and OKR suppression). Paired *t*-tests were used to assess differences in these behavioral parameters.

Functional imaging data

The functional imaging data were analyzed using statistical parametric mapping software (SPM 2, distributed by the Wellcome Department of Cognitive Neurology, University College London, UK) implemented in MATLAB (Version 6.5, Mathworks, Sherborn, MA, USA). For both experiments, motion correction and co-registration were done according to the methodology provided by SPM2 (Tzourio-Mazoyer et al., 2002). Brain volumes were normalized to the standard space defined by the Montreal Neurological Institute (MNI) template. The normalized data had a resolution of $3 \times 3 \times 3 mm^3$ and were spatially smoothed with a three-dimensional isotropic Gaussian kernel, with a full-width-half-maximum of 10 mm.

Statistical parametric maps were calculated for each subject. Movement parameters resulting from the realignment pre-processing were included as

regressors of no interest to further reduce motion artifacts. The model was estimated with a high-pass filter with a cut-off period of 128 s. For each subject and for each experiment, a *t*-contrast map was calculated between the motion condition and the baseline condition (motion > baseline).

The individual *t*-contrast maps were used for second level random effects (group) analysis. One sample *t*-tests were performed for both experiments separately: $[\text{motion} > \text{baseline}]_{\text{smooth pursuit}}$ and $[\text{motion} > \text{baseline}]_{\text{OKR suppression}}$. To investigate the differences in brain activation between smooth pursuit eye movement and OKR suppression conditions corrected for the baseline activation, we used a paired *t*-test $[\text{motion} > \text{baseline}]_{\text{smooth pursuit}}$ versus $[\text{motion} > \text{baseline}]_{\text{OKR supppression}}$ and vice versa. All tests were thresholded at $P < 0.05$ with false discovery rate (FDR) correction for multiple comparisons and at a minimum cluster size of 10 voxels. Anatomic labeling of the observed areas of activation in SPM was done using the macroscopic anatomic parcellation procedure of the Montreal Neurological Institute (MNI) MRI single-subject brain (Tzourio-Mazoyer et al. 2002).

Results

Behavioral data

In five of the 22 subjects, inspection of the eye movement responses showed an improper performance during the experiments. One subject moved her eye position away from the tracker during scanning. One subject failed to complete the OKR suppression experiment. In three subjects, the eye movement recording failed during the smooth pursuit experiment.

All remaining 17 subjects were able to perform the tasks and keep the target on the fovea (Fig. 1). The motion conditions of the smooth pursuit eye experiment evoked typical smooth pursuit eye movements and the moving background was effectively ignored in the motion conditions of the OKR suppression experiment. In all baseline conditions subjects fixated the dot properly.

The statistical analysis of the behavioral data (Table 1) showed that the average number of saccades per 20 second block was low, and did not differ between the motion and experiment and the motion condition of the OKR suppression experiment. The eye movement gain was about one in the motion condition of the smooth pursuit experiment and about zero in all other conditions. The number of blinks did not differ between the conditions or experiments.

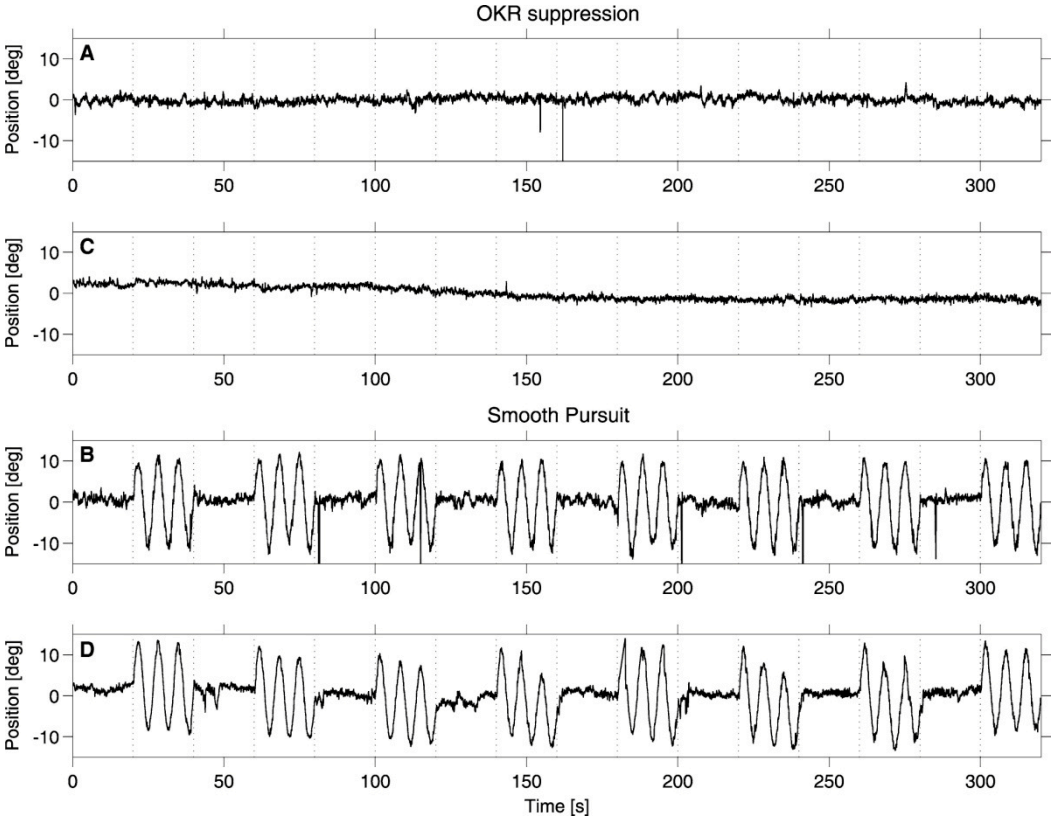

Figure 1. Examples of the 320 s recording of the eye movements made by two subjects in the OKR suppression experiment (**a**, **b**) and in the smooth pursuit experiment (**c**, **d**). The *vertical dotted lines* indicate the beginning of each block.

Smooth pursuit

Random effects group analysis of the smooth pursuit experiment with the contrast [motion > baseline]$_{\text{smooth pursuit}}$ revealed bilateral activation in the precentral gyrus [frontal eye fields (FEF)], unilateral activation in the left supplementary motor area [supplementary eye fields (SEF)] and bilateral activation in the superior and inferior parietal gyrus [parietal eye fields (PEF)]. Unilateral activation was also found in the right middle temporal gyrus [motion-sensitive area (MT/V5)]. Bilateral activation was also found in the cerebellar lobule VI and crus I and centrally in vermis VI. Furthermore, bilateral activation was observed in the inferior occipital, middle occipital, superior occipital, lingual, and calcarine gyri (primary visual area V1) as well as in the fusiform gyrus (visual area V4). (Table 2; Fig. 2).

OKR suppression

Random effects group analysis of the OKR suppression experiment with the contrast [motion > baseline]$_{\text{OKR suppression}}$ revealed bilateral activation in the precentral gyrus (FEF), in the superior and inferior parietal gyrus (PEF), and in the middle temporal gyrus (MT/V5). Furthermore, bilateral activation was observed in the inferior occipital, middle occipital, superior occipital, lingual gyri and unilateral activation in the right calcarine gyrus (primary visual area V1). Bilateral activation was found in the fusiform gyrus (visual area V4). There was no significant activation in the supplementary motor area (SEF) and in the oculomotor areas of the cerebellum (Table 3; Fig. 3).

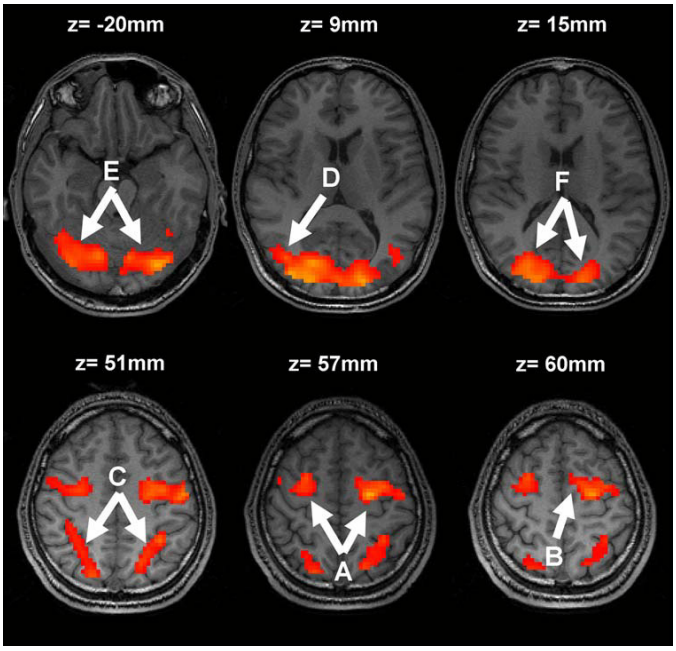

Figure 2. Activated clusters for smooth pursuit experiment. All areas were thresholded at $P < 0.05$ with FDR correction for multiple comparisons and with a minimum cluster size of 10 voxels. *A* FEF, *B* SEF *C* PEF, *D* MT/V5, *D* cerebellar lobule VI, *F* PVA/V1.

Comparison between smooth pursuit and OKR suppression

When the activation in the smooth pursuit eye movement experiment was compared with the OKR suppression experiment [(motion > baseline)$_{\text{smooth pursuit}}$ versus (motion > baseline)$_{\text{OKR suppression}}$], at $P < 0.05$ with FDR correction no activation of interest was seen. Random effects analysis with a threshold at $P < 0.05$ corrected for multiple comparisons at cluster level and a minimum cluster size of 10 voxels revealed activation in the right cerebellar lobule VI.

Furthermore, unilateral activation was found in the right lingual and calcarine gyrus (Table 4; Fig. 4).

When the activation in the OKR suppression experiment was compared with the activation in the smooth pursuit eye movement experiment [(motion > baseline)$_{\text{OKR suppression}}$ versus (motion > baseline)$_{\text{smooth pursuit}}$], at $P < 0.05$ with FDR correction, no activation of interest was seen. Random effects analysis thresholded at $P < 0.05$ corrected for multiple comparisons at cluster level also revealed no activation of interest. A summary of the results is presented in Table 5.

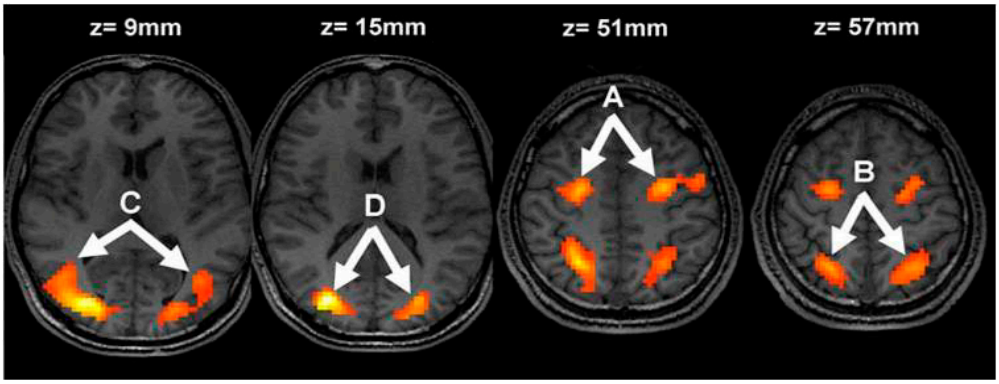

Figure 3. Activated clusters for OKR suppression experiment. All areas were thresholded at P < 0.05 with FDR correction for multiple comparisons and with a minimum cluster size of 10 voxels. *A* FEF, *B* PEF, *C* MT/V5, d PVA/V1.

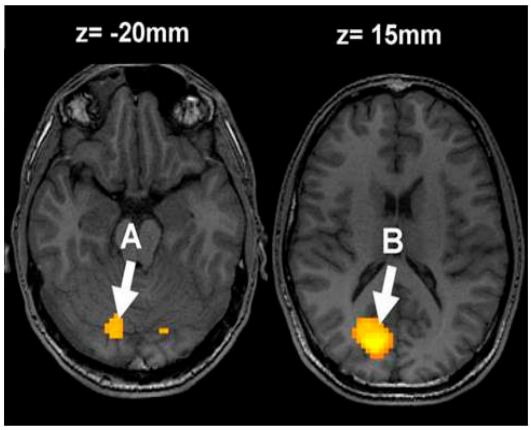

Figure 4. Activated clusters for the direct comparsion (smooth pursuit vs OKR suppression). All areas were thresholded at $P < 0.05$ with correction for multiple comparisons at cluster level and with a minimum cluster size of 10 voxels. **a** Cerebellar lobule VI, **b** PVA/V1

Discussion

The aim of this study was to investigate possible differences in brain activation patterns evoked by smooth pursuit eye movements and fixation suppression of the OKR. Both oculomotor behaviors were evoked by the same retinal stimulation, in which a background pattern of stripes and a target were moving relative to each other. In both experiments the (relative) motion of the background needed to be ignored, while the target was to be kept on the fovea. The eye movement recordings which were acquired during the fMRI scans showed that all subjects were able to perform the tasks accurately, and could keep the target on the fovea continuously. Therefore, the alleged differences in brain activation between the two types of oculomotor behaviors are likely to be related to the differences between smooth pursuit eye movements and fixation suppression of OKR, rather than to differences in retinal stimulation, or to the number of saccades or blinks.

The fMRI data revealed differences in brain activation patterns between the two oculomotor behaviors. Smooth pursuit eye movements induced activation in the FEF, the SEF, the PEF, the MT/V5, and lobules VI and vermis VI of the cerebellum. Fixation suppression of the OKR also induced activation in the FEF, the PEF, MT/V5, but not in the SEF or the cerebellum.

Smooth pursuit

During smooth pursuit the eyes follow a slowly moving target by matching its velocity (motion condition), whereas during fixation, gaze is maintained at a stationary target (baseline condition). The observed activation in our first experiment is likely to be evoked by the motion of the dot and the associated smooth pursuit response. Previous fMRI studies on smooth pursuit eye movements all used homogeneous backgrounds without any features (Berman et al., 1999; Petit and Haxby, 1999; Tanabe et al., 2002; Konen et al., 2005), whereas in the present study smooth pursuit was induced by a small moving target on a background with features, similar to natural smooth pursuit during daily life. Although the use of a cluttered background during smooth pursuit stimulation could have influenced our results, the activated areas in the present smooth pursuit experiment (FEF, SEF, PEF, MT/V5 and cerebellum) are in good accordance with previous fMRI studies (Berman et al., 1999; Petit and Haxby, 1999; Tanabe et al., 2002; Konen et al., 2005). All these areas are known to be involved in pursuit oculomotor behavior. For instance, the FEF and PEF contain neurons that are responsive to foveated tracking (Fukushima et al., 2004; Fukushima et al., 2006). Lesions of the FEF impair predictive and visually guided

smooth pursuit (Keating, 1991; Leigh and Zee, 1999). The SEF are likely to play a role in planning and timing of voluntary movements, such as smooth pursuit (Kandel et al., 2000). Neurons in the middle temporal gyrus (MT/V5) encode visual motion (Albright, 1984; Duffy and Wurtz, 1991; Kandel et al., 2000; Muri et al., 2000), which information can be used to guide pursuit of a small moving target (Dursteler and Wurtz, 1988; Newsome et al., 1988). Patients with unilateral cortical lesions in the MT/V5 area exhibit impairments in smooth pursuit behavior, showing reduced eye movement gains and an increase in the number of catch-up saccades (Leigh and Zee, 1999).

Fixation suppression of OKR

The main difference between the two conditions (motion and baseline) in the fixation suppression of OKR experiment was the motion of the background. Since the subjects were making no eye movements as they continuously fixated on the stationary dot, the induced brain activation is likely due to the suppression of the moving pattern of stripes, rather than to eye movements. The observed activation (FEF, PEF, MT/V5) is congruent with the observations of previous fMRI studies, which studied small-field optokinetic stimulation with and without fixation (Bucher et al., 1997; Dieterich et al., 1998). These studies reported that fixation suppression of the optokinetic response induced FEF, SEF, and PEF activation, albeit weaker than with optokinetic stimulation without suppression that induced optokinetic nystagmus. The activation of MT/V5 observed in the present study was previously reported in a PET study comparing OKR suppression with baseline (Bense et al., 2006). As mentioned above, neurons in MT/V5 are sensitive to the presence of visual motion.

Smooth pursuit eye movement versus fixation suppression of OKR

When we directly compared the two experiments, the only difference in activation was observed in one cluster of activation spanning the cerebellum and primary visual areas when the smooth pursuit experiment was compared to the fixation suppression of OKR experiment. But this activation was present only with a more lenient statistical threshold at cluster level. This outcome suggests that smooth pursuit eye movement and fixation suppression of OKR activate overlapping cortical pathways. The difference in cerebellar activation is likely to be induced the presence of an active eye movement component in the smooth pursuit experiment. The differences in activation in the right lingual and calcarine gyri do suggest differences in the visual input and subsequent visual processing of the stimuli between the two experiments. Although the in-scanner recordings did

not reveal significant differences in eye movement behavior, it should be noted that small eye movements (such as micro-saccades and small drifts) cannot be detected with the present equipment. Such small differences in eye movement behavior could nonetheless lead to small differences in visual input and processing.

Note that in the present study, using sinusoidal stimulation, no differences in saccadic activity was observed. This could explain the absence of differential activity between the two experiments in saccadic related areas like the FEF. Furthermore, there was no difference in activation of area MT/V5 between the two experiments. Cells in MT/V5 are sensitive to visual motion (Albright, 1984; Duffy and Wurtz, 1991; Bremmer et al., 1997; Kandel et al., 2000; Muri et al., 2000). The comparable activation of MT/V5 in the two experiments supports the notion that the visual motion stimulation between the two experiments was indeed very similar.

The majority of activation was found to appear bilaterally, but activation was found to be unilateral in the right MT/V5 area in the smooth pursuit experiment. This right-sided lateralization of activation suggests a right hemispheric dominance in oculomotor performance, which may be related to the predominant role of the right hemisphere in spatial visual attention processes (Dieterich et al., 1998).

Conclusion

In conclusion, our imaging results suggest that under similar retinal stimulation conditions smooth pursuit eye movement and fixation suppression of the optokinetic reflex activate overlapping pathways. The increased activity in the oculomotor areas of the cerebellum during active smooth pursuit is probably due to the presence of an active eye movement component.

Tables

Table 1. Behavioral eye movement parameters.

Behavioral parameter	Condition	Smooth pursuit	OKR suppression
Number of saccades	Baseline	0.24 ± 0.31	0.14 ± 0.26
	Motion	0.48 ± 0.55	0.05 ± 0.10
Eye movement gain	Baseline	0.04 ± 0.03	0.03 ± 0.02
	Motion	0.97 ± 0.13	0.03 ± 0.01
Number of blinks	Baseline	2.28 ± 2.32	3.07 ± 3.52
	Motion	1.18 ± 1.68	1.59 ± 1.94

Number of saccades per 20 s block, eye movement gain and number of blinks per 20 s block across the 17 subjects measured in the two experiments (smooth pursuit and OKR suppression) and two conditions (baseline and motion) during the fMRI session.

Table 2. Smooth pursuit eye movement experiment with (smooth pursuit > fixation)

Cluster size	T-value	MNI coordinates			Anatomical area	Side	% (*)	Functional area
		x	y	z				
3700	12.97	42	-72	-3	Superior parietal gyrus	R	2.68	PEF
					Inferior parietal gyrus	R	1.16	PEF
					Middle temporal gyrus	R	2.92	MT/V5
					Inferior temporal gyrus	R	1.89	
					Cerebellum VI	R	5.35	Oculomotor area
					Cerebellum VI	L	4.24	Oculomotor area
					Cerebellum crus I	L	2.19	Oculomotor area
					Vermis VI		1.73	Oculomotor area
					Cerebellum crus I	R	1.7	Oculomotor area
					Lingual gyrus	R	8.92	PVA/V1
					Lingual gyrus	L	7.89	PVA/V1
					Calcarine gyrus	R	6.05	PVA/V1
					Middle occipital gyrus	L	5.84	PVA/V1
					Calcarine gyrus	L	5.84	PVA/V1
					Middle occipital gyrus	R	5.46	PVA/V1

					Superior occipital gyrus	R	4.19	PVA/V1
					Inferior occipital gyrus	L	3.38	PVA/V1
					Inferior occipital gyrus	R	3.22	PVA/V1
					Superior occipital gyrus	L	2.14	PVA/V1
					Fusiform gyrus	R	6.27	
					Fusiform gyrus	L	3.84	
407	7.03	-21	-9	57	Precentral gyrus	L	47.42	FEF
					Postcentral gyrus	L	13.76	
					Superior frontal gyrus	L	10.32	
					Middle frontal gyrus	L	6.63	
					Supplementary motor area	L	1.72	SEF
253	5.4	-33	-45	51	Superior parietal gyrus	L	49.41	PEF
					Inferior parietal gyrus	L	32.02	PEF
					Postcentral gyrus	L	9.88	
					Precuneus gyrus	L	1.58	
226	4.84	27	3	57	Superior frontal gyrus	R	31.42	
					Middle frontal gyrus	R	28.32	
					Precentral gyrus	R	26.55	FEF

All areas were thresholded at $P < 0.05$ with FDR correction for multiple comparisons and with a minimum cluster size of 10 voxels. L Left hemisphere, R right hemisphere; FEF frontal eye fields, SEF supplemental eye fields, PEF parietal eye fields, MT/V5 motion-sensitive area, PVA/V1 primary visual areas (V1). (*) The unassigned areas for each cluster are not listed in the table

Table 3. Areas of activation for the fiation suppression of OKR (OKR suppression > fixation)

Cluster size	**T-value**	**MNI coordinates**			**Anatomical area**	**Side**	**% (*)**	**Functional area**
		x	**y**	**z**				
937	10.37	33	-78	12	Middle temporal gyrus	R	15.58	MT/V5
					Inferior temporal gyrus	R	10.03	
					Middle occipital gyrus	R	21.24	PVA/V1
					Inferior occipital gyrus	R	11.95	PVA/V1
					Superior occipital gyrus	R	6.94	PVA/V1
					Calcarine gyrus	R	4.8	PVA/V1
					Lingual gyrus	R	4.38	PVA/V1

					Lingual gyrus	L	1.07	PVA/V1
					Fusiform gyrus	R	8.11	
315	7.82	27	-54	51	Superior parietal gyrus	R	42.86	PEF
					Inferior parietal gyrus	R	13.97	PEF
					Postcentral gyrus	R	8.25	
					Supra marginal gyrus	R	3.49	
					Superior occipital gyrus	R	2.86	
					Angular gyrus	R	1.9	
					Precuneus	R	1.27	
568	7.62	-27	-78	21	Middle temporal gyrus	L	7.04	MT/V5
					Middle occipital gyrus	L	41.2	PVA/V1
					Inferior occipital gyrus	L	16.2	PVA/V1
					Superior occipital gyrus	L	10.92	PVA/V1
					Fusiform gyrus	L	9.86	
175	5.91	-27	-3	51	Precentral gyrus	L	54.86	FEF
					Middle frontal gyrus	L	16	
					Superior frontal gyrus	L	12	
112	5.79	27	-6	51	Superior frontal gyrus	R	30.36	
					Precentral gyrus	R	21.43	FEF
					Middle frontal gyrus	R	16.96	
215	5.16	-27	-57	57	Superior parietal gyrus	L	57.21	PEF
					Inferior parietal gyrus	L	29.77	PEF
					Precuneus	L	3.72	
18	4.05	-12	-21	45	Middle cingulum gyrus	L	50	
15	3.9	-54	-39	33	Supra marginal gyrus	L	80	
					Inferior parietal gyrus	L	20	

All areas were thresholded at $P < 0.05$ with FDR correction for multiple comparisons and with a minimum cluster size of 10 voxels *L* Left hemisphere, *R* right hemisphere; *FEF* frontal eye fields, *PEF* parietal eye fields, *MT/V5* motion-sensitive area, *PVA/V1*primary visual areas (V1). (*) The unassigned areas for each cluster are not listed in the table.

Table 4. Areas of activation for the direct comparison of the two experiments (smooth pursuit > OKR suppression) with cluster size, t-values of local maximum, MNI coordinates, anatomic labels, percentage of cluster size and functional area

Cluster size	T-value	MNI coordinates			Anatomical area	Side	% (*)	Functional area
		x	y	z				
175	5.64	21	-75	9	Cerebellum VI	R	6.86	Oculomotor area
					Calcarine gyrus	R	60.57	PVA/V1
					Lingual gyrus	R	28.57	PVA/V1

All areas were thresholded at $P < 0.05$ corrected for multiple comparisons at cluster level and with a minimum cluster size of 10 voxels *R* Right hemisphere; *PVA/V1* primary visual areas (V1). (*) The unassigned areas for each cluster are not listed in the table

Table 5. Activation in the areas of interest for each of the experiment (smooth pursuit and fixation suppression of OKR) separately and for the direct comparison between the two experiments .

Functional area	Anatomic location	Smooth pursuit	OKR suppression	Smooth pursuit vs OKR suppression	OKR suppression vs Smooth pursuit
Frontal eye fields (FEF)	Precentral gyrus	B	B	-	-
Supplementary eye fields (SEF)	Supplementary motor areas	L	-	-	-
Parietal eye fields (PEF)	Inferior and superior parietal gyrus	B	B	-	-
Visual area 5 (MT/V5)	Middle temporal gyrus	R	B	-	-
Oculomotor vermis	vermis Cerebellum VI	B	-	- (R)	-

All areas were thresholded at $P < 0.05$ with FDR correction for multiple comparisons and with a minimum cluster size of 10 voxels. Scores between brackets are the results thresholded at $P < 0.05$ corrected for multiple comparisons at cluster level. *B* Bilateral; *R* right hemisphere, *L* left hemisphere, – indicates no significant activation.

Chapter 5

Eye movements as a marker for cerebellar damage in paraneoplastic neurological syndromes

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J.N. van der Geest

Parkinsonism and Relat Disord 2007; 13: s296–s300

Abstract

Cerebellar disturbances can induce a variety of motor deficits, ranging from severe ataxia to mild deficits of fine motor control. Although motor disturbances appear as an important clinical feature in many neurological disorders, mild disturbances are often difficult to assess properly. Eye movement recordings using video-oculography in a group of patients with a paraneoplastic neurological disorder revealed subtle saccadic and smooth pursuit deficits when compared to controls. We conclude that an easy quantification of eye movement control may assist in the diagnosis and follow-up of mild motor disturbances in patients with neurological disorders, especially when such signs are not overt during clinical neurological examination.

Introduction

The human cerebellum is a distinct part of the brain, located below the occipital lobe in the posterior cranial fossa (Figure 1A). It has a critical role in the smooth execution and the precise timing of movement. Furthermore, the cerebellum is essential for the adjustment of inaccurate movements and motor learning (Bastian, 2006). Therefore, cerebellar disturbances often induce deficits in motor behavior.

At a macroscopic level, the cerebellum can be divided into three distinct regions (Figure 1B) (Voogd J, 1996). The combination of the vermis, positioned in the midline, and the neighboring intermediate hemispheres is referred to as the spinocerebellum. This part receives somatosensory input from the body and is involved in motor coordination. Secondly, the cerebrocerebellum comprises the lateral hemispheres. This region receives input from the cerebral cortex and projects to the (pre)motor and prefrontal cerebral cortex. It is involved in the planning of movement. Thirdly, the flocculonodular lobe, also known as the vestibulocerebellum, receives input from primary vestibular afferents and projects to the lateral vestibular nuclei. It is involved in the control of balance and reflexive eye movements.

At a cellular level, Purkinje cells (PC) are considered as the principal cells within the cerebellum (Figure 1C) (Voogd J, 1996). Parallel and climbing fibers that contact a Purkinje cell provide extensive information about movement planning, motor commands and feedback. For a single Purkinje cell, a multitude of parallel fibers provide sensory information from the periphery as well as information from the cerebral cortex. In addition, a Purkinje cell receives input from a single climbing fiber, which originates in the inferior olivary nucleus. Climbing fibers convey fibers, visual and cerebral cortical information (Simpson JI, 2003).

Within the cerebellar circuitry, Purkinje cells are the only source of output from the cerebellar cortex to the cerebellar nuclei. This organization is important, as the cerebellum is thought to compare efference copies of motor commands with an internal representation of the outside world (Wolpert and Miall, 1996). When proprioceptive feedback indicates that a movement is not accurate, a correction can be made to the ongoing movement. Furthermore, repetitive inaccuracy leads to adjustment of the internal representation and therefore changes in subsequent movements (Frens and van Opstal, 1994; Diedrichsen et al., 2005). Changes within the cerebellar circuitry are effected by modifications of synaptic excitability

by processes such as long-term depression (LTD) (Coesmans et al., 2003; Coesmans et al., 2004). LTD describes the decrease in synaptic strength between parallel fibers and a Purkinje cell when activity of these parallel fiber synapses is accompanied by simultaneous activity of the climbing fiber projecting to the same Purkinje cell. Therefore, the climbing fiber signal can be referred to as a "teacher signal", which tells the Purkinje cell which parallel fibers convey relevant information (Simpson JI, 2003).

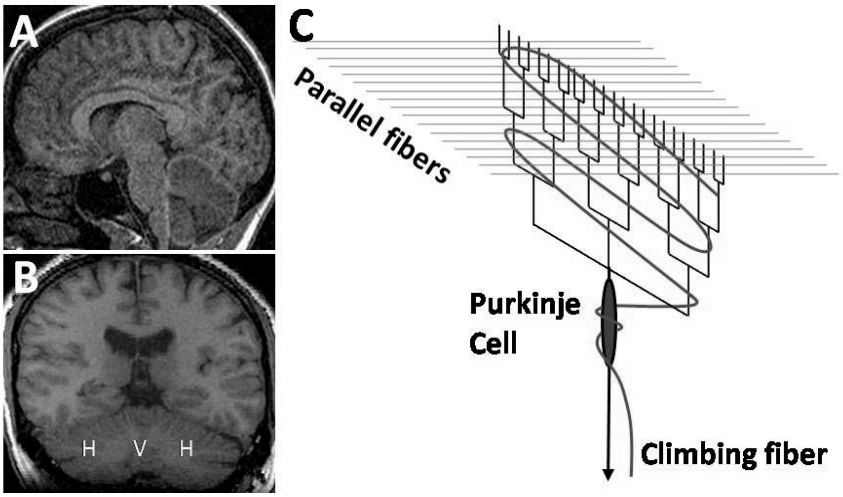

Figure 1. (A) Mid-sagittal view of the human brain. (B) Coronal view with the cerebellar vermis [V] and hemispheres [H]. (C) Schematic overview of the cerebellar circuitry, in which a Purkinje cell receives input from a single climbing fiber and a multitude of parallel fibers, and provides output to the cerebellar nuclei.

In cerebellar and other neurological disorders, motor disturbances often are important clinical features. Severe disturbances of the cerebellum are clinically characterized by ataxia, i.e., the loss of spatial and temporal accuracy of movements. Obvious symptoms in this respect are deficits in balance and gait. Mild motor disturbances, however, can be quite difficult to assess properly. Since the cerebellum is also involved in oculomotor control, the quantitative analysis of eye movements, which are relatively easy to measure, may provide a useful tool in the diagnosis of (cerebellum-mediated) motor dysfunction. In this paper we describe measurements of eye movement in a group of elderly patients affected by a paraneoplastic neurological syndrome (PNS).

PNS denotes the remote effects of cancer on the nervous system, and most types of PNS have an incidence of less than 0.01% in patients with cancer (Darnell

and Posner, 2003). The neurological effects are not caused by direct interactions of the neoplasm or metastases with the surrounding tissues (e.g., by infiltration) and do not appear as a side-effect of medical treatment, but are most probably induced by immunological factors instead. Although the pathogenesis of PNS has yet to be unraveled fully, it is presently thought that the immune system attacks an onconeural antigen that is expressed ectopically by the tumor. These onconeural antigens are normally expressed only in the nervous system. Therefore, the immune response directed against the particular antigen also affects the healthy neurons expressing this antigen (de Beukelaar and Sillevis Smitt, 2006). The affected parts of the nervous system range from a single cell-type, such as Purkinje cells in paraneoplastic cerebellar degeneration (PCD) (Coesmans et al., 2003), to combinations of neural systems as for instance in paraneoplastic encephalomyelitis (PEM). The clinical presentation and, in particular, the severity of motor disturbances of patients with PNS can therefore be quite variable.

Disturbances of the cerebellum, as in PCD, may lead to specific disturbances in the oculomotor system, as this brain structure is critically involved in the proper execution of certain types of eye movements such as smooth pursuit and saccades (Leigh and Zee, 1999). Saccades are very fast and accurate eye movements that serve to move the point of gaze quickly toward a new position in the visual field. Smooth pursuit is used to keep the point of gaze fixed on a small moving target. Cerebellar dysfunction can result in reduced accuracy of these types of eye movement, yielding saccade dysmetria, an increased number of corrective eye movements, and an increase in intrusive saccades during smooth pursuit (Leigh and Zee, 1999). Clinically, however, mild disturbances in oculomotor control are often difficult to assess properly.

Video-oculography (VOG) allows for a quantitative assessment of oculomotor disturbances in smooth pursuit and saccades (van der Geest et al., 2004). The goals of the present study are (1) to investigate the feasibility of VOG recordings in disabled PNS patients using a mobile presentation and recording unit at the bedside and (2) to determine the value of eye-movement quantification as a clinical tool in PNS patients with and without overt cerebellar signs at clinical examination.

Methods

Subjects

Informed consent for this study, which was approved by the ethical review board of the Erasmus MC, was obtained from 11 patients with various types of PNS between 53 and 75 years of age (5 males and 6 females) and six healthy age-matched controls between 50 and 62 years of age (2 males and 4 females). Patient characteristics are presented in Table 1. The control subjects were recruited from the hospital personnel and had no neurological or visual deficits other than minor refractive anomalies. None of the subjects wore spectacle correction but all reported good visual acuity during the eye movement recordings.

Apparatus

Subjects were seated in a hospital bed. Movements of the head were restrained by means of a vacuum cushion. Stimuli were presented on a computer screen (30 × 21 cm, resolution 1024 × 768 pixels) which was positioned at 50 cm distance in front of the eyes of the subject on a movable hospital table. Monocular position of the right eye was registered with infrared video-oculography (ViewPoint EyeTracker & QuickClamp System, Arrington Research, USA) at a 50-Hz sampling rate. The recording camera was mounted on an adjustable positioning arm, and was positioned below the right eye to ensure proper image quality without blocking the line of sight. Eye position was calibrated using a built-in calibration routine with a fixation grid of 9 positions. Stimulus presentations and eye-movement recordings were handled by a single PC.

Stimuli

In both tasks the subject was instructed to look at a yellow dot (0.5° in diameter of visual angle), which was presented on a black background. The dot was continuously visible throughout each task. In the saccade task the yellow dot jumped back-and-forth every three seconds between two positions that were aligned to the centre of the screen with a horizontal separation of 12.5° of visual angle. The saccade task lasted for 60 seconds. In the smooth pursuit task the yellow dot moved sinusoidally between the same two positions (amplitude 12.5°, frequency 0.25 Hz, peak velocity 20°/s). The smooth pursuit task lasted for 24 seconds, yielding six complete movement cycles.

Analysis

The eye-movement data were analyzed off-line. The eye position recordings were imported into Matlab and eye movement velocity was calculated using the two-point central difference method. Saccades were extracted semiautomatically using a velocity criterion of 50°/s and minimum amplitude of 2°. The data were then manually checked for proper inclusion of small correction saccades.

In the saccade task, the first saccades toward the target dot after it had jumped to the other position were marked as primary saccades. Primary saccades with a horizontal amplitude of less than 9° or with a vertical component larger than 5° were discarded. The amplitude gain of the primary saccades was defined as the relative distance travelled by the saccade, i.e. the saccade amplitude divided by the target amplitude (12.5°). For each subject, the average amplitude gain and the standard deviation in amplitude gain were calculated. Secondly, for each subject, the average number of correction saccades following a primary saccade until the target was fixated was determined. Note that a gain of 1 and zero correction saccades therefore indicates that the primary saccade directed the eye precisely onto the target.

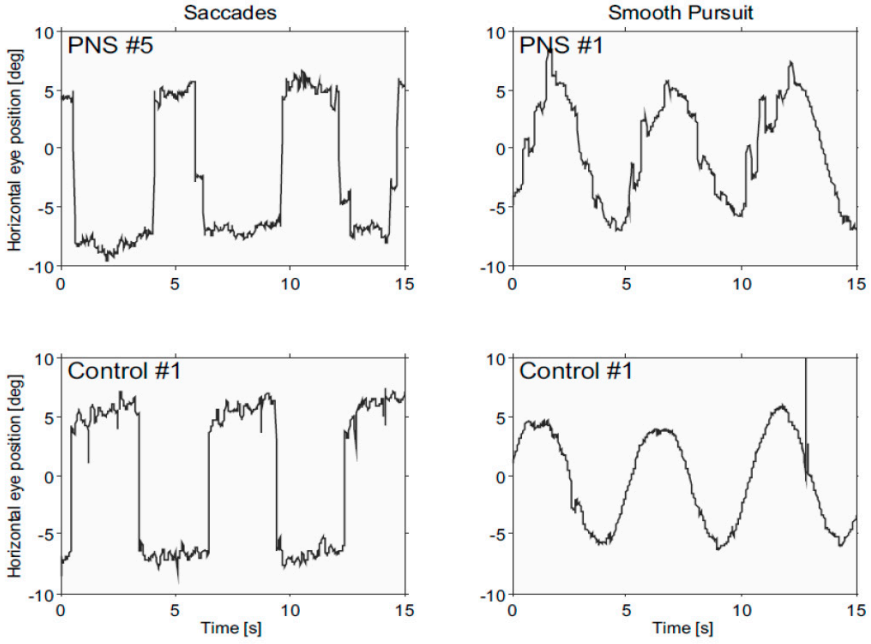

Figure 2. Exemplary eye movement behaviour for 15 seconds in the saccade and the smooth pursuit tasks (positive is to the right). Note the variability in primary saccades toward the left for PNS patient #5 and the ample saccadic intrusions during smooth pursuit in PNS patient #1, when compared to the control subject.

In the smooth pursuit task, the first movement cycle (4 seconds) was discarded. The average number of intermittent (catch-up) saccades per movement cycle and the smooth pursuit gain was calculated for each subject. The gain of the smooth-pursuit eye movement was defined as the amplitude of the sinusoid that was fitted through the eye velocity divided by the peak velocity of the target motion (20°/s).

For each group and each parameter, the between-subject variabilities were calculated using the absolute differences with the average of the group. Statistical differences in the oculomotor parameters between the group of PNS patients and the group of control subjects were assessed using Mann-Whitney U-tests.

Results

Typical eye-movement recordings of a PNS patient and a healthy subject are shown in Figure 2. Qualitatively, the recorded eye-movement behavior of PNS patients yielded an increased variability in saccadic amplitudes in the saccade task, and an increased number of saccadic intrusions during smooth pursuit, compared to healthy controls.

The quantitative analysis of the saccade task showed that the average amplitude gain of the primary saccades was similar for patients with PNS (0.95 ± 0.09 standard deviation) and control subjects (0.92 ± 0.05, $p = 0.73$). Also, the variability in amplitude gains (i.e., the within-subject standard deviation of amplitude gains) was, on average, not significantly different between the two groups (PNS patients: 0.11 ± 0.05 vs. control subjects: 0.08 ± 0.02, $p = 0.35$). However, as can be seen in Figure 3A, the between-subject variability for these two saccadic parameters was greater between PNS subjects than between control subjects (amplitude gain: 0.55 ± 0.04 vs. 0.35 ± 0.02, $p = 0.04$; gain variability: 0.038 ± 0.030 vs. 0.014 ± 0.010, $p = 0.03$). Three PNS patients had an average amplitude gain that was larger than 1, which was not found in any of the control subjects.

The number of correction saccades following the primary saccades was larger in the group of PNS patients (1.18 ± 0.80 correction saccades/primary saccade) than in the group of control subjects (0.40 ± 0.19 correction saccades/primary saccade, $p = 0.02$), showing that half of the PNS patients needed more than two (primary and subsequent correction) saccades to fixate the target dot (Figure 3B). Also, the number of correction saccades was more variable between PNS patients (0.58 ± 0.53) than between control subjects (0.17 ± 0.07, $p = 0.02$).

The quantitative analysis of the smooth pursuit task (Figure 3C) showed that, on average, the number of saccadic intrusions during smooth pursuit eye movements was not significantly larger in PNS patients (1.67 ± 2.61 saccades/cycle) than in control subjects (0.13 ± 0.21 saccades/cycle, p = 0.40).

However, this number of saccadic intrusions was again more variable between PNS patients (2.14 ± 1.33) than between control subjects (0.18 ± 0.07, p = 0.001). Smooth pursuit gain was similar between PNS patients (0.85 ± 0.07) and control subjects (0.87 ± 0.05, p = 0.46).

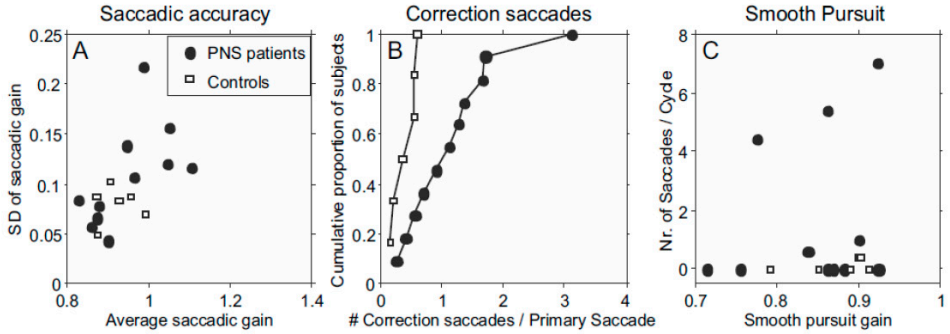

Figure 3. Quantitative oculomotor parameters: (A) variability versus average gain of primary saccades in saccade task; (B) cumulative proportion of subjects for number of correction saccades per primary saccade; (C) number of saccadic intrusions per smooth pursuit cycle versus average smooth pursuit gain. In all panels, each dot represents a single subject.

Individual disturbances in oculomotor behavior for each PNS patient were assessed by comparing the quantitative oculomotor parameters of the two tasks to the outcomes of the control group. This shows that at least one saccadic parameter was deviant in eight PNS patients and at least one smooth pursuit parameter was deviant in four PNS patients (Table 1).

Discussion

Our results show that video-oculograpic eye movement recordings at the bedside of disabled patients provide a feasible tool for the quantitative evaluation of mild oculomotor disturbances. The quality of the obtained data was sufficient to quantitatively compare a group of 11 patients with various types of paraneoplastic neurological syndromes (PNS), and heterogeneous clinical signs, to a group of six age-matched control subjects.

Our data showed that saccadic accuracy was more variable between PNS patients, and that they needed more saccades to reach the target than controls.

The number of intrusive correction saccades during smooth pursuit was also larger in some PNS patients. In eight of the 11 PNS patients deficits in saccadic accuracy were prominent, as reflected by an increase in the number of correction saccades, an average saccadic amplitude gain larger than 1 (which is almost never seen in healthy subjects and is regarded as a hallmark for cerebellar disturbances (Leigh and Zee, 1999; van der Geest et al., 2004)), and/or a marked increase in saccadic amplitude variability. Smooth pursuit disturbances, reflected by an increased number of intrusive catch-up saccades and/or a reduced smooth pursuit gain, were less prominent in our patient group. In four patients with PNS marked smooth pursuit deficits were observed.

Only five of the 11 PNS patients presented with overt clinical signs of cerebellar disturbances during the standard neurological examination. In all of these five subjects, saccadic and/or smooth pursuit deficits were also observed. Furthermore, deficits in these types of eye movements were also observed in four subjects without overt clinical signs of cerebellar deficits. In two PNS subjects without cerebellar signs, normal saccadic and smooth pursuit behavior was recorded.

Although the power of the statistical analyses is limited due to the small number of subjects, we conclude that an easy quantification of eye movement control may aid significantly in the diagnosis and follow-up of mild motor disturbances in patients with neurological disorders, especially when such signs are not overt during clinical neurological examination.

Tables

Table 1. Characteristics of the 11 PNS patients, including gender and age, the clinical syndrome, the overt signs of cerebellar disturbances as assessed in a clinical neurological examination and the quantitative signs of saccadic and smooth pursuit disturbances as assessed by video-oculography.

Patient number	Gender	Age (years)	Clinical syndrome (*)	Overt cerebellar signs	Oculomotor disturbances	
					Saccades	Smooth Pursuit
1	F	69	PEM	no	unclear	yes
2	M	61	PCD	yes (truncal ataxia)	yes	no
3	M	66	PSN	yes (truncal ataxia)	yes	no
4	F	55	PSN & ON	no	yes	unclear
5	M	75	PSN	yes (nystagmus)	yes	yes
6	M	61	PSN	no	no	no
7	F	64	PSN	no	yes	unclear
8	F	61	MND	yes (saccadic pursuit)	yes	yes
9	F	53	PSN	no	unclear	no
10	M	66	PCD	yes (nystagmus, saccadic pursuit, limb and truncal	yes	yes
11	F	70	PSN	no	yes	unclear

(*) PEM: paraneoplastic encephalomyelitis; PCD: paraneoplastic cerebellar degeneration; PSN: paraneoplastic sensory neuronopathy; ON: optic neuropathy; MND: motor neuron disease.

Chapter 6

Human chorionic gonadotropin treatment of anti-Hu associated paraneoplastic neurological syndromes

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ABSTRACT

Paraneoplastic neurological syndromes associated with anti-Hu antibodies (Hu-PNS) are mediated by a T-cell immune response that is directed against the Hu antigens. In pregnancy, many Th1 mediated autoimmune diseases such as rheumatoid arthritis and multiple sclerosis regress. We hypothesized that this decreased disease activity during pregnancy may be related to high human chorionic gonadotropin (hCG) levels.

We treated 15 Hu-PNS patients in a prospective, uncontrolled and unblinded trial with 10,000 IU daily of hCG administered by intramuscular (i.m.) injection during 12 weeks. Primary outcome measures were functional improvement defined as a decrease of one or more points on the modified Rankin Scale (mRS) or stabilization in patients with mRS $\leq$ 3 and improvement of neurological impairment assessed with the Edinburgh Functional Impairment Tests (EFIT). Secondary endpoints included the change in activities of daily living (ADL) as evaluated by means of the Barthel Index (BI).

Seven out of 15 patients (47%) improved on the mRS or stabilized at mRS $\leq$ 3. Four patients (27%) showed significant improvement of neurological impairment as indicated by an overall EFIT score of $\geq$1 point. Five patients improved on the BI (33%).

Comparison with previous studies suggests that hCG may have immunomodulatory activity and may modify the course of Hu-PNS although well established confounding factors may have contributed in this uncontrolled trial.

Introduction

The anti-Hu antibody (Hu-Ab) is the most prevalent paraneoplastic antibody (Graus et al., 2004). The etiology of paraneoplastic neurological syndromes (PNS) associated with Hu-Ab (Hu-PNS) is believed to be autoimmune and the outcome is generally poor despite aggressive anti-tumor and immunosuppressive treatment (Sillevis Smitt et al., 2002; de Beukelaar and Sillevis Smitt, 2006; Dalmau and Rosenfeld, 2008). Circumstantial evidence suggests that T-cell reactivity to the onconeural antigen HuD is pivotal for the development of PNS and the consensus in the field is that Hu-PNS can be classified as Th1 mediated organ specific autoimmune disorders, much like diabetes mellitus and rheumatoid arthritis (Dalmau and Rosenfeld, 2008).

In many patients with Th1 mediated autoimmune diseases such as rheumatoid arthritis (Nelson et al., 1993) and multiple sclerosis (Confavreux et al., 1998), clinical symptoms or number of relapses regress during pregnancy. A prominent feature of early pregnancy is the presence in serum and urine of high levels of human chorionic gonadotrophin (hCG), a heterodimeric glycoprotein hormone that is secreted by syncytiotrophoblast cells of the chorion (an antecedent of the placenta). Because hormonal changes precede and accompany the immune changes in pregnant women, an immunomodulatory role for hCG was hypothesized. Khan et al. (Khan et al., 2001) tested the effect of hCG on the development of diabetes, a Th1 mediated autoimmune disease, in nonobese diabetic (NOD) mice. They showed that treatment of NOD mice with hCG lowered the increased blood glucose levels, reversed the established inflammatory infiltrate of pancreatic tissue, inhibited the development of diabetes for prolonged time and induced profound inhibition of the functional activity (i.e. production of IFN-gamma) of Th1 cells. The mechanism of action of hCG is probably related to its effects on the activation of NF-kappaB (Manna et al., 2000), a transcription factor that plays a central role in regulating immune responses, and is down-regulated in pregnancy (McCracken et al., 2007).

We hypothesized that hCG induced inhibition of Th1 cells in Hu-PNS patients would result in clinical improvement or stabilization. hCG has been previously administered in clinical trials treating HIV-related Kaposi's sarcoma (Tavio et al., 1998). From these studies, we concluded that the daily intramuscular (i.m.) administration of 10,000 IU of hCG was safe. In this uncontrolled and unblinded trial, we treated 15 Hu-PNS patients with daily i.m. injections of 10,000 IU of hCG for 12 weeks.

Materials and Methods

Patients

Inclusion criteria for this prospective uncontrolled and unblinded single center study included high serum titers of Hu-Ab (>400) and progression of neurological symptoms over the last three weeks, defined as new symptoms or progression of existing symptoms confirmed by objective neurological examination. From February 2005 through March 2007, we identified 46 new patients with high titer Hu-Ab, 15 of whom were included in the study. The main reasons for not including the remaining 31 patients were poor clinical condition (patient admitted to ICU for mechanical ventilation or patient already in nursing home or palliative unit), lack of progression of neurological symptoms over the last 3 weeks and declining participation by the patient or family. Clinical characteristics of the 15 included patients are summarized in Table 1. All patients were evaluated throughout the study by one of two clinical investigators (JEB, PSS). The study was approved by the Erasmus University Medical Center Institutional Review Board, and all participants gave written informed consent.

Treatment

The treatment consisted of hCG (Pregnyl, Organon, Oss, Netherlands; lot 685406) 10,000 IU daily administered by intramuscular (i.m.) injection during 12 weeks. Only Pregnyl batches that effectively suppressed IFN-γ production by CD4+ T cell cultures of NOD mice following stimulation were used (Khan et al., 2001).

Outcome measures

The primary endpoints of the study were the functional and neurological improvement after 12 weeks of hCG. Functional outcome was considered 'successful' when a patient with RS ≤ 3 improved or stabilized (i. e. remained ambulatory) and when a patient with RS≥ 4 (bedridden patient) improved to ≤ 3 (ambulatory), after the 12th week of hCG as compared to baseline, as defined by Keime-Guibert et al. (Keime-Guibert et al., 2000) .

Improvement of neurological impairment was assessed with the Edinburgh Functional Impairment Tests (EFIT) that incorporate objective measures of upper and lower limb function, memory and a rating scale for dysphasia (Clyde et al., 1998). An overall EFIT = 0 indicates no change, EFIT > 0 indicates significant neurological improvement and EFIT < 0 indicates significant neurological deterioration.

Secondary endpoints included CSF protein and WBC, the Hu-Ab titers in serum and CSF, and the change in activities of daily living (ADL) as evaluated by means of the Barthel Index (BI) (Mahoney and Barthel, 1965).

Laboratory evaluations

Serum and CSF were sampled at baseline and after four weeks of hCG treatment. Serum was additionally sampled at weeks 8, 12 and 20. The concentration of hCG was determined routinely in the clinical chemistry laboratory (normal value < 1.9 IU/l). IgG titers of the Hu-Ab were determined, as described (Sillevis Smitt et al., 2002).

Statistical analysis

We compared WBC, total protein concentration and Hu-Ab titers in baseline CSF and CSF obtained after 4 weeks of treatment by means of the Wilcoxon matched pairs test. We used the same test to compare Hu-Ab serum titers at baseline and end of study. P-values were two-sided and a significance level $\alpha = 0.05$ was used. All statistical analyses were performed using GraphPad Prism version 5 software (GraphPad Software, Inc., San Diego, CA).

Results

No adverse or serious adverse events related to hCG were reported. Eight patients received tumor treatment overlapping hCG treatment without complications (Table 2). None of the patients received any other immunomodulatory or immunosuppressive drugs while on study treatment. Two patients received immunomodulatory treatment in the weeks preceding hCG administration. Both received one course of IVIg (2 g/kg over 5 doses), 6 weeks (patient 7) or 3 weeks (patient 14) prior to inclusion in the study. Both patients got worse both functionally and neurologically after IVIg. The serum and CSF levels of hCG were negligible at baseline. During the study, the CSF level increased to a median 7 IU/l (range 3.4 – 14 IU/l) while the serum level was median 365 IU/l (range 43 – 963 IU/l) at end of study. Eleven of the 15 patients received hCG treatment for the scheduled 12 weeks while four patients did not complete the treatment (Table 2).

Primary endpoints

Functional outcome was 'successful' in seven out of 15 patients (47%). Six of twelve patients with mRS ≤ 3 at baseline stabilized (n=5) or improved (n=1) while one of the three patients with a baseline mRS ≥ 4 improved to ≤ 3 (Table 2).

Improvement of neurological impairment, as indicated by an overall EFIT score > 0, was demonstrated in four patients (Table 2). The two patients who improved one point on the mRS also had improvement of neurological impairment.

Secondary endpoints

Improvement in ADL was seen in patients 1, 2, 4, 5 and 11. In all patients the change in BI was minimal (+5 points). The laboratory evaluations revealed a median of 2 WBC/µl (range 0.3 – 38) at baseline versus a median of 5 WBC/µl (range 0.3 – 31) after 4 weeks of treatment. The median CSF protein concentration was 0.6 g/l (range 0.13 – 2.15) at baseline and 0.7 g/l (range 0.14 – 1.89) after 4 weeks while the Hu-Ab titers changed in CSF from median 128 (range 8 – 4096) to median 528 (range 8 – 2048). None of these changes were significant. Also, the change in serum titer from baseline (median 6400, range 400 – 25600) to end of study (median 2400, range 800 – 25600) was not significant. Hu-Ab titers did not correlate with baseline mRS nor with the change in mRS during the study (data not shown).

Discussion

With a positive response defined as stabilization at mRS ≤ 3 or improvement, (Keime-Guibert et al., 2000) treatment was 'successful' in seven out of 15 patients (47%). Four other studies have reported treatment results in Hu-PNS patients employing the same functional outcome criterion. In two retrospective studies, IVIg treatment was successful in six of 17 (35%) (Uchuya et al., 1996) while treatment with IVIg, cyclophosphamide and methylprednisolone was successful in two of nine (22%) (Keime-Guibert et al., 2000) evaluable Hu-PNS patients. Vernino et al. prospectively treated five Hu-PNS patients with plasma exchange combined with either cyclophosphamide or chemotherapy based on the absence or presence of an underlying tumor. Treatment was successful in three of these five Hu-PNS patients (60%) (Vernino et al., 2004). Shams'ili et al. (Shams'ili et al., 2006) prospectively treated eight Hu-PNS patients with rituximab and observed improvement or stabilization at mRS ≤ 3 in four of them (50%).

These studies suggest that immunomodulatory therapy, including hCG, may modify the course of Hu-PNS and obtain clinically useful stabilization in patients with Hu-PNS. However, the patient numbers are small and confounding factors may explain the favorable outcome in some of our patients in this uncontrolled study. Four patients were successful on both primary outcome measurements (patients 1, 2, 4 and 11). Several studies have demonstrated that effective treatment of the tumor is important to at least stabilize Hu-PNS (Graus et al., 2001; Sillevis Smitt et al., 2002). The successful outcome in patients 1 and 4 may have been confounded by the partial and complete tumor remissions that were achieved during the trial. Patient 2 presented with limbic encephalitis and sensory neuronopathy. During hCG treatment she regained initiative and her memory subjectively improved. Spontaneous remission of Hu-Ab associated limbic encephalitis has been described and could also have occurred in this patient (Sillevis Smitt et al., 2002). Finally, a long interval from symptoms to diagnosis, as observed in patients 1, 2 and 11 (11 -16 months), is also associated with a favorable outcome (Sillevis Smitt et al., 2002).

The pathogenesis of Hu-PNS is now believed to be mediated by cytotoxic T-cells (Dalmau and Rosenfeld, 2008). Hu-Ab are considered a marker of the immune response rather than being pathogenic and Hu-Ab titers do not correlate well with disease severity nor with its course, as confirmed in this prospective study. In conclusion, seven out of 15 (47%) Hu-PNS patients either functionally improved or stabilized at mRS $\leq$ 3 after treatment with hCG. Comparison with previous studies suggests that hCG may have immunomodulatory activity and may modify the course of Hu-PNS. However, well established confounding factors may have contributed in this uncontrolled trial.

Tables

Table 1. Patient characteristics

Patient Nº	Age/Sex	Antibody	Syndrome	Sympt.-Diagnosis (months)	Tumor	Sympt. - Tumor (months)	Tumor treatment	Response tumor	Sympt. - last FU (months)	Dead / Alive	Cause of Death
1	64/M	Hu	PSN	16	Prostate[1]	16	Chemo	PR	26	Dead	Tumor
2	51/F	Hu	PEM	11	SCLC	31	Chemo and RT	CR	52	Alive	-
3	70/F	Hu	PSN	1	SCLC	3	Chemo	CR	8	Dead	PNS
4	75/M	Hu, Amp	PSN	2	SCLC	1	Chemo and RT	CR	44	Alive	-
5	66/M	Hu	PSN	2	NSCLC	6	Chemo	CR	44	Alive	-
6	55/F	Hu, CV2	PSN + ON	7	SCLC	7	Chemo and RT	CR	25	Dead	Tumor
7	53/F	Hu	PSN	3	SCLC	4	Chemo and RT	CR	43	Alive	-
8	61/F	Hu	PEM	0	SCLC	0	Chemo	CR	1	Dead	Tumor
9	69/F	Hu	PEM	5	SCLC	7	Chemo	PR	11	Dead	PNS
10	66/M	Hu	PCD	3	SCLC	4	No	-	15	Dead	Tumor
11	66/F	Hu	PSN	11	NSCLC	14	Chemo and RT	CR	47	Alive	-
12	61/M	Hu	PCD	4	SCLC	11	Chemo and RT	PD	20	Dead	Tumor
13	68/F	Hu	PSN	2	SCLC	4	Chemo and RT	CR	23	Dead	Tumor
14	61/M	Hu	PSN	2	SCLC	4	Chemo and RT	CR	29	Dead	PNS
15	75/M	Hu	PCD	7	Lung[2]	7	No	-	8	Dead	PNS

[1] Small cell cancer of the prostate

[2] Large mediastinal mass on CT-scan

Amp, anti-amphiphysin, PSN, paraneoplastic sensory neuronopathy; PEM, paraneoplastic encephalomyelitis; ON, optic neuropathy; PCD, paraneoplastic cerebellar degeneration; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; RT, radiotherapy; PR, partial remission; CR, complete remission; PD, progressive disease; FU, follow-up

Table 2. Primary endpoints: Functional and neurological outcome

Nº	Weeks HCG	Chemo during HCG	Successful functional outcome[1]	mRS baseline	Change mRS	Neurological outcome[2]	EFIT baseline	EFIT overall
1	12	Yes	Yes	3	0	**Improve**	2	2
2	12	No	**Yes**	3	**-1**	**Improve**	2	2
3	12	Yes	No	3	1	Worse	2	-2
4	12	Yes	**Yes**	4	**-1**	**Improve**	2	2
5	12	No	Yes	3	0	Stable	2	0
6	12	Yes	Yes	3	0	Stable	2	0
7	12	Yes	No	2	1	Worse	2	-2
8	1	Yes	Died	5	1	N.e.	2	
9	4	No	No	3	1	N.e.	3	
10	4	No	Yes	3	0	Worse[3]	3	-1
11	12	No	Yes	2	0	**Improve**	2	1
12	12	No	No	2	1	Worse	2	-1
13	12	Yes	No	2	1	Worse	2	-2
14	12	Yes	No	3	1	Worse	2	-2
15	1	No	No	5	0	N.e.	4	

mRS, modified Rankin Scale; EFIT, Edinburgh Functional Impairment Tests; N.e., not evaluable. Improvement on mRS and EFIT in bold.

[1] Functional outcome was successful when a patient with RS ≤ 3 improved or stabilized when a patient with RS ≥ 4 improved to ≤ 3(Keime-Guibert et al., 2000)

[2] Neurological outcome is improved when the overall EFIT > 0; stable when overall EFIT = 0; worse when overall EFIT < 0(Clyde et al., 1998)

[3] Overall EFIT score determined at week 4

Chapter 7

General discussion

The general aim of this thesis was to provide more insight in the neurophysiological aspects of saccadic oculomotor control and the value of eye movement abnormalities in a clinical setting. In the previous chapters, several methods were used to pursue these aims. Experience builds knowledge. Inevitably, this means that during and after the execution and analysis of these experiments, new insights were acquired. In the present chapter I will discuss some of these insights and provide suggestions for improvement of this kind of research and ideas for next steps in this line of work.

Neurophysiological Aspects of Saccadic and Smooth Pursuit Oculomotor Control

The first three expeirmental chapters dealt with cerebral and cerebellar processing with repsect to saccadic eye movements and smooth pursuit. We used functional magnetic resonance imaging (fMRI) to visualize patterns of activity in the brain. As fMRI is a relatively young tool in oculomotor research (Gaymard and Pierrot-Deseilligny, 1999; Pierrot-Deseilligny et al., 2004), not all the ins and outs of this tool are known. Most of the research using fMRI focuses on the cerebrum. Other areas of the brain, such as the cerebellum and the brainstem are commonly ignored. To achieve our goal, i.e. visualization of cerebellar activity, we had to perform a number of pilot studies to tweak the scanner for our needs. Repeating the same experiment with every time slightly different parameters was the only way to go, in order to arrive at an optimal fMRI setup for the two experiments on saccadic eye movement control described in chapter 2 and 3.

In **chapter 2,** we set out to investigate the hypothesis that specific cerebellar regions are involved in the processing of post-saccadic errors. We measured healthy subjects, while they performed in a saccadic eye movement task. During the task we imaged brain activity and recorded their eye movement behavior. In the task at hand, the visual target of saccades made by the subject shifted during the saccade. This shift goes unnoticed, but is registered unconsciously as a saccadic error (McLaughlin, 1967). Theoretically, the cerebellum is the prime suspect for dealing with such movement errors (Ito, 2000). Current knowledge about cerebellar involvement in saccades and saccadic adaptation would predict that the oculomotor vermis is likely to play a role here (Hopp and Fuchs, 2004). However, the involvement of other areas outside the cerebellum, for instance in the cerebrum or in the brainstem, cannot be excluded beforehand. In the experiments described in chapter 2, we did not find an increase in cerebral activity related to random post-saccadic errors. This lack of increase in cerebral activation

might be related to limitation in spatial and temporal resolution increases. On the other hand, our data suggested that there is an increase in activity in the cerebellum related to an increase in post-saccadic errors. More specifically, this activity was found in vestibulocerebellar areas, rather than activity in the oculomotor vermis which is commonly implied in saccadic control. In other words, our results suggest a role for cerebellar areas outside the oculomotor vermis in the processing of visual post-saccadic errors. The vestibulocerebellum has been implied in other type of eye movements, such as the vestibulo-ocolar and the optokinetic reflexes, and smooth pursuit. Although to our knowledge never proposed, activity related to errors in this cerebellar area might therefore be a common characteristic between all these types of eye movements.

Saccadic errors and retinal slip are both potent driving forces for the initiation of corrective eye movements or corrections of ongoing movement. Saccadic errors represent a mismatch between target position and eye position after an initial saccade. Retinal slip serves as an error signal that tells the smooth eye movement systems that the current speed of the moving eye is not adequate. One could view the saccadic errors are relatively big errors which drive the saccadic system to make a correction saccade. Similarly, retinal slip can be viewed as a continuous stream of small visual errors on which the smooth eye movement systems reacts and corrects the eye movement velocity. The vestibulocerebellum, including the flocculus and paraflocculus is no part of the traditional cerebellar saccadic eye movement areas. However, the flocculus and paraflocculus do receive retinal slip signals during optokinetic stimulation. In addition, lesions of the vestibulocerebellum prevents optimal processing of retinal slip signals from the inferior olive and therefore result in severely reduced gain in both smooth pursuit and the optokinetic reflex. These lesions also impair adaptation of the vestibulo-ocular reflex. In other words, the vestibulo-cerebellum is involved in processing eye movement errors related to retinal slip. Our results suggest that it may also be involved in processing saccadic eye movement errors.

This is not incongruent with the theory of Marr and Albus (Albus, 1971; Marr, 1969) who hypothesized that motor error signals are relayed through the cerebellar climbing fibers, which originate in the inferior olive. Activity of these cerebellar climbing fibers results in so-called complex spikes in the cerebellar Purkinje cells. Catz & Thier (2005) showed that the size of the saccadic errors and the occurrence of complex spikes in the cerebellar oculomotor vermis are not related. This relationship would be expected based on the theory proposed by Marr and Albus. Our data may suggest that the saccadic error signal is not primarily relayed to the oculomotor vermis, but (also) to the vestibulo-cerebellum.

In **chapter 3**, the aim was to investigate possible differences in brain activation patterns between reflexive and self-paced voluntary saccadic eye movements. We hypothesized that areas in the cerebrum and in the cerebellum might be specifically involved in one of the two types of saccades. In particular, we looked for differences in cerebellar activation patterns between reflexive and voluntary saccades. This was inspired by the observation that the amplitude change after saccadic adaptation, in which the cerebellum is critically involved (Botzel et al., 1993; Desmurget et al., 1998; Barash et al., 1999), does transfer between reflexive and voluntary saccades (Alahyane et al., 2007).

Our results showed stronger activation in several cerebral areas during reflexive saccades than during voluntary saccades. In the cerebellum no differences in activation were seen between the two conditions. This could indicate that functional difference in maintaining the accuracy of the two types of saccades is mediated on a cerebral, rather than a cerebellar level. This is in accordance with current the theory that programming and execution of a saccade is property of the cerebrum and the cerebellum is responsible for maintaining accuracy of the saccadic vector alone. In other words, the cerebellum simply sees no difference between voluntary and reflexive saccades. One alternative explanation for this lack of difference in cerebellar activation might be that the there is an overlap in activated areas, but that different subsets of cerebellar neurons in the same area are activated during the different types of saccades. Another explanation might be in the various efferent connections from the frontal and parietal eye fields to the cerebellum. For instance, the parietal eye fields are more activated in reflexive saccades than in voluntary saccades (Gaymard et al., 1998; Schraa-Tam et al., 2009). It might well be that the adaptation of voluntary saccades is mediated by changing the efferent connections between the parietal eye fields and the cerebellum. This change is not affecting the efferent connection between the frontal eye fields and the cerebellum, leaving reflexive saccades unaffected. Electrophysiological studies are imperative to investigate these alternative explanations.

A final alternative explanation is that the alleged difference in cerebellar activation patterns is not noticeable in our setup. As compared to the cerebrum, the cerebellum is a relatively small part of the brain; consequently, the different functional areas within the cerebellum are even smaller. The activity in these areas might not reach the threshold for significant activation during analysis. Higher spatial and temporal resolution of new MRI machines with higher fieldstrengths and/or new tools for analysis might provide a more detailed pattern of cerebellar activity.

In **chapter 4**, we set out to clarify neuronal pathways underlying smooth pursuit and suppression of optokinetic nystagmus using similar retinal stimulation. Smooth pursuit is commonly evoked by a small moving target whereas fixation is used to foveate a stationary target. For these oculomotor behaviors to be effective, the motion of the visual background relative to the target has to be ignored. Neurophysiological studies in monkeys suggest that smooth pursuit and fixation suppression of OKR are two behavioral phenomena that may be generated by overlapping but distinct pathways. We wanted to investigate whether direct comparison of smooth pursuit and fixation suppression of OKR might yield differences related to the type of oculomotor behavior that is generated while the retinal stimulation is kept the same.

Our imaging results suggest that under similar retinal stimulation conditions smooth pursuit eye movement and fixation suppression of the optokinetic reflex activate overlapping pathways. Smooth pursuit did increase activity in the oculomotor areas of the cerebellum as compared to fixation suppression of optokinetic nystagmus. As described in the introduction, lesions affecting the pons and cerebellum can lead to deficits in fixation. A key structure in fixation is the neural integrator that is situated in the pons (Moschovakis, 1997). As the cerebellum is thought to play a role in correction of ongoing movement, it is possible that we did not find strong cerebellar activity as the neural integrator is functioning properly in the healthy subjects that participated in our study. An active movement as smooth pursuit might need more cerebellar input in order to achieve a smooth movement. Performing this experiment with a focus on the cerebellum as in chapter 2 and 3 might reveal more subtle differences on cerebellar level.

Eye Movement Studies in a Clinical Setting

The last two experimental chapters deal with the study of oculomotor behavior in a clinical setting. In particular we investigated patients affected by paraneoplastic syndromes (PNS) that induces behavioral impairment that are possibly related to cerebellar functioning. First of all, we wanted to investigate the feasibility of video-oculographic recordings (VOG) in (severely) disabled PNS patients using a mobile presentation and recording unit at the bedside. We hypothesized that we could use VOG to distinguish patients with cerebellar damage although they did not show overt cerebellar signs at clinical examination. As described in **chapter 5**, eye movements are delicate movements that can show disturbances that are not visible to the naked eye. We were able to record eye

movement behavior of disabled PNS patients, and could inspect these recordings for small disturbances in detail.

Although satisfactory for these studies, improvement of the eye movement recordings could provide a more precise characterization of the eye movements and their disturbances. In chapter 4, we only looked at gross disturbances of eye movement control. Higher temporal resolution of the recordings could provide more details, allowing for the detection of smaller disturbances that might have been present could not be picked up by the current setup. Furthermore, additional tests as a saccade adaptation paradigm, as described in chapter 1, could be included with a more advanced setup. These improvements could lead to a better appreciation of the eye movement disturbances and to a more specific localization and delimitation of the extend of affected brain areas.

Aside from the setup, the patients' physical state obviously plays an important role in this study. As a result of the disease, stress, tumor treatment or other medication the patients in this study are often tired. Although this did not show as an identifiable effect in the results, shorter test time could lessen the burden of these tests on patient. This might be accomplished by using fewer tests, which are directed specifically to the disturbances found in this study. Also, not all patients were comfortable with the position in bed and head restraint we used. This was necessary in order to keep the setup calibrated. A more advanced setup with which less time per test is needed due to calibration could shorten the test time dramatically. This can be achieved by using a setup which can correct for head movement while performing the test.

In conclusion, VOG may aid significantly in the diagnosis and follow-up of mild motor disturbances in patients with neurological disorders, especially when such signs are not overt during clinical neurological examination.

For the particular group of PNS patients described in chapter 4, no treatment is available to counteract the neurological deterioration (de Beukelaar and Sillevis Smitt, 2006). Although most PNS are probably immune mediated, the precise pathogenesis has yet to be elucidated. Based on the current understanding of the disease process and recent research on the immunomodulatory effects of human chorionic gonadotrophin (hCG) (Khan et al., 2001), we hypothesized in **chapter 6** that hCG would induce inhibition of Th1 cells in Hu-PNS patients and result in clinical stabilization and possible even functional improvement. In seven out of 15 patients was the treatment considered to be successful. These results may indicate that hCG or similar compounds do interact at a certain level with the disease process. However, an alternative explanation for the result could be given

in four of these patients and inherent to the rareness of the disease, the number of patients in this study is small. This makes drawing firm conclusions difficult.

In general, Hu antibody associated paraneoplastic neurological syndromes have proven to be difficult to manage. So far, only radical tumor resection can stabilize or reduce the neurological deterioration in these patients (de Beukelaar and Sillevis Smitt, 2006; Dalmau and Rosenfeld, 2008). Several trials with immunomodulatory medication have not yet shown unequivocal results (Uchuya et al., 1996; Vernino et al., 2004; Shams'ili et al., 2006). As the continuous research will provide new pieces of the puzzle, further understanding of the pathogenesis and treatment options will arise.

Concluding remarks

The general aim of this thesis was to provide more insight in the neurophysiological aspects of oculomotor control and the use of eye movement abnormalities in a clinical setting. To an outsider, these two topics probably seem to be closely related. However, in a practical sense, the fields of basic research and clinical research are often not so close. Especially with the growing amount of knowledge and advanced specialization, professionals from both fields sometimes seem to grow away from each other. The departments in which the research described in this thesis was conducted provided an excellent cooperative environment. The conclusions drawn from this thesis underline the importance of collaboration of basic research scientists and clinicians, both with their own expertise. Combining this knowledge is imperative to expand our understanding of the human body and make advances in human medicine possible

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Summary/Samenvatting

Summary

Accumulating knowledge from electrophysiological, behavioral and imaging studies has led to a quite detailed understanding of the neuronal pathways of the oculomotor system. Extensive knowledge about the oculomotor system can serve as an example for and thus aid in the understanding of other complex neurological systems. Eye movements exhibit specific abnormalities when certain brain areas are affected by disease or trauma. For these reasons, understanding the oculomotor system can be of great value in research as well in a clinical setting.

Eye movements can be recorded and quantified with relative ease. Current technology enables us to record eye movements in healthy adults, children and patients, as well as in challenging environments such as a MR-scanner. The two specific eye movements considered in this thesis are saccades and smooth pursuit. Saccades are fast eye movements that direct the point of regard to a target in the visual field. Smooth pursuit is used to keep the point of gaze on an object that moves across the visual field.

The neuronal circuitry of saccadic eye movements has been studied elaborately. An interesting feature of saccades is the amplitude-adaptation when circumstances ask for adaptation. A crucial part in adaptation is the detection of saccadic errors. **Chapter 2** describes a functional imaging study into the involvement of the cerebrum and the cerebellum in the processing of saccadic errors. This study showed significantly more cerebellar activation in vermis VIII, lobules VIII-X and the left lobule VIIb with random saccadic errors. This suggests a possible role for areas outside the oculomotor vermis of the cerebellum in the processing of saccadic errors.

The saccadic eye movements can grossly be divided into two main types: the reflexive and the voluntary saccade. Reflexive saccades are driven by visual stimulation whereas voluntary saccades require volitional control. Behavioral and lesion studies suggest that there are two separate mechanisms involved in the generation of these two types of saccades. The functional imaging study described in **chapter 3** investigated differences in cerebral and cerebellar activation between reflexive and self-paced voluntary saccadic eye movements. The execution of reflexive saccades induced a stronger activation in the frontal eye fields, parietal eye field, the motion-sensitive area (MT/V5), the precuneus (V6), the angular and the cingulate gyri than the execution of self-paced voluntary saccades. No significant difference was found in the cerebellum. This suggests that the alleged

separate mechanisms for saccadic control of reflexive and self-paced voluntary are mainly observed in cerebral rather than cerebellar areas.

Chapter 4 describes a functional imaging study on brain activation patterns evoked by smooth pursuit and by fixation suppression of the optokinetic reflex (OKR) using similar retinal stimulation. Subjects performed two tasks: 1. a smooth pursuit task in which the moving target was presented on a stationary pattern of stripes and 2. a task in which a stationary target was presented on a moving pattern of stripes, the subjects had to fixate on the target while ignoring the moving background (fixation suppression of the OKR). Smooth pursuit evoked activation in the frontal eye fields (FEF), the supplementary eye fields (SEF), the parietal eye fields (PEF), the motion-sensitive area (MT/V5), and in lobules and vermis VI of the cerebellum (oculomotor areas). Fixation suppression of OKR induced activation in the FEF, PEF, and MT/V5. Comparison of the activity between the two tasks showed during smooth pursuit more activation in the right lobule VI of the cerebellum and in the right lingual and calcarine gyri than during fixation suppression of OKR. These results show that smooth pursuit and fixation suppression of the OKR appear to activate largely overlapping pathways.

Chapter five and six of this thesis are more clinically oriented. In **chapter 5** we assessed the feasibility of using video-oculography as a tool in the evaluation of patients affected by a Hu-antibody associated paraneoplastic neurological syndrome (Hu-PNS). Paraneoplastic neurological syndromes are 'remote effects' of cancer that are not caused by the tumor or its metastasis nor by infection, ischemia or metabolic disruptions. The symptoms of Hu-PNS result from an auto-immune reaction against the nervous system, secondary to a reaction of the immune system against a tumor. Although motor disturbances can appear as an important clinical feature in Hu-PNS, mild disturbances are often difficult to assess. When the cerebellum is affected, patients can show motor deficits ranging from severe ataxia to mild deficits of fine motor control. Saccades and smooth pursuit exhibit specific abnormalities when the cerebellum is affected. Video-oculography can be used to record these eye movements and subsequent analysis of the recordings can reveal even small abnormalities. The eye movement recordings of the patients with Hu-PNS showed subtle saccadic and smooth pursuit deficits. Therefore, we concluded that an easy quantification of eye movement control may assist in the diagnosis and follow-up of mild motor disturbances in patients with neurological disorders, especially when such signs are not overt during clinical neurological examination.

The T-cell immune response that is directed against Hu antigens appears to be responsible for the neurological symptoms in Hu-PNS. During pregnancy many Th1 mediated autoimmune diseases such as rheumatoid arthritis and multiple sclerosis regress. This observation led to the hypothesis that the decrease in disease activity might be related to high human chorionic gonadotropin (hCG) levels. **Chapter 6** describes a trial in which hCG was used as a drug for the treatment of fifteen Hu-PNS patients. Seven patients improved or stabilized at ≤ 3 on the modified Rankin Scale. Four patients showed significant improvement as indicated by an overall EFIT-score of ≥ 1 point. Five patients improved on the Barthel Index. Comparison with previous studies suggests that hCG may have immunomodulatory activity and may modify the course of Hu-PNS.

Overall, this thesis provides new insights in the neurophysiology of oculomotor control, with special attention for cerebellar contributions. Moving from basic research in the laboratory to the clinical applications, this thesis confirms the value of eye movement analysis in a clinical setting. This thesis also underlines the importance of further research into the pathophysiology and treatment options of paraneoplastic neurological disorders.

Samenvatting

De toegenomen kennis uit electrofysiologische, gedrags- en beeldvormende studies hebben geleid tot een gedetailleerd inzicht van de neuronale paden binnen het oculomotore systeem. Een goed inzicht in dit systeem kan dienen als een voorbeeld voor en daardoor helpen bij het begrijpen van andere complexe neurologische systemen. Oogbewegingen vertonen specifieke afwijkingen als bepaalde hersengebieden worden geraakt bij een trauma of bij ziekte. Daarom kan een gedegen kennis van het oculomotore systeem van grote betekenis zijn voor zowel de research als de kliniek.

Oogbewegingen kunnen relatief gemakkelijk worden geregistreerd en gequantificeerd. De huidige techniek maakt het mogelijk om oogbewegingen te registreren bij gezonde volwassenen, kinderen en in een technisch meer uitdagende omgeving zoals de MRI-scanner. De twee specifieke oogbewegingen die bestudeerd worden in dit proefschrift zijn de saccade en smooth pursuit. Saccades zijn snelle oogbewegingen die het fixatiepunt naar een specifiek object in het visuele veld verleggen. Smooth pursuit wordt gebruikt om het fixatiepunt op een object te houden dat door het visuele veld beweegt.

Het neurale circuit van de saccades is uitgebreid bestudeerd. Een interessant kenmerk van saccades is dat de amplitude van een saccadische oogbeweging wordt aangepast, geadapteerd, als de omstandigheden daarom vragen. Een cruciaal onderdeel hiervan is de registratie van de fout in de amplitude van de saccade. **Hoofdstuk *2*** beschrijft een functionele MRI studie naar de betrokkenheid van het cerebrum en het cerebellum bij het het verwerken van saccadische fouten. Deze studie liet een significant grotere activatie zien van de vermis VIII, lobjes VIII-X en het linker lobje VIIb van het cerebellum, bij het maken van willekeurige saccadische fouten. Dit wijst op een mogelijke rol van cerebellaire gebieden buiten de oculomotor vermis bij het verwerken van saccadische fouten.

De saccadische oogbeweging kan grofweg ingedeeld worden in twee typen: de reflexieve en de vrijwillige saccade. Reflexieve saccades worden gedreven door visuele stimulatie, terwijl vrijwillige saccades onder willekeurige controle worden uitgevoerd. Uitkomsten van gedrags- en laesiestudies wijzen naar twee aparte mechanismen die betrokken zijn bij het genereren van deze twee typen saccades. In de functionele MRI studie beschreven in **hoofdstuk *3*** werden de verschillen in cerebrale en cerebellaire activatie tussen reflexieve en zelf-getimede vrijwillige saccades onderzocht. Het uitvoeren van reflexieve saccades induceerde een

sterkere activiteit van de frontale oogvelden, het pariëtale oogveld, het bewegings-sensitieve gebied MT/V5, de precuneus (V6), de angulaire en cingulaire gyri dan bij vrijwillige saccades. Er werd geen significant verschil in het cerebellum waargenomen. Dit duidt erop dat de verschillen in het mechanisme voor het genereren van reflexieve en zelf-getimede vrijwillige saccades zich met name bevinden in het cerebrum en niet in het cerebellum.

Hoofdstuk 4 beschrijft een functionele MRI studie naar de activatie van hersengebieden tijdens smooth pursuit en fixatie-suppressie van de optokinetische reflex (OKR) bij gelijke retinale stimulatie. Proefpersonen voerden twee verschillende taken uit: 1. een smooth pursuit taak waarbij het bewegend doel werd gepresenteerd op een achtergrond van stationaire strepen en 2. een taak waarbij werd gefixeerd op een stationair doel, waarbij een bewegende achtergrond moest worden genegeerd (fixatie-suppressie van de OKR). Smooth pursuit zorgde voor activatie in de frontale oogvelden, de supplementaire oogvelden, de pariëtale oogvelden, het bewegings-sensitieve gebied MT/V5 en in de lobjes en vermis VI van het cerebellum (oculomotore gebieden). Fixatie-suppressie van de OKR zorgde voor activatie in de frontale oogvelden, de pariëtale oogvelden en MT/V5. Vergelijking van de activiteit tijdens deze twee taken liet tijdens smooth pursuit meer activatie zien in het rechter lobje VI van het cerebellum en in de rechter linguale en calcarine gyri dan tijdens fixatie-suppressie van de OKR. Deze resultaten laten zien dat smooth pursuit en fixatie-suppressie van de OKR grotendeels overlappende neurale paden activeren.

De **hoofdstukken 5** en **6** van dit proefschrift zijn meer klinisch georiënteerd. In **hoofdstuk 5** wordt het gebruik van video-oculografie als hulpmiddel bij de evaluatie van patiënten met een Hu-antilichaam geassocieerd paraneoplastisch neurologisch syndroom (Hu-PNS) onderzocht. Paraneoplastische neurologische syndromen zijn 'effecten op afstand' van kanker die niet veroorzaakt worden door de tumor zelf, noch door metastasen, infectie, ischemie of metabole ontregelingen. De symptomen van Hu-PNS zijn het resultaat van een auto-immuun reactie tegen het zenuwstelsel, secundair aan een reactie van het immuunsysteem tegen een tumor. Motorische stoornissen kunnen als een belangrijk klinisch kenmerk optreden bij Hu-PNS, maar milde motorische stoornissen zijn vaak moeilijk te beoordelen. Als het cerebellum is aangedaan kunnen patiënten stoornissen in de motoriek vertonen die variëren van ernstige ataxie tot milde stoornissen in de fijne motoriek. Saccades en smooth pursuit laten specifieke afwijkingen zien als het cerebellum erbij is betrokken. Video-oculografie kan gebruikt worden om deze oogbewegingen te registreren. Analyse van de opnames kan kleine afwijkingen in de oogbolmotoriek aan het licht brengen. De oogbewegingen van Hu-PNS

patiënten lieten kleine afwijkingen zien van saccades en smooth pursuit. Hieruit concluderen we dat quantificatie van de oogbolmotoriek kan helpen bij het diagnostiseren en de follow-up van milde stoornissen in de motoriek van patiënten met neurologische aandoeningen, met name wanneer deze stoornissen niet duidelijk zichtbaar zijn tijdens klinisch neurologisch onderzoek.

De T-cel immuunrespons gericht tegen Hu antigenen lijkt verantwoordelijk voor de neurologische symptomen in Hu-PNS. Tijdens de zwangerschap nemen veel Th1 gemedieerde auto-immuunzieketen zoals rheumatoïde artritis en multiple sclerose in activiteit af. Deze observatie leidde tot de hypothese dat deze afname van ziekte-activiteit het gevolg zou kunnen zijn van hoge humaan choriongonadotrofine (hCG) spiegels in het bloed. **Hoofdstuk 6** beschrijft een trial waarin humaanchoriongonadotrofine (hCG) als medicinale therapie voor patiënten met Hu-PNS werd geëvalueerd. Vijftien patiënten met Hu-PNS werden behandeld met hCG. Zeven patiënten lieten een verbetering zien of stabiliseerden met een score ≤ 3 op de gemodificeerde Rankin Schaal. Vier patiënten verbeterden significant met een EFIT-score ≥ 1. Vijf patiënten verbeterden op de Barthel Index. Vergelijking van deze studie met eerdere studies lijkt te wijzen op een immunomodulerende werking van hCG. Als medicatie zou hCG het beloop van Hu-PNS positief kunnen beïnvloeden.

Samenvattend beschrijft dit proefschrift nieuwe inzichten in de neurofysiologie van oogbewegingen, met speciale aandacht voor de rol van het cerebellum. Vanuit het basaal onderzoek in het laboratorium naar de klinische toepassingen, bevestigt dit proefschrift ook de waarde van de analyse van oogbewegingen in de kliniek. Verder wordt het belang van onderzoek naar de pathofysiologie van en de behandelingsopties voor paraneoplastische neurologische syndromen onderstreept.

List of Abbreviations

ADL	activities of daily living
BBG	brainstem burst generator
BI	Barthel Index
BOLD	blood oxygenation level dependent
CF	climbing fiber
CN	cerebellar nuclei
CSF	cerebrospinal fluid
DLPN	dorsolateral pontine nuclei
EEG	electroencephalography
EFIT	Edinburgh Functional Impairment Tests
EPI	echo-planar imaging
FDR	false discovery rate
FEF	frontal eye field
FL	flocculus
fMRI	functional magnetic resonance imaging
FSPGR	fast spoiled gradient-echo
hCG	human chorionic gonadotropi
Hu-Ab	Hu-antibody
IFN-γ	interferon-γ
IVIg	intravenous immunoglobulins
LTD	long-term depression
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
MST	medial superior temporal visual area (V5)
MT	middle temporal visual area
NOD	nonobese diabetic
NRTP	nucleus reticularis tegmentis pontis
OKN	optokinetic nystagmus
OKR	optokinetic reflex
OMV	oculomotor vermis
PC	Purkinje cell
PCD	paraneoplastic cerebellar degeneration
PEF	parietal eye field
PEM	paraneoplastic encephalomyelitis
PET	positron emission tomography
PF	parallel fiber
PFL	paraflocculus
PNS	paraneoplastic neurological syndromes
PPRF	parapontine reticular formation
PVA	primary visual area (V1)
riMLF	rostral interstitial nucleus of the medial longitudinal fasciculus
SC	superior colliculus
SCLC	small cell lung cancer
SEF	supplementary eye field
SPM	statistical parametric mapping
TE	echo time
Th1	T helper 1
TI	inversion time
TMS	transcranial magnetic stimulation
TR	repetition time
V1	primary visual cortex
V5	visual area V5
VN	vestibular nuclei
VOG	video-oculography
WBC	white blood cell count

List of Publications

van Broekhoven PC, Schraa-Tam CK, van der Lugt A, Smits M, Frens MA, van der Geest JN (2009) Cerebellar Contributions to the Processing of Saccadic Errors. Cerebellum *In press*

van Broekhoven PC, de Graaf MT, Bromberg JE, Hooijkaas H, van den Bent M, de Beukelaar JW, Khan NA, Gratama JW, van der Geest JN, Frens MA, Benner R, Sillevis Smitt PA (2009) Human chorionic gonadotropin treatment of anti-Hu associated paraneoplastic neurological syndromes JNNP *In press*

Schraa-Tam CK, van Broekhoven PC, van der Geest JN, Frens MA, Smits M, van der Lugt A (2009) Cortical and cerebellar activation induced by reflexive and voluntary saccades. Exp Brain Res 192:175-187.

Schraa-Tam CK, van der Lugt A, Smits M, Frens MA, van Broekhoven PC, van der Geest JN (2009) Differences between smooth pursuit and optokinetic eye movements using limited lifetime dot stimulation: a functional magnetic resonance imaging study. Clin Physiol Funct Imaging 29:245-254.

Schraa-Tam CK, van der Lugt A, Smits M, Frens MA, van Broekhoven PC, van der Geest JN (2008) fMRI of optokinetic eye movements with and without a contribution of smooth pursuit. J Neuroimaging 18:158-167.

Schraa-Tam CK, van der Lugt A, Frens MA, Smits M, van Broekhoven PC, van der Geest JN (2008) An fMRI study on smooth pursuit and fixation suppression of the optokinetic reflex using similar visual stimulation. Exp Brain Res 185:535-544.

van Broekhoven PC, Frens MA, Sillevis Smitt PA, van der Geest JN (2007) Eye movements as a marker for cerebellar damage in paraneoplastic neurological syndromes. Parkinsonism Relat Disord 13 Suppl 3:S296-300.

Dankwoord

Observatie leert ons dat een groot deel van de proefschrift-lezers op deze plaats beginnen te lezen. Sommigen haken ook al na een pagina of twee weer af. Daarom wil ik hier graag stellen dat de rode draad, die door de hoofdstukken in dit proefschrift loopt, wordt gevormd door de lijn die loopt van de basale wetenschap naar de kliniek.

Ik heb het als een voorrecht ervaren om als aankomend arts en promovendus in deze omgeving van de neurowetenschappen mijn onderzoek te mogen doen. Dit is mede te danken aan de samenwerking tussen de afdelingen Neurowetenschappen, Radiologie en Neurologie/Neuro-Oncologie binnen het Erasmus Medisch Centrum. Aan iedereen die betrokken is bij deze samenwerking wil ik dan ook mijn grote dank uitspreken.

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