Chapter Six
Experimental Protocol

6.1 Introduction

Two studies are presented in the current project. The kinematic characteristics of the saccadic eye movements of normal humans and humans with cerebellar atrophy were examined in the first study (table 6.1). In the second study, the kinematic characteristics of the normal human aVOR in response to passive and active head impulses were examined with the aid of an aVOR model (table 6.1). Although the data from the second study were collected as part of a previous project (Thurtell 1997; Thurtell et al. 1999), details of the subject characteristics, experimental design, stimulus paradigms, and procedures for *both* studies will be presented.

Table 6.1 Organization of experimental studies. Studies 2A and 2B are from Thurtell et al. (1999).

Study	**1A**	**1B**	**2A**	**2B**
Subjects	Normal (n=5)	Patient (n=5)	Normal (n=8)	Normal (n=8)
Stimulus	Head fixed	Head fixed	Passive head impulse	Active head impulse
Target	Moving between 2 of 4 targets	Moving between 2 of 4 targets	Fixed in 1 of 3 target positions	Fixed in 1 of 3 target positions

6.2 Subjects

6.2a Study One: Saccades

Five normal adult human subjects (N1-5, 24-58yrs, 43 ± 12yrs, mean ± SD) and five adult human subjects with cerebellar atrophy (CA1-5, 54-69yrs, 63 ± 7yrs) were

studied. Each subject gave informed consent before each test. The Royal Prince Alfred Hospital Human Ethics Committee approved the experimental protocols.

All subjects were clinically examined. None of the normal subjects had symptoms or signs of cerebellar or ocular motor disease. Clinically, the subjects with cerebellar atrophy were noted to have downbeat, horizontal gaze-evoked, and rebound nystagmus, in addition to other abnormalities of ocular motor and peripheral motor function (see section 8.1). Many of the subjects had abnormal saccadic metrics, with either hypometric or hypermetric saccades being observed. The specific clinical findings from each patient are summarized in table 6.2. The diagnosis of cerebellar atrophy in the patients was verified with magnetic resonance imaging (MRI) of the brain (see figure 6.1). A definite cause for the cerebellar atrophy was not determined for any of the patients, with none of them testing positive for any of the inherited spinocerebellar atrophies on genetic testing.

Figure 6.1 Sagittal T1-weighted MRI of a patient with cerebellar atrophy (CA5), showing striking atrophy of the cerebellum. Lobules VI and VII were severely atrophied in this patient (white arrow).

Table 6.2 Clinical characteristics of the subjects with cerebellar atrophy.

Patient	CA1	CA2	CA3	CA4	CA5
Age	69	56	54	68	67
Sex	Male	Male	Male	Female	Female
Disorder Inheritance	Sporadic	AD	Sporadic	AD	AD
Gaze-evoked nystagmus	+	+	+	+	++
Downbeat nystagmus	+	+	++	++	++
Rebound nystagmus	+	(+)	+	+	++
Fractured smooth pursuit	+	+	+	+	+
Absent aVOR suppression	+	+	+	+	+
Upper limb ataxia	-	+	(+)	+	+
Lower limb ataxia	+	+	+	+	+
Gait ataxia	++	++	++	+	++
Dysarthria	-	+	+	(+)	(+)
Head impulse sign	+	-	+	-	-

Severity indicated by: -, absent; (+), mild/equivocal; +, moderate; ++, severe. Other abbreviations: aVOR, angular vestibulo-ocular reflex; AD, autosomal dominant.

6.2b Study Two: Angular Vestibulo-Ocular Reflex

The subjects tested in the second study had no history or signs of vestibular, cerebellar, or ocular motor disease. All subjects gave informed consent and the Royal Prince Alfred Hospital Human Ethics Committee approved the experimental protocols.

Eight normal human subjects (P1-8, 26-55yrs, 37 ± 11yrs, mean ± SD) were tested in the first experiment of the second study (passive head impulses). In the second experiment (active head impulses), eight normal human subjects (A1-8, 21-50yrs, 33 ± 10yrs) were tested. Six of the subjects tested in the second experiment had also participated in the first.

6.3 Stimulus Paradigms

6.3a Study One: Saccades

In the first study, saccadic eye movements were recorded while the subject, who had their head fixed in space, attempted to fixate the laser target as it moved between four different locations, the corners of an imaginary square (see figure 6.2). The sides of the square were located 20° up, down, left, and right of centre. Four saccadic paradigms were tested in sequence. Firstly, the laser target (see section 5.3) moved repeatedly between the bottom two corners of the square (figure 6.2, A), then between the top two corners of the square (figure 6.2, B). Following this, the target moved between the left-sided corners (figure 6.2, C), and then between the right-sided corners of the square (figure 6.2, D). In each paradigm, the target moved at a random time every 2-3s, so that the subject was unable to predict when it was going to move. The target was always illuminated, so that there was no gap in illumination when the target changed position.

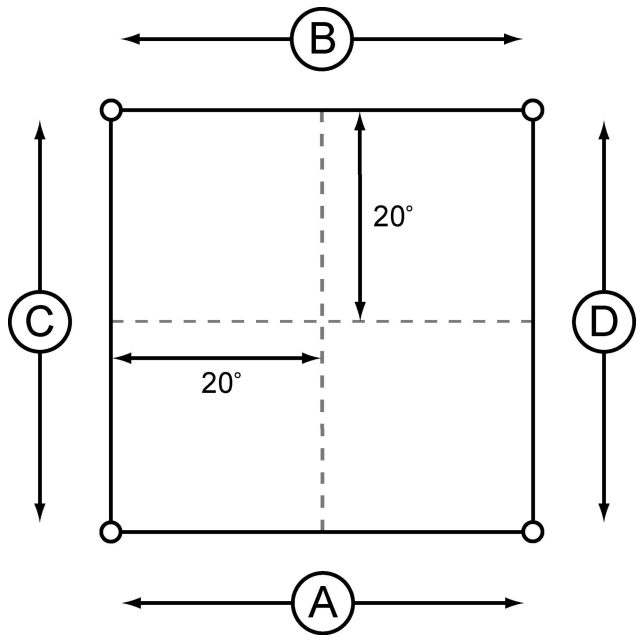

Figure 6.2 Saccadic eye movements were measured while the subject attempted to fixate a laser target as it moved between the corners of an imaginary square. In the experiment, the target moved between the bottom corners (A), the top corners (B), the left corners (C), and the right corners (D).

The saccadic stimuli were designed to give rise to a constant eye velocity axis tilt during each paradigm. For example, the saccadic eye movements made between the square corners located 20° up would be expected to give rise to a backward tilt of the eye velocity axis in the pitch plane, whereas those made between the square corners located 20° down would be expected to give rise to a forward tilt of the eye velocity axis. The difference in vertical eye position eccentricity in the given example is 40°. If the half-angle rule is obeyed, the difference in the tilts of the eye velocity axes in the pitch plane would be 20°. Dividing this value by the difference in eccentricity gives a measure of how well the half-angle rule is obeyed (in the above example 20°/40° = 0.5, so the half-angle rule is perfectly obeyed). Even if the primary position for the subject lies outside of the imaginary square and the eye velocity axis tilts are in the same direction, the above findings would remain the same.

Both groups of subjects were tested with the same paradigm. As a result, a measure of how well the half-angle rule is obeyed in normal subjects could be obtained and compared with a measure of how well it is obeyed in humans with cerebellar atrophy.

6.3b Study Two: Angular Vestibulo-Ocular Reflex

The responses to two different head rotation stimuli were recorded in the second study. The responses to passive (manually-generated) yaw head impulses were considered in the first experiment, while the responses to active (self-generated) yaw head impulses were considered in the second.

The passive yaw head impulse is a manually-imposed, unpredictable horizontal head-on-neck rotation that characteristically has an amplitude of 15-25°, peak velocity of 150-350°/s, and peak acceleration of 4000-6000°/s^2 (Halmagyi and Curthoys 1988; Halmagyi et al. 1990; Aw et al. 1996a). The protocol for delivering a passive yaw head impulse is described in section 6.4i. The passive yaw head impulse has an axis that approximately aligns with the z-axis of the head, making it a predominantly horizontal head rotation (see figure 6.3).

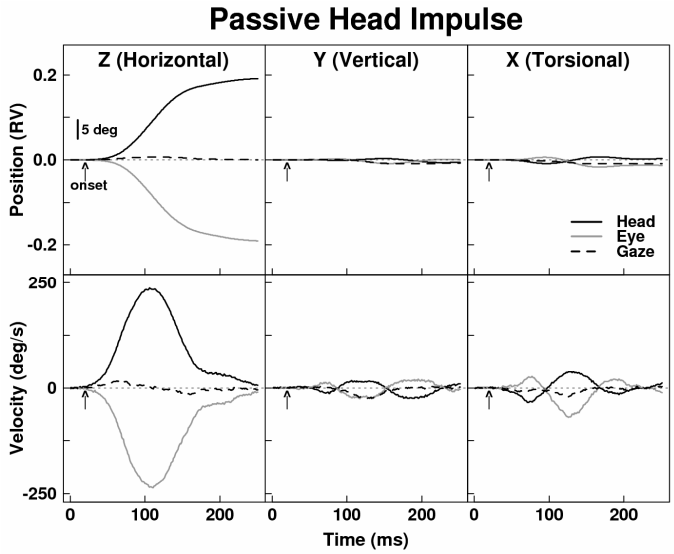

Figure 6.3 The position components (rotation vectors) and velocity components (velocity vectors) of the response to a passive yaw head impulse in a normal human subject fixating on a centrally-placed target. Horizontal, vertical, and torsional components of head-in-space (head), eye-in-head (eye), and eye-in-space (gaze) are plotted in the time domain. The arrow indicates the time of impulse onset. The head rotation is mostly horizontal, with small vertical and torsional components. The eye trace approximately mirrors the head trace, resulting in stabilization of gaze (from Thurtell 1997).

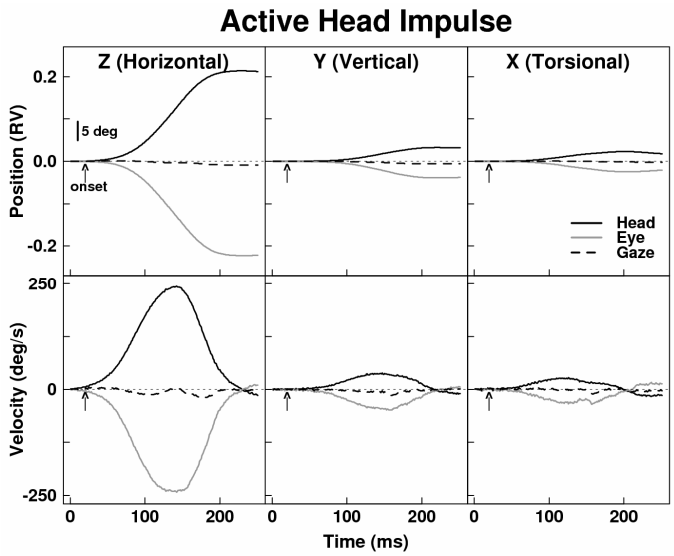

Figure 6.4 The position and velocity components of the compensatory response to an active yaw head impulse, from the same subject as in figure 6.3. The head rotation profile is similar to that of the passive head impulse, with the eye trace once again mirroring the head trace (from Thurtell 1997).

Active yaw head impulses are self-generated, high velocity horizontal head-on-neck rotations (Thurtell et al. 1999; Halmagyi et al. 2003; Black et al. 2005). The profiles of the head rotations during active yaw head impulses were similar to those observed during passive yaw head impulses (see figures 6.3, 6.4, and 6.5), with the head rotation velocities achieved during the active head impulses being similar to those for the passive head impulses (see figure 6.5).

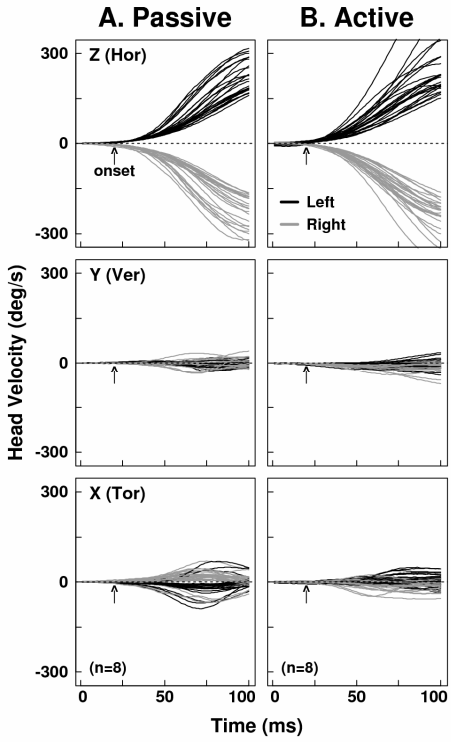

Figure 6.5 A. Mean horizontal, vertical, and torsional head velocity traces from each subject during passive head impulses. **B.** Mean head velocity traces from each subject during active head impulses. The head velocity profiles of the two stimuli were noted to be very similar (from Thurtell et al. 1999).

During both the passive and active head impulses, gaze was fixed on a target in one of three different vertical positions, each in a separate test. During each test, the target was displayed constantly, and the subject was instructed to fixate on it. The first test was conducted with the laser target positioned centrally (directly in front of the subject's eyes) on the display screen. During the passive head impulses, this paradigm was identical to that of Aw et al. (1996a). The second and third tests were completed with the laser target positioned 20° up and 20° down from centre, respectively, on the display screen.

6.4 Procedure

The procedure by which eye and head rotation data were collected is described in this section. To minimize the amount of artifact in the signals from the search coils, all but essential metallic objects were removed from the vicinity of the magnetic fields. All equipment used during the tests was constructed from wood or plastic, with the exception of the search coils, the leads connecting the search coils to the amplifiers, the signal amplifiers (surrounded by mu-metal shielding; see section 5.2e), and the leads from the signal amplifiers passing to the phase detectors.

6.4a Sterilization of Search Coils

The eye search coil was sterilized prior to each test, using the STERRAD 100 sterilization system (Johnson and Johnson, Sydney, Australia). The STERRAD 100 system uses a combination of radio waves and hydrogen peroxide, at low temperatures (<50°C), to create a gas plasma that destroys micro-organisms.

The eye search coil was handled with aseptic technique prior to placement on the conjunctiva of the subject's left eye. Before the *in vitro* calibration, it was placed into a search coil holder that had also been sterilized using the STERRAD 100 system. It remained in this search coil holder during and following the *in vitro* calibration, until it was due for placement on the eye.

6.4b In Vitro Calibration

Both the eye and head search coils were calibrated *in vitro* using a Perspex Fick gimbal (figure 6.6a) that was positioned in the centre of the magnetic fields (figure 6.6b). The eye search coil was secured in the centre of the gimbal, while the head search coil was secured 2cm in front of the eye search coil to approximate its position on the head. Prior to each test, the gain of each search coil was adjusted, for rotation in each dimension, so that a +4V signal was elicited when the search coil was positioned at +20°.

During the *in vitro* calibrations for the first study, the gimbal was moved in 5° steps from –25° to +25° in the horizontal, vertical, and torsional planes. During the *in vitro* calibrations for the second study, it was moved from –20° to +20° in each plane, once again in 5° steps. At each calibration position, the data acquisition system was triggered to collect approximately 500 data samples from each search coil. Each sample was saved to the hard disk of the PC for later off-line analysis. The method by which the *in vitro* calibration data were used to scale the test data is described in section 7.2.

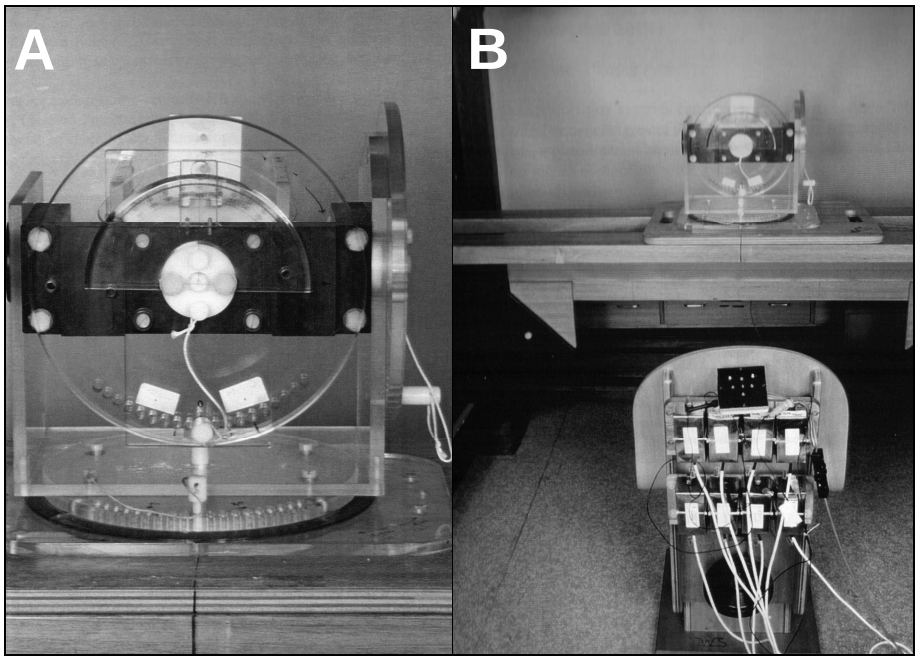

Figure 6.6 A. The *in vitro* calibration data were obtained using a Fick gimbal. **B.** The data were collected within 1.9m × 1.9m × 1.9m magnetic field-generating coils (from Aw 1996).

6.4c Subject Preparation

At the beginning of each test, the subject was seated comfortably, so that the anterior surface of the cornea was positioned 94cm from the tangent screen and the pupils were positioned at the same vertical level as the laser target. Two drops of a topical ophthalmological anaesthetic, proxymetacaine (5mg/mL, Alcon Laboratories, Sydney, Australia), were then placed in the left eye. The spectacle frame, with the head search coil attached, was firmly secured to the subject's head. Neither the position of the head search coil nor that of the spectacle frame was readjusted at any stage during the experiment.

To confirm that the anaesthetic had taken effect, the corneal reflex was tested 3-5 minutes after the anaesthetic had been applied. If the reflex was present, further anaesthetic drops were placed in the eye. If it was absent, the search coil was placed on the eye with the exiting wire positioned nasally, and the copper leads were connected to the preamplifier leads (which had been secured to the subject's garments). If the subject complained of discomfort following the application of the search coil, the eye was examined for evidence of trauma. If there was evidence of trauma, the search coil was removed immediately. If there was no evidence of trauma and the subject was agreeable, further drops of anaesthetic were added to the eye and the test was continued. None of the subjects in the current series of experiments sustained ocular trauma as a result of placement or removal of a search coil.

To keep the head fixed in space following placement of the search coil on the eye (see section 6.4e), the subject was asked to bite onto a bite bar attached to the magnetic field-generating coil frame. Alternatively, the head was positioned and maintained in a ±1° software window by the experimenter (see figure 6.7 and section 6.4e). The subject's head was fixed using one of these two methods during *in vivo* calibrations and all other parts of the experiments not involving head movement.

Following the completion of each experiment, the search coil was removed from the eye using a sterile Jobson-Horn probe to gently break the seal between the search coil and the conjunctiva. The search coil was rinsed in sterile water and then sent for re-sterilization.

The subject's eye was irrigated with a small amount of fluorescein (2%, Chauvin Pharmaceuticals, Romford, UK) to facilitate examination of the conjunctiva and cornea for abrasions using the cobalt beam of the slit lamp. None of the subjects were found to have a conjunctival or corneal abrasion after removal of the search coil. Following examination with the slit lamp, the subject's eye was irrigated with Celluvisc eye drops (Allergan, Sydney, Australia) to prevent conjunctival and corneal drying while the anaesthetic was wearing off.

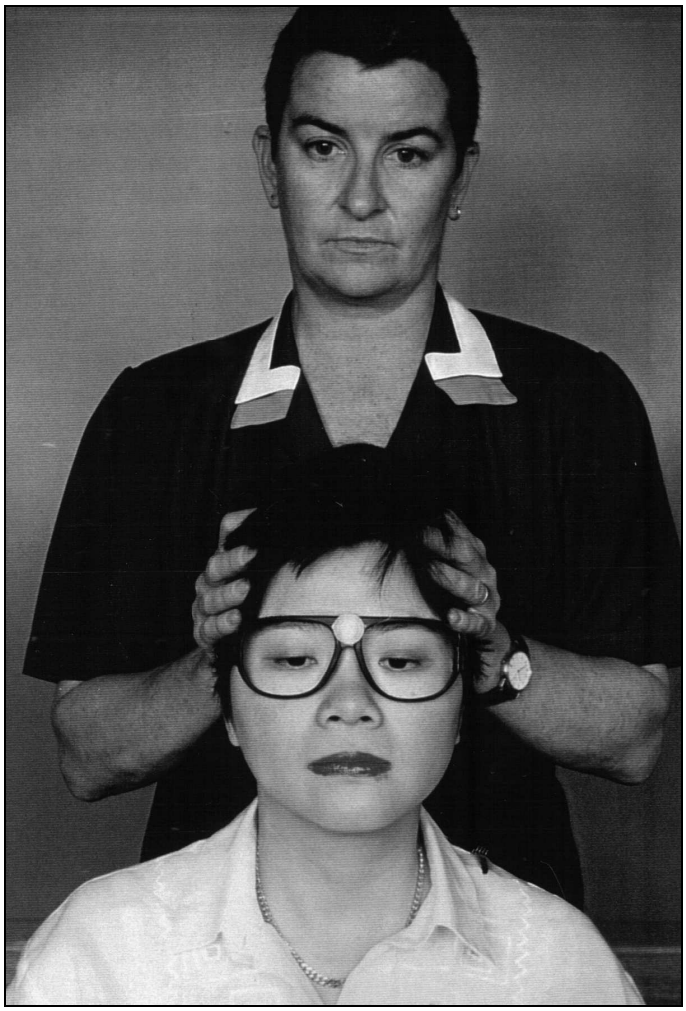

Figure 6.7 An experimenter positioning a subject's head in the ±1° software window. Neither subject nor operator would normally be wearing jewellery in the magnetic fields (from Aw 1996).

6.4d Search Coil Lead Position

Tethering of the eye search coil was avoided by allowing adequate slack on the copper leads from the coils, as it was observed to degrade the quality of the signal from the search coil and also resulted in increased slip of the search coil on the conjunctiva. Data from experiments where there was evidence of search coil tethering were discarded. The eye search coil lead was also positioned so that it did not catch in the eyelashes. The leads from both the head and eye search coils were taped to the face and neck to maintain the lead positions during the test. Taping was noted to prevent movement of the leads relative to the magnetic fields and decreased the likelihood of the leads being broken during the test.

6.4e Head Positioning

To ensure that the reference position of the head remained consistent during all parts of the experiment, the head was maintained in a set position using one of two methods. In the first study, the subject's head was held fixed, in most cases, using a bite bar that was attached to the magnetic field-generating coil frame. The bite bar was made of rigid plastic and filled with an impressionable but non-adhesive dental mould (President Putty, Coltène, Mahwah, New Jersey) that set firmly in the shape of the subject's occlusion after a short period of time. Once the dental mould was set, little movement of the head was possible when the subject was biting onto the bite bar. The head therefore remain fixed in the same position for the duration of the test.

When testing with a bite bar was not possible (for example, if the subject had false teeth or delicate dental prostheses), the subject was tested after the head was gently positioned into a ±1° window in software (see figure 6.7). Some of the subjects in the first study were tested using this method of fixing the head, while all subjects in the second study were tested using this method. The software window was included in the *LabVIEW* program responsible for logging the data. While positioning the head, the experimenter received real-time feedback from a touch screen monitor positioned away from the magnetic fields. Once the yaw, pitch, and roll head signals were in the software window, the data acquisition system could be triggered.

6.4f In Vivo Calibration

In vivo calibration data were gathered while the subject was fixating on a centrally-positioned laser target. Once the head was fixed, the data acquisition system was triggered and about 1000 samples (1s) of data were recorded. The calibration was accepted if the real-time display did not show any blinks or saccades.

The *in vivo* calibration was repeated at regular points during each experiment to control for slip of the eye search coil on the conjunctiva, which was most likely to occur between the stages of the experiment or while further drops of anaesthetic were being

applied to the eye. The calibration files were also used to adjust for eye and head search coil misalignment in the data files obtained immediately following the calibration (as in Tweed et al. 1990; see section 7.4).

6.4g Listing's Plane

Listing's plane data were collected following the first *in vivo* calibration, with the subject fixating on the laser target as it moved from 0° to 20° in 5° steps along horizontal, vertical, and diagonal radial spokes originating from the centre of the tangent screen. During this part of the test, the subject's head was held fixed. The laser remained at each target position for 2.25 s. Approximately 90s of data were collected (see figure 4.1, for an example of a full data set).

6.4h Study One: Saccades

Saccadic data were collected while the head was fixed in space (see section 6.4e). The subject was instructed to fixate the laser target as it moved between four different positions, the corners of an imaginary square (see figure 6.2). Data were collected during four different saccadic paradigms, as described in section 6.3a. Prior to each paradigm, *in vivo* calibration data were obtained. Up to fifteen saccades in each direction were then obtained during each paradigm. The subjects were instructed to avoid blinking whilst making saccades. Data sets in which there was evidence of blinking during saccades were discarded.

6.4i Study Two: Angular Vestibulo-Ocular Reflex

The passive yaw head impulse was the stimulus used in the first experiment of the second study (see section 6.3b). For each vertical eye position (0°, 20° up, and 20° down), at least eight head impulses to each side were recorded. The experimenter, who stood behind the subject in the magnetic fields (see figure 6.7), delivered the stimulus. The subject's head was firmly grasped and positioned so that all head signals were in the ±1° software window. Data acquisition was commenced and the head impulse was delivered by

quickly rotating the head to one side or the other. Data acquisition was ceased a few seconds after the end of the head impulse. Great care was taken to avoid nudging the spectacle frame while delivering the impulses. Any data that showed artifact suggesting that the spectacle frame had been nudged, such as a sudden departure from the usual bell-shaped velocity profile of the head impulse (see figure 6.3), were discarded. To confirm that rotation of the spectacle frame was tightly coupled to rotation of the head, the data from a search coil attached to a bite bar were compared with the data from the search coil attached to the spectacle frame. It was assumed that rotation of the bite bar was coupled very closely with rotation of the head. The difference between the bite bar and spectacle frame search coil velocities was found to be very small (see figure 6.8).

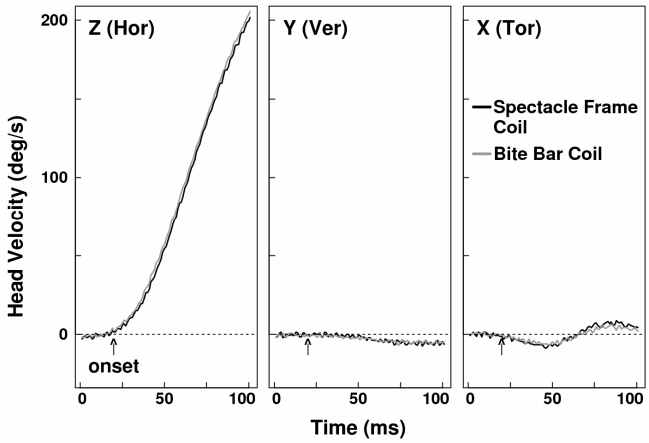

Figure 6.8 Head velocity, as recorded by search coils mounted on a spectacle frame and on a bite bar, during a passive head impulse. The recorded head velocities are almost identical, indicating that movement of the spectacle frame was closely coupled to that of the head (from Thurtell et al. 1999).

In the second experiment of the second study, the active head impulse stimulus was used. The experiment was otherwise identical to the first experiment. A minimum of eight active head impulses to each side were recorded for each vertical eye position. The experimenter positioned the head so that the head signals were in the ±1° software window, and then removed their hands from the vicinity of the head, at which point the subject was required to maintain that head position to the best of their ability. The subject was then instructed to quickly rotate their head to one side or the other, whilst maintaining visual fixation on the laser target. Some subjects moved their head out of the ±1° software window before the start of the active head impulse, while others made anticipatory

movements in the opposite direction beforehand. Consequently, all subjects were carefully tutored before the experiment, and were given ample time to practice before the search coil was placed on the eye. The subjects were given verbal feedback during the experiment, encouraging them to keep their head still and to avoid making anticipatory movements before the start of each active head impulse. Data sets that showed any slow head movement before the onset of the impulse were excluded from further analysis.

6.5 Summary

In the current project, two studies were conducted. In the first study, the saccadic eye movements of normal humans and humans with cerebellar atrophy were recorded using the magnetic search coil technique. Horizontal and vertical saccadic eye movements were recorded while the subjects attempted to maintain fixation on a target that moved between the corners of an imaginary square. The orientation of Listing's plane was determined, in each subject, from recordings of fixations and horizontal, vertical, and oblique saccades.

In the second study, eye and head rotations of normal human subjects were recorded, using the magnetic search coil technique, during passive and active yaw head impulses. The rotations were recorded while the subjects attempted to maintain fixation on each of three different vertical targets, positioned centrally, 20° up, and 20° down. The orientation of Listing's plane was also routinely determined for each subject. The data from this study were collected as part of a previous project (Thurtell 1997; Thurtell et al. 1999). The head rotation and initial eye position data were utilized in the current study as the input for a model of the aVOR; the eye position and velocity simulations from the model were compared with the actual eye position and velocity data obtained from the human subjects in the study (see chapter 9).

The data were saved to the hard disk of the data acquisition PC and were later copied to the hard disk of a DECstation 5000/240 for off-line analysis. Data analysis and modelling techniques are described in the following chapter.