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Oculomotor abnormalities in a sheep (*Ovis aries)* model of Huntington's disease: Towards a biomarker for assessing therapeutic efficacy

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Key words: Huntington's Disease, sheep, oculometer, eye movement

Abstract

Background

Huntington's disease (HD) is characterised by a loss of control of motor function that causes the presence of abnormal eye movements at early stages.

Objective

Here we measured eye movements in a sheep (*Ovis aries)* model of HD using a purpose-built, head-mounted sheep oculometer. This device allows us to measure saccades in sheep without the need for either behavioural training or head fixation. At the age of testing (6 years old), the HD sheep were pre-manifest.

Results

We found small but significant differences in eye movements between normal (control) and HD sheep during vestibular ocular reflex (VOR)- and vestibular-based post-rotational nystagmus (PRN)-based tests.

Conclusions

Two measures were identified that could distinguish normal from HD sheep; these were the number of PRN oscillations when tested in the dark and the gain (eye movement to head movement ratio) during the VOR when tested in the light. This is the first study, to our knowledge, in which eye movements have been quantified in sheep. It demonstrates the feasibility of measuring and quantifying human-relevant eye movements in this species. The HD-relevant deficits show that even in 'premanifest' sheep there are measurable signs of neurological dysfunction that are characterised by loss of control of eye movements**.**

1.0 Introduction

Abnormal eye movements are characteristic of many human neurological disorders, including corticobasal degeneration, frontotemporal dementia, Huntington's disease (HD), Kufor-Rakeb syndrome, Niemann-Pick type C, neuronal intranuclear inclusion disease, Gaucher's disease, and Whipple's disease [1]. HD is a progressive inherited neurodegenerative disease in which degeneration starts in the neostriatum and eventually spreads to most other parts of the brain [2, 3, 4]. Dominant symptoms are loss of motor control, cognitive decline, psychiatric disorder and sleep disturbance. While chorea is the most obvious motor sign, abnormal eye movements are also characteristic of HD [5, 6] and one of the first clinical signs in this disease [5]. Oculomotor abnormalities in patients in the early stages of HD include problems with initiating voluntary saccades, over-shoot of saccades and diminished fixation [7]. These impairments are thought to arise indirectly from neurodegeneration of the basal ganglia nuclei (caudate and substantia nigra pars reticulata) that have high modulatory control over the premotor oculomotor brainstem system (lateral vestibular nucleus, superior vestibular nucleus and superior colliculus) [8, 9, 10]. In the long term, all major types of oculomotor control, including saccades, smooth pursuits, vergence, vestibulo-ocular reflex (VOR), optokinetic nystagmus, fixation, acuity and gaze holding are functionally impaired in HD [11, 12, 13, 14, 15, 16] (Table 1).

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Table 1 near here

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Although eye movements are relatively easy to measure in humans, one of the main challenges of using such oculomotor tests for patients with neurological disease is conveying the instructions needed for the specific test requirements of gaze fixation and initiating voluntary saccades [7]. This is even more problematic with animal models of disease, since non-verbal animal species cannot understand complex verbal commands. This difficulty is further compounded by fundamental differences in eye movement control systems between mice and humans [17]. For example, mice consistently do not make saccades because their eyes are adapted for small-space nocturnal rather than wide-space diurnal vision [18]. Furthermore, mice do not make coupled (human-like) eye movements unless their heads are restrained but instead make more complex non-conjugate eye movements often moving in opposite directions [19]. For these reasons, despite there being excellent mouse models of HD, study of eye movement abnormalities such as gaze fixation and initiation of voluntary saccades have not been achieved in mice.

To overcome some of these difficulties with using mice, in this study, we used sheep (*Ovis aries)* as a model species. First, we used normal sheep to determine if the measurement of eye movements in this species was possible. Then we compared eye movements in normal versus HD transgenic sheep. Whilst this model of HD does not show overt motor symptoms up to the age of 10 years, it does show aggregate brain pathology [20], early circadian behavioural

abnormalities [21], abnormal melatonin levels [22], abnormal sleep [23, 24] and abnormal metabolism [20]. This model is therefore ideally positioned for studying the earliest stages of HD and for determining whether or not oculomotor deficits are part of the phenotype of the disease in sheep.

We used a novel custom-built head-mounted sheep oculometer, to measure oculomotor responses. Initially, we attempted to train sheep to fixate and follow visual stimuli. Whilst we were successful in establishing for the first time (to our knowledge) that sheep produce saccades, these data were too variable to be quantifiably useful. In the rest of the study, therefore, we focussed on measuring the vestibular ocular reflex (VOR) and vestibular-based post-rotational nystagmus[1] (PRN), neither of which are voluntary in nature. Both of these oculomotor responses are affected during HD, with patients exhibiting reduced gain in the slow component of VOR [25, 26] and abnormalities in the slow component of the PRN [27, 28]. We quantified VOR and PRN responses in sheep during rotation in both dark and light conditions. We found differences in response between normal and HD sheep. This proof-of-principal study shows that, not only can eye movements be reliably quantified in sheep, but also that HD sheep show subtle early changes in the control of eye movements. This represents, to our knowledge, the first demonstration of impairment of control of movement in this model.

2.0 Methods

2.1 Sheep

Pilot studies were conducted using 2 Welsh mountain female sheep aged 3 years from farm stock. The main study animals were 21 female merino sheep, 10 of which were normal, the other 11 were transgenic for the human HD transgene carrying 73 CAG repeats [29]. These sheep were reared in a large flock on open pasture at a livestock research facility in South Australia and imported to the University of Cambridge, UK at 4 years of age. The sheep were aged 6 years old when they were used in this study. All sheep were kept outside with *ad libitum* access to fresh forage and shelter. All procedures were conducted in accordance with the UK Sheep Scientific Procedures Act (1986) and the University of Cambridge Sheep Welfare and Ethical Review Bodies (AWERB).

2.2 Experimental Recording

All recordings were carried out indoors within a climate and light controlled testing room (3m x3m) in which all walls and ceiling were painted black to ensure maximal darkness for the dark testing phase.

Sheep were tested while supported in a veterinary sling (Figure 1A). All sheep had been trained previously to sit in the veterinary sling with their feet off the ground (See Nicol and Morton (2021) for the training procedure)[30].

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Figure 1 near here

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Oculomotor testing (VOR and PRN) was conducted using all 21 sheep over a 2 day period. Each test took approximately 15 minutes. Eye movements were recorded using a novel custom-designed, purpose-built head-mounted ovine telemetric oculometer (Figure 1B; Ober Consulting, Poznan, Poland). The oculometer was an adaptation of an instrument designed for humans [31]. Fitting the oculometer onto the sheep's head involved 1) moving the adjustable orbit loops horizontally until there was no lateral movement of the device and 2) inserting a Velcro sponge wedge under the front of the oculometer such that there was no vertical movement of the device. Once secured, the adjustable infra-red sensor was aligned with the pupil of the eye and then fixed at that point. The oculomotor uses direct infra-red oculography sampled at 1 kHz with two infra-red sensors (one for each eye) to monitor the velocity of eye movement directly in front of the eye. Data were collected directly onto JazzRecorder (version 3.19). The sheep oculometer measures horizontal and vertical eye movement using infrared oculography. The measurable ranges for horizontal and vertical eye movements were ±35° and ±25° respectively. The noise level (along the horizontal axis) was equivalent to 6 min of visual angle. Data from the oculometer for all metrics of eye movement are presented as an average of the movement of both eyes [32].

VOR and PRN testing were carried out using a single frequency (0.5Hz) rotation around a fixed point axis whilst the sheep were held in the veterinary sling (for time line, see Table 2). Sheep were manually rotated and timed using a metronome set at 1 s intervals to indicate the point of 180º rotation. Manual rotation was practiced by two handlers until a rotation of 360 º over 2 s could be reliably and consistently achieved. Rotation was for 60 s in a clockwise direction (to induce VOR), followed by a static phase for 60 s (to measure PRN) that was

155 followed by an anti-clockwise rotation for another 60 s (to induce VOR in the opposite
156 direction) and finally a static phase for another 60 s (to measure PRN again). Both tests were
157 carried out under both dark (0 lux as measured using a lux meter but with a red light) and light
158 (250 lux) testing conditions (Table 2).

159 Oculometer recordings were made continuously throughout each test. For the PRN test,
160 the number of nystagmus oscillations (total and the number/10 s) as well as the mean duration
161 and mean peak velocity (degrees/ms) of each oscillation (illustrated in Figure 2) that occurred
162 post-rotation (clockwise and anti-clockwise) in the static phase during both light and dark
163 conditions were recorded using the oculometer. During the VOR part of the test, gain (ratio of
164 head to eye movement) was estimated by measuring the position amplitude of each eye
165 oscillation during rotation (clockwise and anti-clockwise). Head movement was set at a
166 constant of 180^{o}/sec for all tests.

167 **

168 **Table 2 near here**

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171 *2.3 Statistical analyses*

172 For the PRN analysis, the parameters measured were the number of oscillations per 10 s, total
173 number of oscillations, the average oscillation velocity and the average oscillation duration
174 during the post-rotational static phase for both clockwise and anti-clockwise rotations in both
175 light and dark. The average gain during VOR in both the light and dark conditions was also
176 compared between groups. Data were checked for parametric assumptions using the

Kolmogorov- Smirnov and the Levenes test and subsequently analysed using general linear model to test for phenotypic difference of the number of oscillations per 10 s, and either the T test (parametric) or Mann Whitney test (non-parametric), depending upon whether parametric assumptions were met, to assess phenotypic differences in the total number of oscillations, average duration and velocity of oscillation and average gain.

Due to reduced data quality, data from one and two sheep were removed from the clockwise data and anti-clockwise data sets respectively.

2.0 Results

3.1 Typical saccade and nystagmus oscillation patterns in sheep

We confirmed that sheep exhibit trackable eye movement in terms of both position and eye velocity (for a typical example, see Figure 2A; Supplementary video 1). The majority of the velocity curves observed in the sheep were asymmetrical. The sheep also demonstrated typical human-like eye oscillation movements during rotational VOR (Figure 2B) and during post-rotational nystagmus (Figure 2C). During VOR, the slow phase movement of the eye was in the same direction as the rotation, with the fast phase in the opposite direction. During PRN, the slow phase was in the opposite direction of the rotation. In the example shown in Figure 2A and Supplementary video 1, concurrent movement of eyes and head with two sequential saccades (1 and 2) are seen before the eyes reached the final position.

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Figure 2 near here

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3.2 Post-rotational nystagmus and vestibulo-ocular reflex testing in dark conditions

The total number of nystagmus oscillations post-clockwise rotation was significantly higher in the normal compared to the HD sheep (t=-2.35, d.f.= 18, p=0.03) (Figure 3A). During the clockwise rotation, the eye oscillation frequency for both groups of sheep exhibited a significant linear reduction over time (F=87.05, d.f.=5, p<0.001) with normal sheep having a significantly higher frequency compared to HD sheep (F=5.55, d.f.=1, p=0.03). A similar reduction over time was observed for nystagmus frequency after anticlockwise rotation (flat exponential) (F=112.61, d.f.=5, p<0.001) but in this instance control sheep tended to show fewer saccades at each time point compared to HD sheep (F=3.67, d.f.=1, p=0.07). Similarly, the total number of oscillations over the 60 s of the anticlockwise test was also significantly lower for the control compared to the HD sheep (t=2.11, d.f.=17, p=0.0496; Figure 3D). Due to there being a difference in the direction of significance between genotypes for clockwise versus anticlockwise rotation, the differential in the number of oscillations between the two types of rotation were compared statistically. There was a significant difference between genotypes for both frequency (F=7.57, d.f.=1, p=0.01); Figure 3E) and also for the total number of saccades (t=-2.94, d.f.=17, p=0.009; Figure 3F).

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Figure 3 near here

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For the PRN test, there was no significant difference between genotypes post-clockwise or post-anti-clockwise rotation for mean oscillation eye velocity (Mann Whitney U =48; p=0.912; Mann Whitney U =37; p=0.549), or mean oscillation duration (t=-1.334, d.f.=18, p=0.201; t=1.48, d.f.=17, p=0.267). There was also no significant difference in mean oscillation gain between genotypes during the VOR (rotation) part of the test for either clockwise or anticlockwise rotation (Mann Whitney U =77; p=0.132; Mann Whitney U =69; p=0.152; Figure 4).

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Figure 4 near here

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3.3 Post-rotational nystagmus and vestibulo-ocular reflex testing in light conditions

There was a more pronounced exponential reduction in oscillation frequency over time during the PRN compared to the VOR test, with both groups of sheep reaching zero oscillations 40 s after clockwise rotation and after 20 or 50 s for the control and the HD sheep, respectively, after anticlockwise rotation (Figures 5A,C). Due to the number of zero oscillation data points within the oscillation frequency data, it was not possible to meaningfully apply statistical tests to assess the reduction over time nor the difference in frequency between groups. Comparisons could, however, be made for the first 10 s frequency and total oscillation values. In this respect, there was no significant difference for the first 10s oscillation frequency (t=-.13, d.f.= 18, p=0.90) nor for the total number of oscillations (t=0.12, d.f.= 18, p=0.91) between control and HD sheep post-clockwise rotation (Figure 5A, B). For the anticlockwise rotation, there was also no significant difference in oscillations frequency (t=-1.05, d.f.= 18, p=0.31) nor

for the total number of oscillations (t=0.78, d.f.= 18, p=0.45) between control and HD sheep (Figure 5C, 5D). There was also no significant difference in the differential value between rotations for the first 10 sec. oscillation frequency (t=-1.15, d.f.= 18, p=0.27) nor for the total number of oscillations (t=-0.55, d.f.= 18, p=0.59) between control and HD sheep (Figure 5E, 5F). Also for the PRN test, there was no significant difference in the values between genotypes post-clockwise or post-anticlockwise rotation for mean oscillation eye velocity (Mann Whitney U =30; p=0.143; Mann Whitney U =31; p=0.165), mean oscillation duration (Mann Whitney U =59; p=0.529; Mann Whitney U =51; p=1.00). There was, however, a significant difference between genotypes for the mean oscillation gain during the VOR (rotation) part of the test for both the clockwise (Mann Whitney U =76; p=0.046)(Figure 6A) and anticlockwise (Mann Whitney U =91; p=0.01)(Figure 6B) rotation.

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Figures 5 and 6 near here

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4.0 Discussion

Eye movement abnormalities are among the earliest manifestations of HD and can be measured and quantified in human patients using specialized equipment, typically an oculometer. The purpose-built sheep oculometers used in this study was able to capture both eye velocity and eye movement data from sheep, allowing a comparison between normal and HD sheep. Saccade velocity profiles were typically asymmetrical with a longer duration of the

descending (deceleration) portion of the saccade than of the ascending. Human saccade velocities tend to be symmetrical in nature [33] but become similarly asymmetrical with increasing amplitude, which is considered to be optimal in reducing motor noise [34]. The asymmetrical nature of normal sheep saccades is interesting in this respect and deserves future study, particularly in the context of previous work that has identified specific relationships between saccade velocity, amplitude and duration for different animal species [35, 36].

Differences in oculomotor control were observed between the control and the HD sheep. During the PRN (static) phase of the test in light conditions, HD sheep initially showed fewer nystagmus oscillations than control sheep after the clockwise rotation, but then a greater number of oscillations after the anti-clockwise rotation. It is not clear why the changes were only in one direction. This may be due to an order effect since we did not randomize the rotational direction. The vestibulo-ocular system is heavily modulated by the cerebellum and the parietal cortex [37] and known to acclimatize after repeated exposure to rotational movement [38]. Thus, the reduced initial response during first rotation followed by the reduced acclimatisation during the static phase of rotation in HD sheep, suggests disease-related neurophysiological alteration of these brain systems. The mechanism underlying this change, however, is unknown.

The total number of nystagmus oscillations observed during the static phase of the light PRN test was substantially less than when it was carried out in dark conditions. This is likely to be due the increased visual input of the visible static scene. There was no significant difference between genotypes for the amount of nystagmus during the static phase of the light test, suggesting that the overriding effect of the visual static scene was equally modulating for both

genotypes. The velocity and duration of eye movements post-rotation (clockwise and anticlockwise) were also not significantly different between genotypes. Previous work has shown that human HD patients have a slower and restricted range of eye movement during volitional saccades [39] which theoretically would translate to differences in both velocity and duration of eye movement during a VOR nystagmus event. The lack of difference observed within this study potentially reflects the differences in underlying circuitry controlling eye movements during volitional saccades versus the VOR [40], The latter is potentially less affected by early-stage HD pathology [9] and thus may reflect the earlier stage of disease in the sheep ovine model compared to when eye movements become clinically relevant for human HD patients [13, 15]. There was, however, a significant increase in the gain eye movement during the rotational element of the light but not the dark VOR test. Previous work in human HD patients has reported a reduction in gain in the slow component of the VOR and in the slow and rapid component of OKN (see Rub et al., 2009, for review), thus, the results for the HD sheep appear contrary to expectation given the human literature. However, other human studies have also reported that the characteristics of the VOR response remains intact until the latter stages of HD pathology [5] . The significant increase in gain during the light rotation in the HD sheep is difficult in explain in either of these contexts but does suggest the existence of some form of neuropathology.

There are several limitations of this study. First, we did not randomise the direction of rotation, therefore it cannot be determined if the differences we saw were due to direction of rotation or to a desensitisation (the latter being more likely in our opinion). Second, we worked to a fixed schedule in terms of timing. If the responses are affected by the time between tests,

we would not have detected this. Third, we only used a single rotational speed. Different rotational speeds may exacerbate (or reduce) the difference between genotypes. Future studies should explore the timing between the tests and include randomisation of the test direction as well as different speeds. Future studies would be better done using a rotational apparatus to control speed, rather than the manual rotation method used here. Finally, a major advantage of the sheep oculomotor system is that the tasks are non-invasive and the responses are reflexive and so repeated testing can be easily conducted. Longitudinal testing in this study was not possible because the project had come to an end. Future studies could be cross-sectional (using different cohorts of younger and older sheep) or longitudinal, using same group over a relevant period of time (that is probably several years, given the slow progression of the disease). A longitudinal study within a closer time period would also be useful in determining the within-animal variability.

Oculomotor signs have been largely overlooked as a biomarker of HD. Given that they occur relatively early in disease and are easy to measure in humans, they have a strong potential to act as a metric for both disease onset and progression, as well as therapeutic efficacy [27, 28, 41, 42, 43, 44, 45]. This study, therefore, opens up possibilities for studying control of eye movements in HD sheep as a valid biomarker of disease progression. In addition, monitoring eye movement during cognitive paradigms that require impulse control may elicit additional phenotypic differences between control and HD sheep given previous work in humans [46] . Since sheep can be trained to perform in impulse control paradigms [47] this may be a useful additional avenue of future research.

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5.0 Conclusion

We set about determining whether it was possible to quantify eye movements in untrained sheep without head fixation and to then use oculomotor measurements to compare control of eye movements in normal and HD sheep. Through the use of highly innovative oculometer device specifically designed and manufactured for use in sheep, in a simple test paradigm (rotation in dark and light environments) we identified two measures that could distinguish normal from HD sheep at a premanifest stage. These were the number of post-rotational nystagmus oscillations within the dark environment and the relative eye-head movement (gain) during rotation (VOR) within the light environment. Future research will assess whether these differences change over time and if they can be used as a biomarker to track disease progression in this large animal model of HD.

6.0 Acknowledgments

We would like to acknowledge Professor Roger Carpenter for his advice and guidance on development of the ovine oculometer methodology. This work was funded by a grant from CHDI *Inc*.

7.0 Conflict of Interest

Sebastian D. McBride, William Schneider and A. Jennifer Morton have no conflict of interest. Jan Ober was Director of Ober Consulting and Jacek Dylack was an employee of Ober Consulting which provided the oculometer equipment to facilitate the collection of data.

8.0 Datasets/Data Availability Statement

The data supporting the findings of this study are available on request from the corresponding author.

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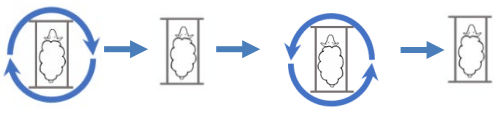
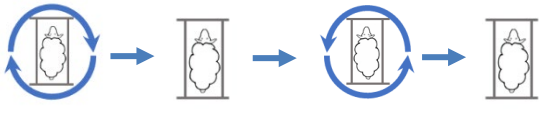
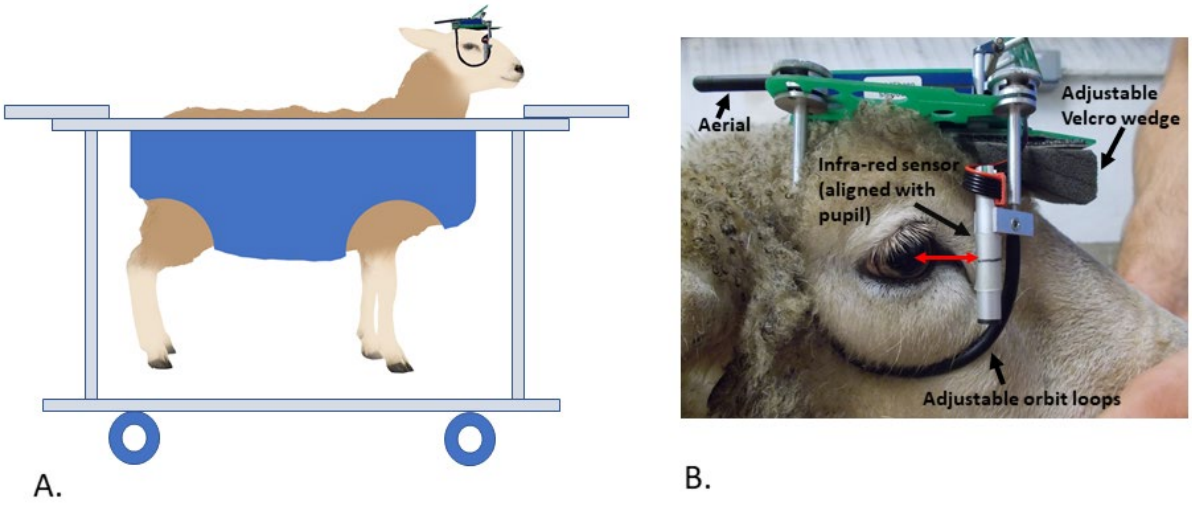
Table 1. Definitions of different types of eye movement

Eye Movement	Definition
Saccade	Jerk-like movements of the eyes that abruptly change the point of fixation. They can be small in amplitude, for example the movements made whilst reading, or large, for example when looking out of a moving vehicle. Saccades occur reflexively whenever the eyes are open.
Vestibulo-ocular reflex (VOR)	Motor response of eyes to move in the opposite direction to a translational or rotational movement of our head. Eye movement is due to activation of the vestibular system.
The optokinetic reflex (OKR)	Occurs when the movement of the large visual field (optokinetic stimulation) induces the eyeball to turn in the same direction as the image motion. Together, VOR and OKR keep the image stationary on the retina with VOR compensating for fast movements and OKR for slower ones.
Nystagmus	Rhythmic eye movements with a slow eye movement that drives the eye off the target followed by a second rapid movement that brings the eye back to the target. Optokinetic nystagmus is the eye movement elicited by the tracking of a moving field

Table 2. Timeline and consecutive testing stages for all sheep.

Test period	**Day 1 (0 lux)**				**Day 2 (250 lux)**			
(light level)	**Stage 1 →**	**Stage 2 →**	**Stage 3 →**	**Stage 4**	**Stage 1 →**	**Stage 2 →**	**Stage 3 →**	**Stage 4**
Measure	VOR	PRN	VOR	PRN	VOR	PRN	VOR	PRN
Direction of rotation	Clockwise	none	Anti-clock wise	none	Clockwise	none	Anti-clock wise	none
Duration (s)	60	60	60	60	60	60	60	60
								

VOR = vestibulo-ocular reflex
PRN= post-rotational nystagmus



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486 **Figure 1. The sheep oculometer.** A cartoon of a sheep held suspended in a veterinary sling

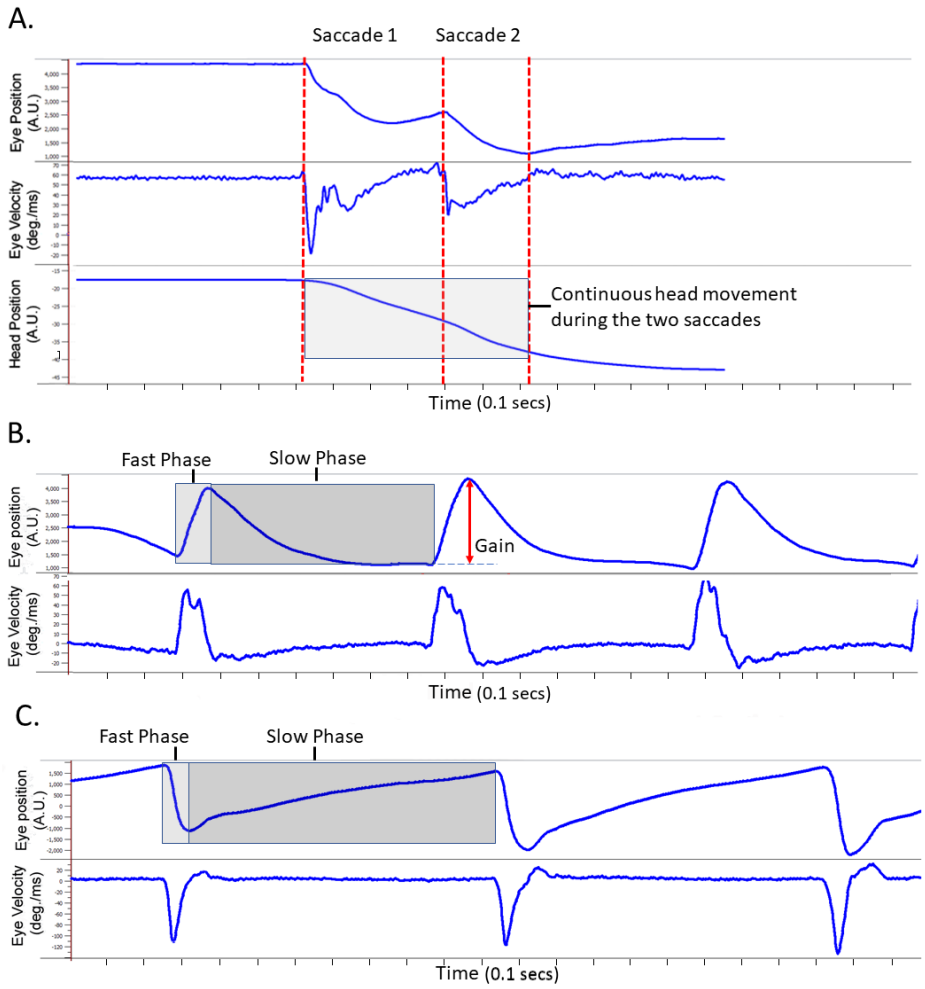
487 wearing the sheep oculometer (A). Photograph of a sheep wearing the head-mounted

488 oculometer (B). The device is held in place by two metal loops that surround the eye and are

489 linked by a movable plastic frame that can be adjusted for different sized sheep. An infrared

490 sensor (arrow) is aligned with the centre of each eye. The distance from the eye (↔) is fixed. -

491



492

493 **Figure 2 Typical examples of sheep eye saccades.**

494 In Panel A, the top, middle and lower traces show the relative eye position, eye velocity and the
495 head position respectively during two typical normal (non-test) eye and head movements. The
496 two sequential eye movement (1 and 2) bring the eye to the final resting position during
497 concurrent movement of the head (see supplementary video 1). Panel B shows the relative eye
498 position and eye velocity vestibular ocular reflex during rotational (clockwise) phase of the test.
499 The arrow indicates the measur5e of gain (eye movement in relation to a constant head
500 movement). Panel C shows relative eye position and eye velocity during post-rotational
501 nystagmus static phase of the same test.

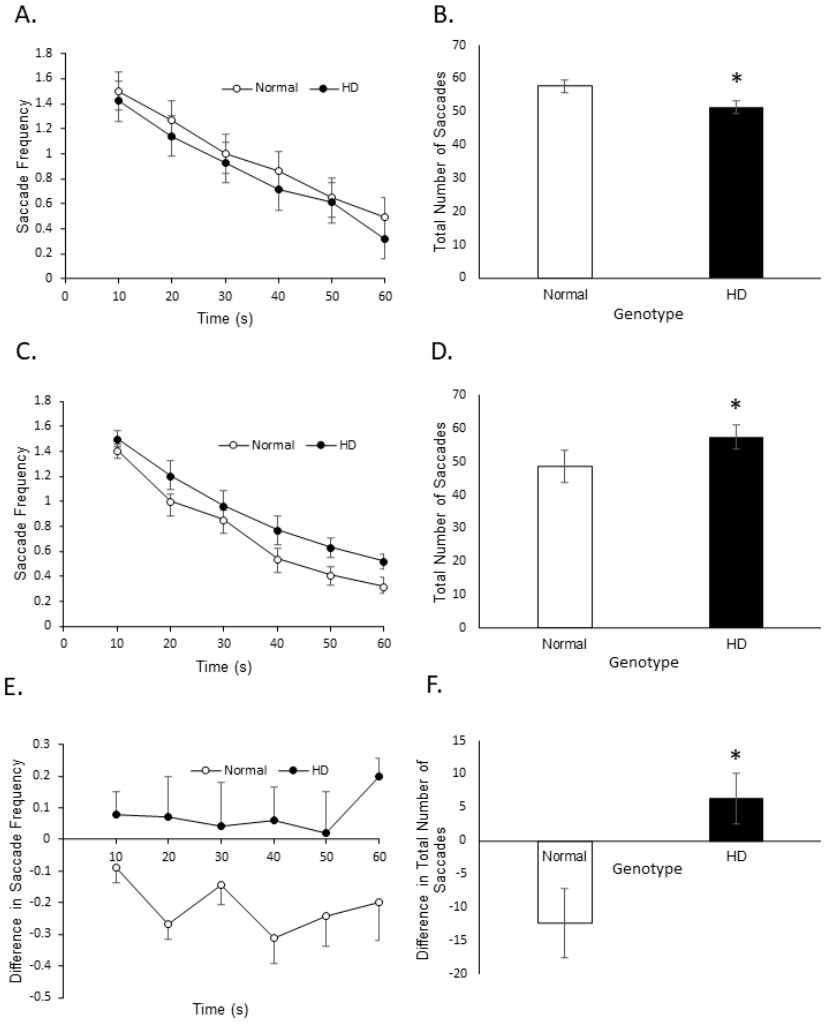
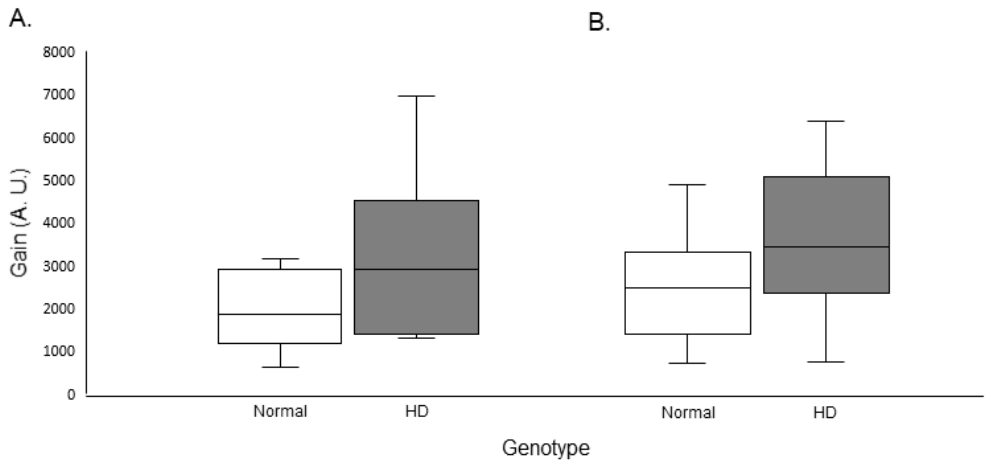


Figure 3. Normal and Huntington's disease sheep oculomotor responses during the post-rotational nystagmus test in the dark. The mean (±SEM) number of oscillations/ 10 s and total number of oscillations during the post-rotation phase during the clockwise (A, B) and anticlockwise (C, D) rotation phase for normal (open symbols and columns) and HD (closed symbols and columns) sheep during the PRN test. The mean (±SEM) difference in oscillation frequency (per 10s) (E) and total number of oscillations (F) between the clockwise and anticlockwise rotations during the post-rotation phase for normal and HD sheep during the PRN test.



511

512 **Figure 4. Eye-head gain values during the VOR test in dark conditions**. Data are median (with
513 interquartile range) gain during the rotation phase during the clockwise (A) and anticlockwise
514 (B) rotations for normal (open columns) and HD (closed columns) sheep during the VOR test in
515 the dark conditions.

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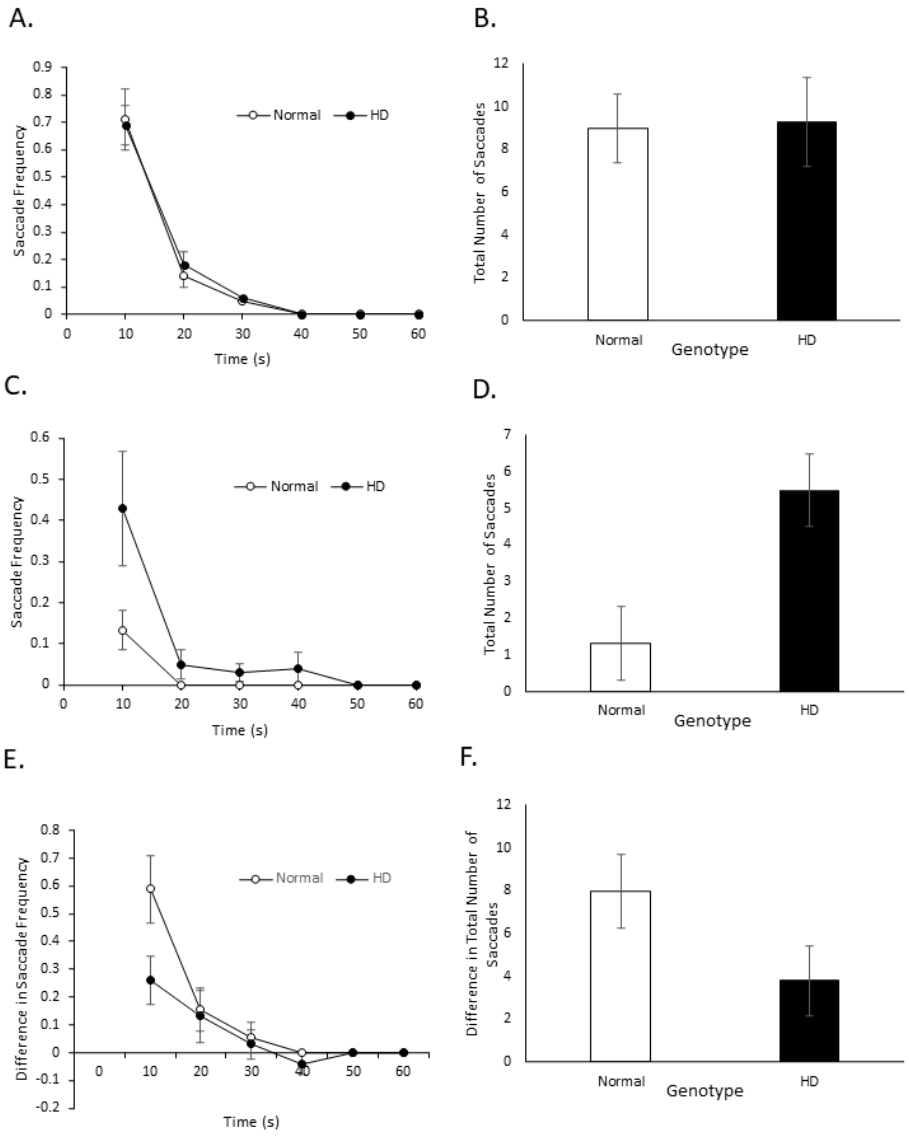

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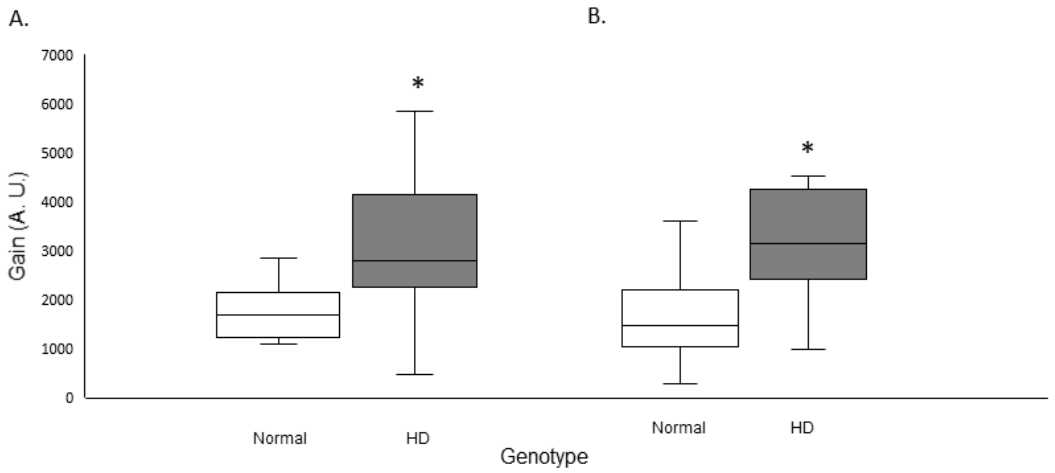
518 **Figure 5 Normal and Huntington's disease sheep oculomotor responses during the post-**
519 **rotational nystagmus test in the light.** The mean (±SEM) oscillation number/ 10 s and total
520 number of oscillations during the post-rotation phase during the clockwise (A, B) and
521 anticlockwise (C, D) rotation phase for normal (open symbols and columns) and HD (closed
522 symbols and columns) sheep during the PRN test. The mean (±SEM) difference in oscillation
523 frequency (per 10s) (E) and total number of oscillations (F) between the clockwise and
524 anticlockwise rotations during the post-rotation phase for normal and HD sheep during the PRN
525 test.



526

527 **Figure 6 Eye-head gain values during the VOR test in light conditions.** The median (with
528 interquartile range) gain during the rotation phase during the clockwise (6A++) and
529 anticlockwise (6B) rotations for normal (open columns) and HD (closed columns) sheep during
530 the vestibulo-ocular reflex test in light conditions (* denoted significant difference ($p<0.05$)
531 between normal and HD sheep.

532