Visual evoked potentials in the horse

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Abstract

Vision is an important sense for horses, both for survival in the wild and when horses are used for work, sport or recreation. However, it is often difficult to diagnose visual impairment in this species. Traditional techniques available in clinical equine practice, are based on subjective evaluations, and their results are many times difficult to interpret. Electrodiagnostic methods, flash electroretinography (FERG) and recording of flash visual evoked potentials (FVEP) are used to objectively evaluate the function of the retinal and post-retinal visual pathways. The electrical potentials generated in response to brief visual stimuli are measured non-invasively. Abnormal function in visual pathways can affect the FERG and FVEP waveforms, peak times and amplitudes. Lesions can thereby be detected, and their approximate localization evaluated. FVEPs are used in human medicine, and occasionally in animal species, but have not been described in the horse. The general aim of this thesis was to establish a technique for recording of FVEPs in horses in clinical practice. The results showed that FVEPs can be readily recorded in sedated horses in a clinical setting. The recorded waveform consisted of a series of positive (P1-P5) and negative (N1-N2) wavelets. The overall appearance of the waveform was shown to be similar in foals, young horses and adult horses. An age-related effect on peak times and amplitudes was observed, but most of the changes occurred early in life. Important data on FVEP variability and repeatability was reported, and it was concluded that P2, N2 and P4 peak times should be included in the evaluation of equine, clinical FVEPs. The large inherent variability of FVEP amplitudes made them less useful, but they occasionally provided support to a clinical diagnosis. In clinical patients, electrodiagnostic testing helped assessing functional impact of potentially visual-threatening diseases. By recording FERGs and FVEPs simultaneously, a subdivision into retinal vs post-retinal dysfunction could be made in many patients, such as horses with optic neuropathies and cortical visual impairment. FVEPs may also be of prognostic value in horses with traumatic optic neuropathy and possibly in cases with cortical visual impairment. The results from this thesis, opens up for the use of the FVEP as an adjunctive, objective method in the evaluation of equine patients with suspected visual impairment and neurological disease, but also for studies of development and function of the visual pathways in this species.

Keywords: visual evoked potential, VEP, electroretinogram, ERG, horse, vision, visual impairment, blindness, retina, optic nerve, visual cortex

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Abstract

Synen är ett viktigt sinne för hästen, både för överlevnad i det vilda och när hästar används för arbete, sport eller rekreation. Det är ofta svårt att diagnostisera synnedsättningar hos häst. De tekniker som används i klinisk verksamhet i dagsläget, baseras på subjektiv utvärdering, och resultaten från dessa undersökningar är ofta svåra att tolka. Elektrodiagnostiska metoder, elektroretinografi (flash-ERG, FERG) och visuella retningspotentialer (flash-VEP, FVEP) kan användas för att objektivt utvärdera funktionen i retina och post-retinala synbanor. De elektriska potentialer som genereras som svar på ljusblixtar registreras icke-invasivt med elektroder. Nedsatt funktion i synbanorna kan påverka latenstider och amplituder i den vågform som registreras. Lesioner kan därmed detekteras och ungefärlig lokalisation fastställas. FVEP används inom humansjukvården, och ibland även på djur, men har inte beskrivits hos djurslaget häst. Övergripande syfte med denna avhandling var att utveckla en teknik för registrering av FVEP hos häst i klinisk verksamhet. Resultaten visade att FVEP kan registreras på häst under klinikförhållanden. Vågformen bestod av en serie av positiva (P1-P5) och negativa (N1-N2) toppar och dalar. Vågformens utseende var i huvudsak lika mellan föl, unga samt vuxna hästar. En åldersrelaterad effekt observerades för latenstider och amplituder, främst under de första åren i livet. Metodens variabilitet och repeterbarhet utvärderades, och latenstiderna för P2, N2 och P4 visades vara användbara vid utvärdering av hästens FVEP. Amplituderna var mer variabla, även om de kunde bidra med kliniskt relevant information vid vissa diagnoser. Elektrodiagnostik visades ge värdefull information vid utvärdering av synnedsättning hos häst. Genom att registrera FERG och FVEP samtidigt, kunde retinal dysfunktion skiljas från post-retinala problem hos många patienter, till exempel vid traumatisk optikusneuropati och vid kortikal påverkan. Resultaten från detta arbete möjliggör för användning av FVEP som en objektiv utvärderingsmetod, tillsammans med övriga undersökningar av hästpatienter med synnedsättningar och/eller neurologisk sjukdom, samt även för användning vid studier av synbanornas normala funktion och utveckling hos detta djurslag.

Keywords: visual evoked potential, VEP, elektroretinogram, ERG, häst, syn, synnedsättning, blindhet, retina, synnerv, synkortex

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To my family

Peter, Gustav, Marcus, Erik och min pappa Sören

"*An imperfect apprehension of the sensory world of animals usually prevents our being able to speak confidently of what the 'vision' of a given species is like. On the other hand, familiarity with the structure and physiological reactions of its eyes, together with an understanding of its behavior and requirements can, perhaps, enable us to make not too foolish a guess.*"

Dr Katherine Tansley in *Vision in Vertebrates*, 1965

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Ström L* & Ekesten B (2016). Visual evoked potentials in the horse. *BMC Veterinary Research,* 12:120.

II Ström L*, Michanek M, Ekesten B (2019). Age-associated changes in the equine flash visual evoked potential. *Veterinary Ophthalmology,* 22 (4):388-397.

III Ström L*, Bröjer J, Ekesten B. Variability, repeatability and test-retest reliability in equine flash visual evoked potentials. Submitted manuscript.

IV Ström L*, Källberg M.E, Nostell K, Ekesten B. Electrophysiological assessment in horses with visual impairment. In manuscript.

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The contribution of Lena Ström to the papers included in this thesis was as follows:

I Participation in the design of the study, acquisition, analysis and interpretation of data, drafting the article, critical revision of the article.

II Participation in the design of the study, acquisition, analysis and interpretation of data, drafting the article, critical revision of the article.

III Participation in the design of the study, acquisition, statistical analysis and interpretation of data, drafting the article, critical revision of the article.

IV Participation in the design of the study, acquisition, analysis and interpretation of data, drafting the article, critical revision of the article.

Abbreviations

BHL: bullet-hole lesion

CI: confidence interval

CR: coefficient of repeatability

CV: coefficient of variation

ERG: electroretinogram/electroretinography

FERG: flash electroretinogram/electroretinography

FVEP: flash visual evoked potential

ICC: intraclass correlation coefficient

IOP: intraocular pressure

ISCEV: International Society of Clinical Electrophysiology of Vision

LOA: limits of agreement

OD: right eye

ON: optic nerve

ONH: optic nerve head

OS: left eye

OU: both eyes

PLR: pupillary light reflex

PVEP: pattern visual evoked potential

RGC: retinal ganglion cell

SD: standard deviation

VEP: visual evoked potential

1 Introduction

1.1 Vision

Vision is an important sense for many animal species, and is considered to be one of the most important factors to trigger evolutionary changes (Nilsson, 2013; Darwin, 1859). The complex process of visual perception has taken millions of years to develop, and adaptations of the eye and visual pathways in different species have evolved to suit each ecological niche. In the intricate interplay between prey species and predators, a well-adapted vision provides advantages for survival and reproduction in a hostile environment.

Visual perception can be described as the ability to perceive and interpret the surroundings using light. The simplest form is the ability in some bacteria to perceive light by using photosensitive pigments (Schuergers *et al.*, 2016; Fishman, 2008). In higher species, vision has evolved to a complex process with eyes, with specific adaptations in different species, connected to the central nervous system through visual pathways (Nilsson, 2013).

In the sophisticated process of visual perception, light reflected by the environment is refracted through the ocular media, reaches the retinal photoreceptors and the light energy is converted to electrical potentials. The potentials generated are processed and transmitted within the neuroretina, and sent further through the optic nerve, optic chiasm, optic tract and optic radiation to the visual cortex and other cortical areas, for processing and interpretation of the visual stimulus (Ofri, 2013). Input from the eyes is also sent through reflex pathways to coordinate pupil size, head, neck and eye movements, in response to visual stimuli (de Lahunta, 2015), and also to control circadian rhythm (Berson *et al.*, 2002; Hattar *et al.*, 2002).

1.2 Vision in the horse

1.2.1 Ocular adaptations

The horse is a prey species and as such, well-adapted vision at all hours has been important, to avoid predators and survive in the wild.

Being a species active both day and night, their eyes and visual pathways have adapted to maintain adequate performance over a wide range of light intensities. The eye of the horse is one of the largest among terrestrial mammals (Duke-Elder, 1958), which is beneficial for several reasons. The pupil is large, horizontally oriented, and has a large dynamic range which mediates good capacity to control the inlet of photons, depending on the light conditions (Banks *et al.*, 2015; Davis *et al.*, 2003).

When constricted, the elongated, horizontal pupil efficiently reduce the retinal illumination in bright daylight, while maintaining a wide visual field. The *corpora nigra (granula iridica)*, outgrowths of iris tissue on the rim of the pupil (Duke-Elder, 1958), function as a shield and provide extra protection from bright light (Banks *et al.*, 2015). They are also thought to enhance depth perception in bright light, by dividing the pupil into two segments when constricted (Murphy & Howland, 1986). To reduce glare in bright daylight, the lens contains yellow pigment (Miller & Murphy, 2016). The yellow pigment also filters out UV-light, and thus protects from photo-oxidation and damage to intraocular structures (Wolffsohn *et al.*, 2000; Zigman & Paxhia, 1988).

In dim light, the horse has been shown to be able to make visual discriminations and negotiate the environment at very low light-intensities, and they outperform humans by far (Hanggi & Ingersoll, 2009). The large equine eye and large pupil, is able to admit much light also at low light-intensities, which is beneficial for preserving visual resolution (Warrant, 1999). The *tapetum lucidum*, a reflective layer beneath the retina, improves retinal sensitivity by reflecting light, and thus provide a second chance for the photoreceptors to catch non-absorbed photons, which also improves vision in dim light (Shinozaki *et al.*, 2013; Ollivier *et al.*, 2004).

The horizontally oriented pupil and laterally placed eyes provide a wide field of view, which is also beneficial for a prey species. When combining the visual fields from both eyes, the total horizontal visual field in a horse is up to 350 degrees, i.e. almost a full circle with only a few minor blind areas (Harman *et al.*, 1999). A consequence of the laterally placed eyes is a limited binocular visual field, which is a disadvantage for depth perception. However, the horse has been shown to also be able to use monocular visual cues to estimate depth (Timney & Keil, 1999; Timney & Keil, 1996).

1.2.2 Visual pathways

After refraction through ocular media, the light reaches the retina, where the photon energy is converted to electrical potentials, and is thereafter carried further along post-retinal visual pathways to the visual cortex in the brain, for further processing and interpretation (Fig 1.).

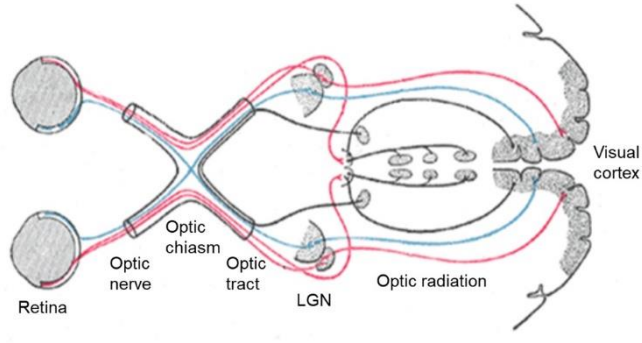

Figure 1. The visual pathways. Modified from Grey, 1918.

The equine retina is divided into ten layers histologically (Fig. 2). The outermost layer, is the supportive retinal pigment epithelium (RPE). The neurosensory retina is formed by nine layers; the photoreceptor layer (PL), outer limiting membrane (OLM), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GC), nerve fiber layer (NFL) and the inner limiting membrane (INL). The major cell types found in the neuroretina are photoreceptors, bipolar cells, horizontal cells, amacrine cells, Müllers cells and retinal ganglion cells (RGCs) (Ehrenhofer *et al.*, 2002; Guo & Sugita, 2002).

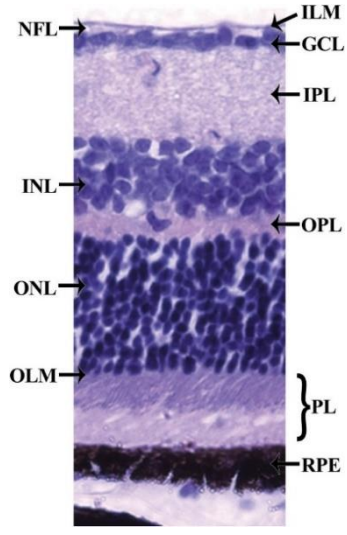

Figure 2. Histology of the retina. The retina can be divided into 10 layers including: the inner limiting membrane (ILM); the nerve fiber layer (NFL); the ganglion cell layer (GCL); the inner plexiform layer (IPL); the inner nuclear layer (INL); the outer plexiform layer (OPL); the outer nuclear layer (ONL); the outer limiting membrane (OLM); the photoreceptor layer (PL), and the retinal pigmented epithelium (RPE) monolayer. The image is oriented from the inner (vitreal) surface (upwards), to the outer (choroid and scleral) surface (downwards).
Available from: https://www.researchgate.net/figure/Histology-of-the-retina-The-retina-can-be-divided-into-10-layers-including-1-the-inner_fig2_262941023 [accessed 30 Jul, 2019].

The first-order neurons in the visual pathway are the photoreceptors, rods and cones. These convert light energy to electrical potentials through a series of biochemical reactions, the phototransduction cascade (Arshavsky *et al.*, 2002). Cones function mainly in bright light, and mediate color vision and sharp visual acuity, whereas rods function in dim light, to detect shape and motion. The horse has a rod-dominated retina, with approximately 95% rod and 5% cone photoreceptors (Wouters & De Moor, 1979). They have two types of cones, mediating dichromatic color vision in the blue-green-yellow spectrum (Carroll *et al.*, 2001).

The second-order neurons include the bipolar and horizontal cells, located within the internal nuclear layer. The bipolar cells connect and send their signals further, to the third-order neurons, whereas the horizontal cells are interconnecting neurons that regulate responses of both photoreceptors and bipolar cells, to increase sensitivity to changes in illumination and color. The amacrine cells, also located mainly in the inner nuclear layer, are interneurons that increase the RGC sensitivity to changes in illumination, and thereby enhance detection of motion, direction and contrast. The RGCs transmit the signals further through their axons, forming bundles in the optic nerve, into the post-retinal pathways. A subgroup of RGCs, the intrinsically photosensitive RGCs (ipRGCs), contain a photosensitive pigment (Hattar *et al.*, 2002). These cells have been shown to contribute to regulation of pupillary light reflexes, circadian behavior and seasonal reproductive rhythm (Markwell *et al.*, 2010; Freedman *et al.*, 1999).

Müller glial cells span the entire thickness of the retina. They modulate neuronal activity, and have important metabolic and supportive functions needed to maintain retinal physiology (Newman & Reichenbach, 1996).

The visual streak is a well-defined, horizontal band in the tapetal area (approximately 2-3 mm dorsal to the optic disc), spanning the whole width of the equine retina, with increased densities of photoreceptors and RGCs for high resolution and maximum visual acuity (Evans & McGreevy, 2007; Ehrenhofer *et al.*, 2002; Guo & Sugita, 2002; Hebel, 1976). In the temporal arm of the visual streak, a weak area centralis has also been described (Guo & Sugita, 2002; Harman *et al.*, 1999; Hebel, 1976). Behavioral, histopathological and electrophysiological studies have shown that the horse has relatively good visual acuity, approximately half that of humans or even better (Timney & Macuda, 2001; Harman *et al.*, 1999; Ver Hoeve *et al.*, 1999; Timney & Keil, 1992). All layers in the equine retina are thinner in the peripheral compared to central areas, and photoreceptor and RGC densities are lower (Evans & McGreevy, 2007; Ehrenhofer *et al.*, 2002; Guo & Sugita, 2002; Harman *et al.*, 1999; Hebel, 1976). Therefore, the ability to produce detailed images in the periphery is limited, although motion and shapes can still be detected.

The axons of the RGCs in the NFL lack myelin to maintain retinal transparency, and myelination of the optic nerve starts at the level of the scleral canal in horses (de Lahunta *et al.*, 2015; Guo *et al.*, 2001). The optic nerve is a white matter tract of the CNS, and consists of about 481 000 – 543 000 myelinated RGC axons (Guo *et al.*, 2001). RGC axons enter the skull of the horse through the optic canals, located in the presphenoid bone, to come together in the optic chiasm. The optic canals are of a considerable length in the horse compared to other species (de Lahunta *et al.*, 2015). About 80% of the axons in the optic nerve decussate at the optic chiasm, and 20% continue on the ipsilateral side (Herron *et al.*, 1978; Duke-Elder, 1958). The optic chiasm is located on the floor of the rostral cranial fossa (presphenoid bone) and rostral to the pituitary gland. After the chiasm, the visual pathway continues as the optic tract and the majority of the optic tract axons synapse in the lateral geniculate nucleus (LGN) in the thalamus (de Lahunta *et al.*, 2015; Karamanlidis & Magras, 1974). Some fibers, including ipRGC axons, leave the optic tract before the LGN, to relay input to the pretectal nucleus, rostral colliculus and suprachiasmatic nucleus (de Lahunta *et al.*, 2015; Karamanlidis & Magras, 1974). The axons from the cell bodies in the LGN, project further into the internal capsule and form the optic radiation, until they synapse in the primary visual cortex, situated at the lateral, caudal and medial aspect of the occipital lobe (Strom & Ekesten, 2016; Takeuchi & Sugita, 2001). In the cortex, the visual input is further processed in several

cortical areas. A disturbance at any location along the visual pathways or in the brain, may result in a visual impairment.

In summary, from the evolutionary perspective, vision has been an important sense for horses. Therefore, they have many specific adaptations of their eyes and visual pathways. Although the threat from predators no longer remains, at least for most horses, vision is still important. Today, many horses are expected to perform tasks where adequate vision is needed, such as when used for work, sport and recreation. Sufficient vision is also important for safety reasons, as handling and riding a large animal with impaired vision may pose a risk for the human, as well as for the horse itself.

1.3 Evaluation of visual impairment in the equine patient

Traditionally, taking the owner's history, testing of vision and performing an ophthalmic examination are procedures used in the evaluation of the visually impaired equine patient. A thorough history, including owner-opinion on performance in bright and dim light conditions, and in familiar and unfamiliar environments, provide important clues for further evaluation. Testing of vision should include neuro-ophthalmic examination, falling cotton-ball testing, and evaluation of performance in an obstacle course. An ophthalmic examination is also performed, to detect and subjectively quantify abnormal findings.

1.3.1 Neuro-ophthalmic examination

The neuro-ophthalmic examination can provide guidance to the site of a lesion causing visual impairment (VI) (de Lahunta *et al.*, 2015; Mayhew, 2010; De Lahunta & Cummings, 1967). Firstly, behavior, mentation and overall posture is assessed from a distance, as well as the function of various cranial nerves important for vision, such as coordination of head, pupils, eye position and movement. Thereafter, evaluation of the dazzle reflex, direct and indirect pupillary light reflexes (PLRs) and the menace response follow. Although non-visual, the palpebral reflex is also assessed, to make sure that a blink response is mediated through the facial nerve when touching the medial and lateral canthus.

Both the dazzle and PLRs are subcortical reflexes induced by sudden retinal illumination. The sensory pathway for the dazzle reflex is through the optic nerve to the rostral colliculus. Input is propagated further to facial nuclei in the medulla oblongata, and the motor pathway is through the facial nerve. The afferent of the PLRs is the optic nerve, sending signals to the pretectal olivary nucleus and further to the Edinger-Westphal nucleus (both ipsi- and contralateral to the stimulus). The efferent signal passes through the oculomotor nerves, and

pupillary contraction is elicited in both eyes (direct and indirect), although only stimulating unilaterally. Being subcortical reflexes, both the dazzle and PLRs test retinal and optic nerve function, but they may still be present in a blind patient.

The menace response is elicited by applying a non-tactile, visual threat to the medial and temporal visual fields of each eye, looking for eyelid closure, and possibly also an evading reaction indicating vision. The response should be evaluated in both bright and dim light conditions. It requires an intact sensory pathway, from the retina and subsequent visual pathways to the visual cortex, but also an intact motor pathway, via the frontal cortex, pons and cerebellum, through the facial nuclei in the medulla and lastly through the facial nerve. However, the menace response is estimated to require a visual acuity of only 20/20 000, approximately 1000 times worse than normal eyesight (Holladay, 1997). Thus, it is still possible to show a positive menace response, although being severely VI. In addition, because the menace response is a cortically mediated response, it can also be suppressed in excited, stressed, agitated or depressed horses. Thus, an absent, weak or intermittent response may not always be due to a VI.

The menace response is an acquired response, and is not developed fully in the foal until about 2-3 weeks of age (Enzerink, 1998; Adams & Mayhew, 1984), whereas the palpebral reflex, dazzle and PLRs are present already at birth (Adams & Mayhew, 1984).

1.3.2 Falling cotton-ball test and obstacle course evaluation

The falling cotton-ball test is performed by letting thin strands or balls of cotton fall in front of each eye, without touching the vibrissae or making noise (Featherstone & Heinrich, 2013). If normal, the horse is expected look at and follow the stimuli, but results from this test are inconsistent, also in horses with normal vision.

Obstacle course testing is performed by letting the horse negotiate a maze or pass over obstacles. The examiner subjectively assesses poor performance such as stumbling, bumping into obstacles repeatedly, or refusal to move. This test is difficult to employ in large ungulates, such as the horse (Cutler, 2002). If VI or blindness is present, it can be hazardous to employ this test in such a large species, both for the horse and the handler. If deemed safe, unilateral evaluation should be performed by covering each eye in turn. The test only provides a crude assessment of vision, and it is difficult to detect partial visual impairments.

1.3.3 Ophthalmic examination

Ophthalmic examination is performed using slit-lamp biomicroscopy. Presence of potential congenital abnormalities, as well as signs of previous or ongoing disease is evaluated. Tonometry to assess intraocular pressures is also performed, and the fundus is evaluated using indirect and direct ophthalmoscopy. The examiner eventually makes a subjective assessment of the impact of abnormal findings on vision.

The techniques described above are traditionally used to evaluate vision in horses. However, most of them are based on subjective evaluations and are many times difficult to interpret in the horse. In addition to the overall clinical assessment, it would thus be valuable to be able to use adjunctive, objective methods to evaluate vision in this species, such as electrophysiological methods.

1.4 Visual impairment in horses

Understanding equine vision is important for the equine practitioner. Clinicians may be asked to assess whether a suspected VI is the cause for undesirable behavior such as shying or startling, or to estimate the visual capabilities of a horse, for example at pre-purchase examination (Lavach, 1992). In clinical patients, assessment of abnormal findings, their localization and their potential impact on vision is expected. Furthermore, the clinician should also be able to evaluate prognosis, and preferably choose the correct treatment to maximize future visual performance, which is not always an easy task.

A number of diseases that may cause reduced vision or blindness can affect horses. Equine recurrent uveitis (ERU) has been reported to be one of the most common causes of blindness (Gerding & Gilger, 2016; Spiess, 1997; Dwyer *et al.*, 1995). Estimations of the prevalence of this disease vary between 8-25% (Gilger, 2010) and 8-10% (Spiess, 2010). Many of these horses suffer from VI, although not all of them will become blind.

An increasing number of reports describe the overall prevalence of ophthalmic lesions in horses (Andrysikova *et al.*, 2019; Malalana *et al.*, 2019; Paschalis-Trela *et al.*, 2017; Rushton *et al.*, 2013; Scantlebury *et al.*, 2013; Ireland *et al.*, 2012; Thangadurai *et al.*, 2010; Hurn & Turner, 2006). However, not all these authors report on the prevalence of VI in their material, and assessment of visual capabilities has been performed differently. Chandler et al. described a prevalence of 8.4% (7/83) in geriatric horses based on owner-reported VI (Chandler *et al.*, 2003). Hurn & Turner reported on potential vision-threatening disease in 7.4% (15/204) of racing horses, based on ophthalmic examination (Hurn & Turner, 2006), and VI was confirmed using menace

response and obstacle course testing in four of these horses, who also had a history of behavioral problems. Malalana et al. described impaired vision in 5.5% of examined horses, as defined by a reduced menace response at veterinary examination (Malalana *et al.*, 2019). They also performed an owner-survey, and found a prevalence of 1.1% (11/974) of owner-reported diminished vision. In a population of army horses in India, a prevalence of VI of 17% was found based on results from neuro-ophthalmic examination, including menace response testing (Thangadurai *et al.*, 2010).

The numbers in these studies must be considered to be more or less gross estimations. In some studies, proper testing of vision was not performed, which makes results unreliable and difficult to assess. In addition, menace response and obstacle course testing (used in some studies) are subjective tests and interpretation is often difficult. Overall, this highlights the need for standardized protocols and additional, objective methods for evaluation of visual abilities in this species.

1.5 Electrophysiology

1.5.1 History

In 1865, Swedish physiologist Fritiof Holmgren found that light stimulus could elicit electrical potentials in the amphibian eye (Holmgren, 1865). Similar findings were seen by other scientists thereafter (Dewar & McKendrick, 1873). In 1880, Holmgren found that the retina was the source of the response (Holmgren, 1880). After years of work studying the electroretinogram (ERG), Ragnar Granit published a detailed study on its ingoing components in 1947 (Granit, 1947). Even today, his description remains the basis for our understanding of the components of the ERG, although with some modifications.

In 1929, Hans Berger described the spontaneous activity in the brain, the electroencephalogram (EEG) (Berger, 1929). Shortly thereafter, in 1934, it was reported that electrical potentials generated in response to flash stimuli, could be recorded from the occipital cortex, the visual evoked potentials (VEPs) (Adrian & Matthews, 1934). Dawson presented a superimposition technique based on signal averaging, to extract the VEP waveform from the EEG (Dawson, 1954) and subsequently, Cigánek described the waveform morphology of the human VEP (Ciganek, 1961). In the years following, further work was performed to explore the human VEP, improve the technique and implement recording of VEPs as a clinical, diagnostic aid.

1.5.2 Clinical use

Abnormal function can affect the waveform, peak times and amplitudes of the ERG and/or the VEP. Thus, lesions can be detected, their functional impact assessed, and their approximate localization can be evaluated.

ERGs are widely used to study retinal function for research purposes, and to evaluate various retinal disorders worldwide (Creel, 2019a; McCulloch *et al.*, 2015). VEPs assess the functional integrity of post-retinal pathways, including the optic nerve, optic tract and optic radiation to the visual cortex (Creel, 2019b; Odom *et al.*, 2016). In human medicine, VEPs are used to evaluate visual impairment due to several pathological conditions, mainly optic neuropathies, such as optic neuritis, multiple sclerosis and optic atrophy, but there are also other indications such as in the evaluation of cortical function as a part of the neurologic work-up of a patient (Creel, 2019b; Young *et al.*, 2012; Walsh *et al.*, 2005).

A discrimination between retinal and post-retinal dysfunction can often be made using ERGs and VEPs simultaneously. The methods allow functional assessment, while imaging techniques, such as magnetic resonance imaging (MRI), computer tomography (CT) and optical coherence tomography (OCT), are mainly used to obtain information about structural integrity in eyes and visual pathways.

1.5.3 Electrodiagnostic testing in medicine

The International Society for Clinical Electrophysiology of Vision (ISCEV) regularly updates the human standard protocols for clinical electrophysiological examinations (McCulloch *et al.*, 2015; Odom *et al.*, 2010). Guidelines are also issued by the American Clinical Neurophysiology Society (American Clinical Neurophysiology, 2006).

An active electrode at the corneal surface is normally used to record ERGs. Full-field flash stimulation (FERG) is mostly used, but there are also other techniques, such as multifocal-ERGs (mf-ERGs) and pattern-ERGs (PERGs) to evaluate the effect of focal lesions and RGC function.

The main components in the FERG waveform are the a-, b- and c-waves, but there are also other known components (Creel, 2019a). The first part of the a-wave represents photoreceptor activity, the b-wave mainly represents activity in bipolar cells, and the c-wave (which requires special recording equipment not used in this thesis) is known to originate in the retinal pigment epithelium. There are also oscillatory potentials (thought to represent activity in amacrine cells) and other minor waves in the ERG that can be observed and evaluated.

VEPs are usually recorded non-invasively using skin electrodes on the scalp overlying the visual cortex. In routine testing, the VEP scalp electrodes are placed according to the International 10/20 system (Jasper, 1958). Midline electrodes over the visual cortex are used to assess prechiasmal function, while additional lateral electrode positions are used to detect asymmetries in chiasmal and postchiasmal dysfunction. Usually one eye at a time is stimulated by flashes of white light (flash-VEP; FVEP) or a reversing, isoluminant pattern (pattern-VEP; PVEP). In human subjects, PVEPs are preferred in most clinical situations due to less variability than the FVEPs. PVEPs will also enable a better quantitative assessment of visual function. However, when using a pattern stimulus it is critical that the subject focuses on the pattern. Therefore, the FVEP can still be more useful in infants and patients with poor cooperation, poor fixation or poor acuity (Creel, 2019b; Odom *et al.*, 2010).

1.5.4 ERGs and VEPs in animals

Flash stimulation is usually preferred when ERGs and VEPs are employed in veterinary medicine, because animals rarely cooperate sufficiently to ensure focus and fixation on the pattern.

In small animal ophthalmology, FERGs are widely used to assess retinal function and disease (Ofri, 2002), both for research purposes and in clinical patients with visual impairment, and there are clinical guidelines for diagnostic FERGs in dogs (Ekesten *et al.*, 2013).

Techniques for recording light-adapted and scotopic FERGs in sedated, healthy horses have also been reported (Ben-Shlomo *et al.*, 2012; Church & Norman, 2012; Komaromy *et al.*, 2003). Diagnostic FERGs in horses have so far mainly been performed to evaluate retinal function in congenital stationary night blindness (CSNB), a hereditary disease affecting rod-function in leopard-spotted horses (Sandmeyer *et al.*, 2012; Sandmeyer *et al.*, 2007; Nunnery *et al.*, 2005; Witzel *et al.*, 1978). However, a few reports on FERGs from horses with diseases such as neuroaxonal dystrophy/equine degenerative myeloencephalopathy (NAD/EDM) (Finno *et al.*, 2012), ivermectin-induced blindness (Pollio *et al.*, 2018), and multiple fundus bullet hole lesions (Allbaugh *et al.*, 2014) have also been published.

Although not as widely used in veterinary medicine as FERGs, FVEPs have been studied in a large number of our domestic species including the cat, dog, cow, sheep and pig (Strain *et al.*, 2006; Padnick & Linsenmeier, 1999; Strain *et al.*, 1991a; Sims *et al.*, 1989; Strain, 1989; Strain *et al.*, 1986b; Mattsson *et al.*, 1978; Creel *et al.*, 1973). Not surprisingly, FVEP amplitudes and peak times have been shown to differ between these species, due to anatomical and

physiological reasons. The FVEP in horses has so far only been very briefly described (Brooks, 1999).

Using FVEPs, it has also been shown that there are substantial differences in maturation of the visual system after birth between species. In many species, including cats (Rose *et al.*, 1972) and dogs (Kimotsuki *et al.*, 2006; Strain *et al.*, 1991b; Myslivecek, 1968), the FVEP has been shown to change considerably during the first weeks and months of life, whereas in other species, such as pigs, cows and sheep (Strain *et al.*, 2006; Strain *et al.*, 1989; Woods *et al.*, 1981), the FVEP is more adult-like at birth, and only minor changes occur during the postnatal period. In dogs, one report also showed that although the overall waveform morphology is unaltered during the adult life-span, some peak times of the canine VEP increase with age, and some amplitudes are gradually reduced (Kimotsuki *et al.*, 2006), similar to what is also seen in humans (Allison *et al.*, 1984; Dustman & Beck, 1969).

FVEPs has mostly been studied in normal animals and there are only a few reports on the use of FVEPs in clinical patients within veterinary ophthalmology. However, Strain et al. reported on scrapie, thiamine-responsive polioencephalomalacia (PEM), suspected listeriosis, and abscessation in the thalamus and cerebral cortex in ruminants (Strain *et al.*, 1990a; Strain *et al.*, 1987; Strain *et al.*, 1986a), and showed that abnormal findings could be detected in these conditions.

2 Aims

The general aim of this thesis was to establish a method for recording of flash visual evoked potentials (FVEPs) in horses in clinical practice. The hypothesis was that FVEPs could provide clinically important objective information about the function of visual pathways, and aid in the differentiation between retinal and post-retinal dysfunction, and thereby help localizing disease causing visual impairment in this species.

The specific aims were;

- To describe a technique for recording FVEPs from normal horses in a clinical setting.

- To define the normal FVEP waveform and evaluate if the recorded response is of post-retinal origin.

- To describe the characteristics of FVEPs with increasing age in horses, and thus to assess whether age-matched normal data are required when evaluating clinical patients.

- To establish normal ranges for FVEP parameters to enable detection of abnormal results in patients and follow-up of individual patients. This included determination of the variability, repeatability and test-retest reliability of FVEPs in normal horses.

- To evaluate if the FVEP, in combination with the FERG, can provide useful clinically useful information, such as differentiation of retinal and post-retinal dysfunction, in equine patients with confirmed or suspected visual impairment of different etiologies.

3 Materials and methods

This section provides a summary on materials and methods used in the separate papers (I - IV) included in this thesis. More detailed descriptions are presented in each of the papers.

3.1 Horses

All studies included in this thesis were approved by the Local Ethical Committee in Uppsala, Sweden. The experiments were carried out following national and institutional guidelines for care and use of animals in research.

The adult horses used in papers I-III, were owned by the Department of Clinical Sciences, SLU, Uppsala, Sweden. The healthy foals and young horses (<3 years) studied in paper II were privately owned. Informed owner consent was obtained. Only one specific breed (Standardbreds) was evaluated in the studies of normal, healthy horses within this thesis (I-III). In paper IV, client-owned horses of different breeds were prospectively recruited at the ophthalmology unit in the Equine clinic at the University Animal Hospital UDS, Swedish University of Agricultural Sciences (SLU). Informed owner consent was obtained. The number of horses included in each of the studies are summarized in table 1.

Table 1. *Total number of horses included in each study (I-IV). The number of horses shown within brackets were those also included in previous studies.*

I	II	III	IV
10	*(10)*	*(10)*	-
	28	*(7)*	-
		17	-
			30

As inclusion criteria for papers I-III, horses had to be clinically healthy, without signs of ocular or neurological disease.

Inclusion criteria for clinical cases with suspected or confirmed visual impairment (paper IV) were; history of presumed visual impairment, abnormal menace response, poor performance in an obstacle course and/or abnormal findings at ophthalmic examination with potential to cause visual impairment. Four main groups were constructed based on the cause of the visual impairment; "Cataracts", "Retinal Disorders", "Optic Neuropathies" and "Cortical Visual Impairment" (CVI). Each group was then divided into subgroups depending on the more precise neuroanatomical site of the lesion. All horses were assigned to a specific subgroup according to specific inclusion criteria for each group.

3.2 Methods of examinations

Physical examinations were performed on all horses. Examinations including falling cotton-ball testing, neuro-ophthalmic examination (menace response testing under both room and dim-light conditions, pupillary light reflexes, dazzle reflexes, palpebral reflexes), slit-lamp biomicroscopy, direct and indirect ophthalmoscopy and rebound tonometry, was performed in all horses.

Prior to electrodiagnostic testing, horses were evaluated in a simple obstacle course under room-light conditions to assess vision. Performance in the obstacle course was considered abnormal (poor), if the horse bumped into the obstacles repeatedly at several passages, or if the horse refused to move through the course. In clinical cases (paper IV), additional tests and diagnostic examinations, such as orthopedic evaluation, neurological examination, blood and

cerebrospinal fluid analyses, endoscopy and diagnostic imaging were also performed if required for the clinical work-up.

3.3 Sedation and preparations

Adult horses were sedated with an intravenous bolus injection of detomidine, 0.01 mg/kg (Domosedan vet., 10 mg/ml, Orion Pharma Animal Health, Sollentuna, Sweden). Maintenance of sedation was achieved throughout the recording sessions using a continuous intravenous infusion of 2% detomidine in physiologic saline solution (Natriumklorid, 9 mg/ml, Fresenius Kabi, Uppsala, Sweden), sufficient to ensure the horse rested its head steadily on a padded headstand (papers I-IV). The dose of sedation was reduced if deemed necessary to establish the appropriate level of sedation in clinical patients (paper IV). Newborn foals were sedated by a 0.4 mg/kg bolus dose of xylazine (Narcoxyl vet., 20 mg/ml, Intervet AB, Sollentuna) intravenously (paper II). In one clinical case with severe neurological signs (paper IV), short-acting intravenous anesthesia was used, following an established protocol (Davidson, 2008) with a combination of xylazine (Rompun® 20 mg/ml, Intervet, Stockholm, Sweden), diazepam (Stesolid® novum, 5 mg/ml, Teva, Helsingborg, Sweden) and ketamine (Ketaminol 100 mg/ml, Bayer Animal Healt, Copenhagen, Denmark) (paper IV). The recordings under both sedation and anesthesia, including recovery, were uneventful in all horses.

Pupils were dilated with tropicamide (Mydriacyl, 0.5%, Novartis, Stockholm, Sweden), and were checked before and during recordings to ensure maximum dilation was achieved and maintained. Akinesia and analgesia of the eyelids and electrode positions were performed to avoid muscle artifacts (Carbocain, 20 mg/ml, AstraZeneca, Södertälje, Sweden), and topical corneal anesthesia was applied (Tetrakain, 1 %, Bausch & Lomb Nordic AB, Stockholm, Sweden). Corneas were kept moist during recordings with repeated instillations of artificial tears (ZilkEye, Evolan Pharma AB, Stockholm, Sweden or Comfort Shield SD, i.com medical GmbH, München, Germany). Cotton wads were placed in the ear canals to reduce auditory stimuli that could influence the level of consciousness.

3.4 Electrophysiological recordings

All recordings, apart from those of newborn foals that were performed at their home stable, were performed in a clinical examination room in the Equine Clinic at the Swedish University of Agricultural Sciences. The examination rooms were kept dark during recordings to avoid stray light.

The human standard for electrophysiological FVEP testing (Odom et al., 2010) by the International Society for Clinical Electrophysiology of Vision (ISCEV) was used as a guideline, when deciding on FVEP settings and procedures.

Light-adapted FERGs and FVEPs were recorded simultaneously. A handheld, dome-shaped, full-field light photo stimulator (Retiport mini-Ganzfeld, an-vision, GmbH, Hennigsdorf, Germany) with a background light intensity of 25 cd/m^2 and a flash intensity of 3 cd/m^2/s was used to light-adapt and stimulate the retina. The stimuli were presented at a frequency of 1.09 Hz. The sweep duration was 500 ms post-stimulus. Responses were amplified, band-pass filtered (0.1-300 Hz for FERGs and 1-100 Hz for FVEPs), stored and analyzed using the RETIport ERG (an-vision, GmbH, Hennigsdorf, Germany) and a laptop computer.

If reduced vision in dim light was suspected, scotopic FERGs were performed, according to the ECVO clinical guidelines for FERGs in dogs (Ekesten et al., 2013) and a previous publication in horses (Ben-Shlomo et al., 2012) (paper IV).

Electrode impedance was kept below 5 kΩ in all FERG recordings, and below 2 kΩ in all FVEP recordings. A minimum of three (papers II-IV) to five (paper I) reproducible replicates were obtained from each eye examined.

3.4.1 FERGs

FERGs were recorded using corneal electrodes (Gold-foil corneal electrodes, CH electronics, Bromley, UK). Metal corkscrew electrodes (Stainless Steel Disposable Corkscrew Electrode, Cephalon A/S, Nørresundby, Denmark) served as reference and ground electrodes, and were placed approximately 3 cm caudal to the lateral canthus and at the forehead of the scalp respectively.

3.4.2 FVEPs

Paper I

Electrode positions were essentially selected based on a modification of the International 10-20 system (Jasper, 1958) for humans. The electrodes were placed at distances relative to bony landmarks on the equine head to adjust for head size (Fig. 3). Metal corkscrew electrodes (Stainless Steel Disposable Corkscrew Electrode, Cephalon A/S, Nørresundby, Denmark) served as active, ground and reference electrodes.

Five electrode positions in the midline (ten horses) starting at the nuchal crest, moving rostrally ($P_{z\text{-}0}$ to $P_{z\text{-}60}$), and two to four lateral positions were evaluated (P_1 to P_4) (five horses) (Fig. 3). Reference and ground electrode positions are shown in figure 3. FVEPs were recorded during uniocular stimulation of left and right eyes respectively, for comparison between eyes (four horses). FVEPs were recorded at two separate sessions (five horses) to assess reproducibility. The minimum number of responses averaged to obtain a waveform with clearly distinguishable wavelets was evaluated (4, 16, 32, 64, 100, 144 and 196 averaged responses) (three horses). Unilateral retrobulbar nerve blocks (two horses) (Gilger & Davidson, 2002), and transection of the optic nerve was performed in a terminal procedure (one horse), to evaluate potential contamination from FERG potentials in the recorded FVEP waveform.

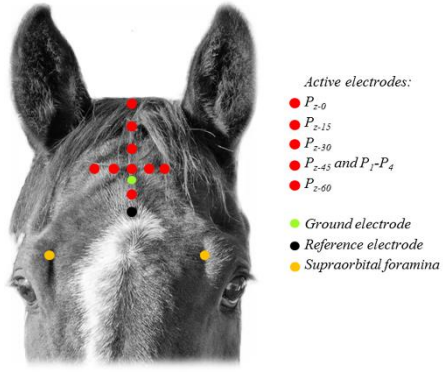

Figure 3. Electrode positions evaluated in Strom & Ekesten (Strom & Ekesten, 2016).

Papers II-IV

Recordings were performed as described in paper I, using the FVEP electrode position at $P_{z\text{-}45}$. The minimum number of responses averaged in each FVEP replicate was ≥100 (100 to 144). Recordings driven from one randomly selected eye were obtained from all horses in paper II. In paper III, unilateral recordings driven from both left and right eyes were obtained in eight horses, and from one randomly selected eye in nine horses. Unilateral recordings from left and right eyes were obtained from most clinical cases, except from three cases with symmetric bullet-hole lesions (cases 7-9), where only one randomly selected eye was stimulated (paper IV). FVEPs were recorded at two separate sessions (2-11 months apart, median 7.5 months) in six horses (paper III), and at two separate sessions, separated by 6 months, in one clinical case (paper IV).

A summary of the procedures performed in each of the studies are presented in table 2.

Table 2. *An overview of the procedures performed in paper I-IV. Numerals represent the number of horses evaluated. All electrode positions in the midline ($P_{z\text{-}0}$ to $P_{z\text{-}60}$) were evaluated in all ten horses, whereas two to four lateral position (P_1 to P_4) were evaluated in only five horses (paper I). ON = optic nerve.*

Study	*Total number of horses*	*One eye*	*Both eyes*	*Two sessions*	*Averaging evaluated*	*Retrobulbar nerve block*	*Transection ON*	*Active electrode positions*
I	10	6	4	5	3	2	1	$P_{z\text{-}0}$, $P_{z\text{-}15}$, $P_{z\text{-}30}$, $P_{z\text{-}45}$, $P_{z\text{-}60}$, P_1, P_3, P_2, P_4
II	28	28	-	-	-	-	-	$P_{z\text{-}45}$
III	17	9	8	6	-	-	-	$P_{z\text{-}45}$
IV	30	3	27	1	-	-	-	$P_{z\text{-}45}$

3.5 Evaluation of FERGs and FVEPs

FERG a- and b-wave amplitudes and peak times were evaluated according to established guidelines (Ekesten *et al.*, 2013; Komaromy *et al.*, 2003) (papers I-IV). The wavelets in the recorded equine FVEP waveform were identified and named according to the nomenclature by Harding (Harding, 1974), a classification that was later also used in veterinary neurophysiology (Strain *et al.*, 1986b). The same examiner (LS) performed all marking of tracings (studies I-IV). In paper II and IV, only peak times and amplitudes for wavelets P2, N2 and P4 were included in the evaluation, since results from papers I and III showed that these wavelets were most consistent and robust.

3.6 Statistical analyses

P-values ≤0.05 were considered statistically significant (papers I-III). Statistical analyses were performed with a statistical software, JMP® Pro 11.0.0 (papers I-II) and JMP® Pro 14.0.0 (study III).

3.6.1 Paper I

Descriptive data; means, standard deviations (SDs) and ranges were reported for all identified FVEP wavelet amplitudes (μV) and peak times (ms). A non-parametric test was used for analysis of data (Kruskal-Wallis one-way analysis of variance by ranks). Parameters were tested for homogeneity of variance using Levene´s test. Bonferroni correction was used to adjust the p-values when multiple comparisons were performed. Results from recordings before and after retrobulbar nerve block (horse I-J) and transection of the optic nerve (horse I) were compared to normal data.

3.6.2 Paper II

Dunn´s method for joint ranking with adults as control was used to compare FVEP results between three age groups; newborn foals (aged 36 to 44 hours), young (aged 6 to 12 months) and adult horses (aged 3 to 28 years).

Regression analyses were used for statistical evaluation of the relationship between FVEP peak times and amplitudes vs age. Non-linear regression models were fitted to the amplitude and peak time data of foals and horses ≤3 years of age (table 2 and figure 4-5 in paper II). Linear regression analysis was performed to evaluate data from horses during the adult life-span (≥3 years of age, table 3 and figure 6-7 in paper II).

3.6.3 Paper III

Data were assessed for normality by visual examination of residual plots prior to analysis. Parameters were tested for homogeneity of variance using Levene´s test. To assess the intra- and inter-variability of the three replicates for each parameter (peak times and amplitudes), a nested mixed-model with random effects was used, and coefficients of variation were calculated.

Only peak times and amplitudes of wavelets observed in 100% of the recordings were used for the remainder of the analyses. An average of the three recorded FVEP replicates was used as the estimate of each parameter.

A paired t-test was used to compare results between eyes (eight horses) and between sessions (six horses). The variability and repeatability for measured parameters between eyes, as well as between sessions were determined by the coefficient of variation (CV) and coefficient of repeatability (CR) (Bartlett & Frost, 2008; Bland, 2000), whereas ICC was used to evaluate test-retest reliability between sessions (Bartlett & Frost, 2008). The guidelines by Cicchetti (Cicchetti, 1994) were used for interpretation of ICC reliability: poor: < 0.40; fair: 0.40 – 0.59; good: 0.60 – 0.74; excellent; 0.75 – 1.00.

To assess and graphically present repeatability between sessions, Bland-Altman plots including 95% CI of upper and lower limits of agreement were also constructed based on measurements from recordings at two separate sessions (Bland, 2006; Bland & Altman, 1986).

3.6.4 Paper IV

Overall waveform morphology was assessed by comparing responses to the typical equine FERG and FVEP waveforms previously described (paper I). The FVEP waveform appearance was judged either as normal or abnormal, where abnormal was defined as a waveform where major landmarks such as the P2, N2 and P4 were not easily discriminated, and/or had biphasic and/or clearly, abnormally broadened peaks. Peak times and amplitudes were measured as described in study I, and an average of the tree replicates was calculated to use as one measurement for each parameter. Wavelets P2, N2 and P4 were included in the evaluation of the FVEP, because these wavelets were previously determined to be most consistent and robust (papers I and III). Waveform morphology, peak times and amplitudes were compared to age-matched groups; young horses <3y and adult horses ≥3y, according to results from paper III. Values for peak times and amplitudes falling outside of the normal range were considered abnormal.

4 Results

This section summarizes the results from the separate papers included in the thesis. More detailed descriptions of the results are presented in each paper.

No findings indicating visual impairment, ocular or neurological disease were observed in the normal horses included in the studies (papers I-III). All clinical cases fulfilled one or more of the inclusion criteria for paper IV.

4.1 The normal Equine FVEP (I)

Light-adapted FERGs and FVEPs were readily recorded from all ten, sedated horses. The waveform of the equine FVEP consisted of a series of positive (P1 to P5) and negative wavelets (N1 to N2) (Fig. 4). Only wavelets N1, P2, N2 and P4 were found in all recordings in all horses.

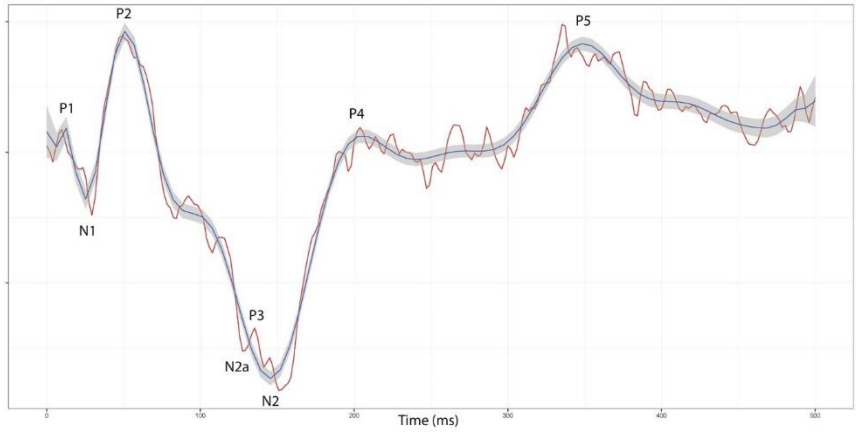

Figure 4. The equine FVEP waveform described in Strom & Ekesten (Strom & Ekesten, 2016). Vertical division = 5 µV, horizontal division = 50 ms.

No significant differences in peak times or amplitudes were found when comparing recordings between sessions ($p>0.05$). There were no significant differences in peak times or amplitudes regardless of eye stimulated.

The waveform morphology did not differ between the electrode positions in the midline ($P_{z\text{-}0}$, $P_{z\text{-}15}$, $P_{z\text{-}30}$, $P_{z\text{-}45}$ and $P_{z\text{-}60}$) (Fig. 3), but the FVEP waveform was somewhat difficult to evaluate at the most caudal positions ($P_{z\text{-}0}$ and $P_{z\text{-}15}$). Peak times did not differ significantly between electrode positions in the midline. However, amplitudes reached maximum at the positions $P_{z\text{-}30}$ and $P_{z\text{-}45}$, and there were significant differences when comparing amplitudes from these two positions to recordings at the most caudal ($P_{z\text{-}0}$) and rostral ($P_{z\text{-}60}$) positions ($p<0.001$).

At lateral positions (P_1 to P_4), both amplitudes and peak times showed a greater variability compared to recordings in the midline (and were more difficult to mark) but there were no significant differences between means of peak times and amplitudes compared to midline positions.

Peak times and amplitudes from tracings with 32, 64, 100, 144 and 196 averaged responses did not differ statistically. However, by averaging no less than 100 responses, the background noise was markedly reduced, which resulted in more easily discernible peaks and reduced number of peaks split by large artifacts.

After retrobulbar nerve block, only a minuscule remnant of P2 was discernible, despite that FERG a- and b-wave peak times were within normal limits and b-wave amplitudes were only marginally reduced. The FVEP was clearly visible again approximately 90 minutes after the nerve block, although peak times and amplitudes were not yet within normal limits. The FVEP disappeared completely when the optic nerve was transected.

4.2 Age-associated changes in the equine FVEP (II)

Reproducible light-adapted FVEPs and FERGs were readily recorded from all subjects. Overall, the FVEP waveform was similar in foals, young and adult horses. Although the shape of the waveform was similar, it was found that amplitudes and most peak times differed significantly between the newborn foal and adult horse.

When performing statistical analysis to compare age-groups (foal, young and adult horses respectively), it was observed that amplitudes for all peaks were significantly higher in newborn foals compared to recordings from adult horses, and also in young horses compared to adult horses. Peak times for N2 and P4 were significantly shorter in newborn foals compared to adult horses. P2 mean

peak time was also shorter comparing newborn and adults, however not statistically significant (p=0.07). Comparing young and adult horses, N2 peak time was significantly longer in young horses, whereas P2 and P4 peak times did not differ significantly.

When fitting non-linear models for data including horses from all age-groups (0-28 years), coefficients of determination were found to be low, which was also obvious from visual inspection of plotted data. Therefore, the material was instead divided into horses ≤3 years and adult horses (≥3 years) to achieve a better fit for models. This is unfortunately not clearly described in paper II, and there is an error in the text. The coefficients of determination for the non-linear analysis including data from all horses was written in the text, with a reference to table 2 and figure 4-5 in paper II, However, these tables and figures show the result from a non-linear regression analysis including only horses ≤3 years, with a better fitted model. This non-linear regression analysis showed that all peak times increased and amplitudes decreased significantly during the observed time-span between newborn to three years of age.

When excluding horses <3 years old, and fitting linear models to the data from adult horses, there was a significant, positive correlation between age and peak time for the P4 wavelet, although the coefficient of correlation was very low, at less than 0.1. Hence, age appears to have a negligible effect on VEP amplitudes and peak times in horses older than three years in our material. However, it should be noticed that we had only few horses older than 20 years of age in our study.

FERG mean amplitudes and implicit times for both a- and b-waves were similar to those reported in other equine studies (Ben-Shlomo *et al.*, 2012; Komaromy *et al.*, 2003).

4.3 Variability, repeatability and test-retest reliability of the equine FVEP (III)

Intra- and inter-individual variability for the three replicates for parameters present in all recordings and all horses were determined. N1, P2, N2 and P4 wavelets were present in 100 % of recordings in all horses, while the remaining peaks (P1, N2a, P3 and P5) were only present in some recordings. In general, intra- and inter-individual CV values were lower for peak times (for P2, N2 and P4: 6-7% and 5-11% respectively) compared to amplitudes (for N1P2, P2N2 and N2P4: 19-30% and 37-64% respectively).

The FVEP waveforms were similar between eyes and there were no statistically significant differences between measurements from right and left eyes (p>0.33). Coefficients of variation (CVs) between right and left eyes were

low for peak times (3-5%) but higher for amplitudes (11-28%). The coefficients of repeatability (CRs) between eyes for each parameter were computed (P2: 5 ms; N2: 18 ms; P4: 18 ms; N1P2. 2.0 µV, P2N2: 1.7 µV; N2P4: 2.3 µV).

FVEP waveforms were similar between recording sessions and there were no statistically significant differences between first and second parameter measurements ($p>0.49$). Coefficients of variation between sessions were low for peak times (3-6%) but higher for amplitudes (24-30%). Coefficients of repeatability between sessions for each parameter were computed (P2: 5 ms; N2: 16 ms; P4: 39 ms; N1P2: 5.1 µV, P2N2: 5.7 µV; N2P4: 6.1 µV). Intraclass correlation coefficients (ICCs), as estimates of test-retest reliability, were assessed to range from fair (N2 peak time), good (P4 peak time, N1P2 and N2P4 amplitudes) to excellent (P2 peak time and P2N2 amplitude).

4.4 Electrodiagnostic findings in horses with visual impairment (IV)

Thirty horses met one or more of the study inclusion criteria. Horses were classified into the four main groups; cataracts, retinal disorders, optic neuropathies and cortical visual impairment (CVI), and then further into subgroups based on the more precise neuroanatomical location of the lesion.

Complete cataracts were diagnosed in two adult horses (two eyes), and four foals (six eyes). In this group, normal light-adapted FERGs were recorded from all eyes, although supranormal amplitudes were observed in two cataractous eyes. Normal FVEPs (normal waveform, peak times and amplitudes) were recorded from three of the horses (one adult, two foals). In one foal, the overall FVEP waveform seemed immature when stimulating the cataractous eye, similar to what was described in newborn foals in paper II. An atypical, although reproducible, FVEP waveform was observed in one foal with bilateral cataracts. Abnormal N2 and P4 wavelets were observed in one horse with a unilateral cataract caused by trauma, indicating post-retinal dysfunction.

Retinal disorders were diagnosed in ten horses (14 eyes). Diagnoses represented in this group were moderate to large numbers of bullet-hole lesions (five eyes), chronic multifocal chorioretinitis (four eyes), generalized retinal degeneration (three eyes) and retinal detachments (two eyes). Light-adapted FERGs were abnormal or extinguished in most affected eyes. FVEP responses driven from all eyes with affected or extinguished FERGs, were subsequently also abnormal or non-detectable. However, in eyes with only moderate numbers of bullet-hole lesions in the non-tapetal fundus, FERGs and FVEPs were normal and functional deficits could not be detected with full-field flash stimulation.

Optic neuropathies were diagnosed in eight horses (12 eyes). Diagnoses represented in this group were glaucoma (three eyes), space-occupying lesions (four eyes), optic atrophy (three eyes) and optic neuritis (two eyes). In eyes with elevated intraocular pressures (glaucomas and also eyes with space-occupying lesions compressing optic nerves and globes), light-adapted FERG b-wave amplitudes were mildly reduced in some cases, when compared to the unaffected fellow eye. However, FVEPs were abnormal, with profoundly delayed P2 wavelets (glaucoma) or completely extinguished responses (compressing lesion). In eyes with optic atrophy, light-adapted FERGs were normal (or with supranormal amplitudes), but FVEPs were extinguished. One horse, mainly assigned to the "CVI" group because of bilateral VI and neurological signs after head trauma, was also diagnosed with right-sided traumatic optic neuropathy, due to a pre-chiasmal fracture. In this case, light-adapted FERGs were normal, whereas FVEPs were non-detectable when stimulating the right eye, and ophthalmoscopic signs of optic atrophy were observed at follow-up examination, six months later. In another case, with signs of acute optic neuritis in one eye after head trauma, light-adapted FERG amplitudes were supranormal. The FVEP waveform was normal, however an apparent reduction of amplitudes was observed. The other eye was diagnosed with optic atrophy ophthalmoscopically, and FVEPs were non-detectable. Overall in this group, light-adapted FERGs were mostly normal (or only mildly affected) whereas FVEPs were profoundly abnormal or non-detectable.

Bilateral VI with acute onset was diagnosed in seven horses with signs of neurological disease of cortical/brainstem origin (CVI). Etiologies and/or diagnoses represented in this group included head trauma, hepatic encephalopathy, parasitic meningitis, severe systemic inflammation (including peritonitis, uveitis and meningitis) and one horse with a cortical and/or brainstem/cerebellar lesion without known etiology. Light-adapted FERGs were mostly normal in this group (only affected in two horses with concurrent uveitis). FVEP waveforms were normal in four horses that later regained vision (head trauma, parasitic meningitis, meningitis). FVEPs were severely affected in three horses (hepatic encephalopathy, meningitis, cortical and brainstem/cerebellar lesion) all of which were eventually euthanized because of clinical deterioration despite treatment, or persistence of neurological signs.

5 General discussion

Vision is important sense for both wild and domesticized horses. It is needed for adequate performance, and normal vision is also required for welfare and safety reasons in this large species. Equine practitioners and ophthalmologists are expected to assess and diagnose a suspected visual impairment. However, with traditional techniques, testing of vision has for the most part been a subjective assessment so far, and objective methods to evaluate the function of visual pathways, such as FERGs and FVEPs, have not been frequently used in equine clinical practice.

In this thesis, a technique to record FVEPs has been adapted for use in horses in a clinical setting (paper I), and the characteristics of the normal equine FVEP in young and adult horses has been described (papers I-II). The variability, repeatability and test-retest properties of the equine FVEP has been assessed, to evaluate the limitations of the technique, and to produce normative data (paper III). Furthermore, the technique has been employed in a case series of equine patients with known or suspected visual impairment, to evaluate its usefulness in equine clinical practice (paper IV).

Electrodiagnostic testing, using FERGs and FVEPs simultaneously, was shown to provide diagnostic and prognostic information in some conditions causing visual impairment in horses. The results from this thesis, opens up for the use of FVEP as an adjunctive, objective method in the evaluation of equine patients with suspected visual impairment and neurological disease, but also for studies of development and function of the visual pathways in this species.

5.1 The normal equine FVEP waveform (I)

FVEPs were readily recorded in sedated horses in a clinical setting, and the waveform was described. As in other mammalian species, the adult equine FVEP waveform consisted of a series of positive and negative wavelets, and

although similarities between species can be observed, peak times and amplitudes differ somewhat, likely due to anatomical and physiological differences. An interspecies comparison of peak times in the FVEP is displayed in table 3.

Table 3. *Interspecies comparison of FVEP peak times (ms). Based on tables from Mattsson et al. (Mattsson et al., 1978) and Strain et al. (Strain et al., 1990b). 1) Electrode positions, based on approximations of the International 10-20 System (Jasper, 1958); O_1, left occipital; F_1, left frontal; F_{pz}, midline frontal; O_z, midline occipital: O_3, left occipital, lateral to O_1; F_3, left frontal, lateral to F_1; P_1, left parietal; P_{z-45}, midline parietal, F_4, right frontal, mirror of F_3; A_1A_2, linked ears. 2) Sources of data: a) Creel et al., (Creel et al., 1973), b) Sims & Laratta, (Sims & Laratta, 1988), c) Fox, (Fox, 1968), d) Howard & Breazile, (Howard & Breazile, 1972), e) Mattsson et al., (Mattsson et al., 1978), f) Strain et al., (Strain et al., 1990b), g) Bichsel et al., (Bichsel et al., 1988) h) Sims et al., (Sims et al., 1989), i) Strain et al., (Strain et al., 1986b), j) Strain et al.,(Strain et al., 1991a), k) Ström et al., (Strom et al., 2019), l) Kraut et al., (Kraut et al., 1985).*

	Electrodes[1)]		**Wavelets**							
Species[2)]	**Positive**	**Negative**	**N**	**P**	**N**	**P**	**N**	**P**	**N**	**P**
Rat (a)	O_1	F_1	16.9	21.2	31.3	49.8	68.3	95.0	162.3	244.4
Guinea pig (a)	O_1	F_{pz}	16.8	24	30.0	40.0	54.8	92.5	128.8	190.0
Cat (a)	O_1	F_{pz}		15.8	24.6	32.2	43.4	101.6	180.6	254.6
Cat (b)	O_1	Chin		17.0	-	44.6	-	65.3	-	91.5
Dog (c)	O_3	F_3				40	85			
Dog (d)	P_1	F_4				58.3				
Dog (e)	O_z	F_{pz}			30	60	95	124	146	195
Dog (f)	O_z	F_{pz}		14.3	29.2	54.5	78.0	98.1		
Dog (g)	O_z	Atlas			55.9	-	72.0	-	86.3	
Dog (h)	O_z	Chin		21.9	-	46.9	-	66.0		
Miniature pig (e)	O_z	F_{pz}				40-60	80	115	160	
Cow (i)	O_z	F_{pz}				45.7	63.9	86.2	105.7	136.9
Sheep (j)	O_z	F_{pz}		35.0	43.1	52.8	64.1	74.5	90.4	112.2
Horse (k)	P_{Z-45}	F_{pz}		15	25	54	99	115	136	213
Monkey (a)	O_1	A_1A_2		31.7	-	-	61.0	92.0	150.0	274.3
Monkey (l)	O_3	F_3		15-18	40	65	95	120		
Man (a)	O_1	A_1A_2		26.4	52.0	60.3	83.6	115.1	154.6	193.3

The horse has a large retina and the potentials generated were sizable. Thus it was important to evaluate if the recorded FVEP consisted of post-retinal potentials only, or if a far-field conducted FERG was included, as has been

described in dogs (Strain *et al.*, 1990b; Bichsel *et al.*, 1988; Malnati *et al.*, 1981). By using retrobulbar nerve block and optic nerve transection, it was shown that the wavelets in the recorded equine FVEP are of post-retinal origin, as there was no contamination from a conducted FERG. This conclusion was further supported by results in study IV, where several clinical cases with VI and normal, or even supranormal FERG amplitudes, were found to have flatline FVEPs due to post-retinal disease.

The FVEP waveform recorded on the scalp, is the summation of potentials generated by neurons beneath. However, the neural generators of VEPs have not been fully understood. In primates, Kraut et al. suggested that early wavelets (with peak times comparable to the P1 and N1 in the equine FVEP) may be generated in the thalamocortical radiations, and using intracortical recordings they concluded that later components (comparable to the equine P2) were generated in the primary visual cortex (Kraut *et al.*, 1985). Subsequent wavelets (>95 ms) were shown to be generated in extrastriate areas. Ducati et al., performed intracerebral recordings in humans, using electrodes that were advanced close to area 17 in the striate cortex. They found that the two positive wavelets found at the surface at 45-60 ms and 80-100 ms were both generated in these cortical areas (Ducati *et al.*, 1988).

In study IV, P2 and subsequent wavelets were delayed (or extinguished) in equine cases with severe retinal disorders and optic neuropathies, whereas in the CVI group, patients were observed with a normal P2, but with delayed or absent later wavelets, most notably P4. The findings may suggest that P2 represent the input signal to the primary visual cortex also in the horse, in accordance to other species, and that later wavelets (at least P4) require higher cortical input.

5.2 Technical aspects (I-IV)

The technique for recording FVEPs in horses was adapted from other species, which required evaluation of suitable electrode positions, number of averages needed to obtain stable waveforms with easily discernible wavelets, and also to describe a suitable protocol for sedation (paper I).

The individual wavelets in the equine FVEP were evident and amplitudes were large, when the active electrode positions $P_{z\text{-}30}$ and $P_{z\text{-}45}$ were used. These positions correspond well to the presumed anatomical location of the visual cortex in the horse (Takeuchi & Sugita, 2001), which was confirmed by identifying the primary visual cortex using stria of Gennari in postmortem specimens (paper I). Comparing peak times and amplitudes, the $P_{z\text{-}30}$ and $P_{z\text{-}45}$ were equivalent. However, the electrodes were more easily applied at position $P_{z\text{-}45}$, compared to $P_{z\text{-}30}$ in most horses, and it was therefore the position of choice

in the subsequent studies (papers II-IV). As expected, the suitable electrode positions differ considerably from the positions recommended in the guidelines for clinical VEPs in human beings (Odom *et al.*, 2010), reflecting the substantial anatomical differences between man and horse.

At lateral electrode position positions (P_1 to P_4), an asymmetry was expected in recordings, due to the high degree of decussation of optic fibers in the horse of more than 80 % (Duke-Elder, 1958). However, wavelets were often difficult to discriminate due to split peaks. This is likely because of the close proximity to the large muscles controlling ear movements in this species (Budras, 2012), which cause muscle artifacts affecting recordings. However, while ear movements may have masked more subtle differences between lateral and midline positions, a method for obtaining FVEPs at lateral positions should be further explored, because these positions may be important for differing between pre- and postchiasmal lesions.

It was found that ≥100 responses should preferably be averaged to suppress noise and obtain curves with easily discernible, reproducible wavelets. The need to obtain easily discriminated wavelets for marking, will always enhance the interpretation of responses, but might be especially important for the inexperienced examiner. However, it is also important to note that 32-64 averages may also produce acceptable results. The advantage of averaging fewer responses, is that the time to perform the examination is shorter, which might be beneficial when recording FVEPs in critically ill patients.

Anesthesia has been shown to affect VEPs to varying extent, depending on type and depth of anesthesia. However, in some situations, it is still needed; for example when recording from uncooperative subjects (Creel, 2019b). Sufficient and stable depth of sedation were shown to enhance recording of equine FVEPs and FERGs in normal foals, young and adult horses (papers I-III), as well as in equine patients (paper IV). This is also in accordance with previous studies where FERGs were recorded in the standing, sedated horse (Ben-Shlomo *et al.*, 2012; Church & Norman, 2012; Komaromy *et al.*, 2003). It was obvious that the depth of sedation influenced the amount of artifacts and the quality of recordings, and the waveform could easily be obliterated if the level of sedation was too light, due to increased attention to surroundings, and ear and head movements causing muscle artifacts. The appropriate level of sedation sought, was to induce a relaxed horse, inattentive to its environment, resting its head heavily on a padded headstand, without being too wobbly.

In patients with neurological disease, one concern was increased sensitivity to sedative agents, and also aggravation of neurological signs during sedation, with risk of injury for both patient and examiner (horses in the “CVI” group, paper IV). Thus, the sedative dose was reduced in some patients, compared to

what is normally used in healthy horses. Nonetheless, a stable level of sedation suitable for electrophysiological recordings was achieved.

However, in one horse, even a low sedative dose resulted in severe aggravation of neurological signs, and electrodiagnostic testing could not be performed. Recordings were instead safely performed under general anesthesia in this horse, with an established equine protocol combining ketamine, xylazine and diazepam (Davidson, 2008). This anesthetic cocktail seemed to have had little or negligible impact on the electrophysiological recordings, as FERGs had normal waveforms and peak times OU, and a normally appearing FVEP with amplitudes and peak times for all wavelets within normal limits was obtained. This is in accordance with studies in other species, where both retinal and cortical responses have been shown to be maintained using combinations of ketamine and xylazine (Michelson & Kozai, 2018; Nair *et al.*, 2011). General anesthesia may therefore still be an option, when performing electrodiagnostic testing in the horse, particularly if the clinical workup includes additional procedures (such as safely obtained cerebrospinal taps and CT-scans) where general anesthesia is required or recommended. However, the potential impact of this anesthetic protocol on the equine FERG and FVEP needs further study.

In summary, FVEPs were easily recorded in normal horses, as well as in equine patients. Electrode positioning at $P_{z\text{-}45}$ was preferred, although $P_{z\text{-}30}$ produced similar results. Averaging ≥100 responses resulted in more easily evaluated waveforms. A level of sedation where the horse was inattentive and was resting its head on a padded head stand, without being wobbly, was suitable for recordings, and the dose of the sedative had to be individually adjusted for each horse. Recordings under general anesthesia, using ketamine/xylazine/ diazepam intravenously may be considered if necessary.

5.3 Age-associated changes (II)

The effect of age on the equine FVEP, starting with foals only hours post-partum up to geriatric horses almost thirty years of age was evaluated in study II. The aim was to assess whether age-matched normal data is required, as has been described in humans (Odom *et al.*, 2016), and also in animal species (Kimotsuki *et al.*, 2006). The overall FVEP waveform was shown to be similar across the life-span of these horses. An increase in some peak times with increasing age, and decrease in amplitudes were observed. Most of the changes occurred during the first years of life.

The appearance of the waveform was similar in newborn foals and adult horses, and the visual system of the foal seems to be fairly well developed

already at birth. These results were not surprising, because the horse is a precocial species where the offspring is born with open eyelids and must be able to see and follow the mother just a few hours post-partum, sometimes also at high speed to avoid predators. The results were also in accordance with what has previously been described in other precocious animals, such as the calf, lamb and piglet (Strain *et al.*, 2006; Strain *et al.*, 1991a; Strain *et al.*, 1989). On the other hand, altricial species such as the cat and dog, have immature FVEPs during the first weeks and months of life (Kimotsuki *et al.*, 2006; Strain *et al.*, 1991b; Rose *et al.*, 1972; Myslivecek, 1968).

In general, peak times were shorter in the newborn foal than in the adult horse, which might reflect the shorter distance between the eye and the visual cortex in the small foal. Surprisingly, N2 peak times were significantly longer in young horses compared to both newborn foals and adults. The delay may reflect a transient period of cortical development, which for some reason preferentially affects the timing of this wavelet in the young horse. Hence, these interesting findings indicated that maturation of FVEPs occur in young horses.

From three years of age and throughout adulthood, a small, but statistically significant, increase in P4 peak times (9 ms per decade) and decrease in amplitudes, N2P4 (1.4 µV per decade), were observed, although with very low correlation coefficients. In paper II, it was discussed that the small increase in peak times may be due to decreased conduction velocity in the optic nerve and optic radiation. However, later findings (paper IV) indicate that P2 may be the input signal to the primary visual cortex, whereas P4, may more likely be the result of processing and feedback from other cortical areas. Hence, other factors related to aging and cortical processing, are probably causing the small delay in P4 peak time and decrease in N2P4 amplitude observed. Regardless, compared to the individual variability in equine FVEPs shown in study III, with intra- and interindividual coefficients of variation (CVs) for P4 peak time at 7 and 11 %, and at 30% and 37% for the N2P4 amplitude respectively, the minor age-associated changes observed during the adult life-span of the horse, were considered to be of limited clinical significance.

Amplitudes of the FVEP recorded from newborn foals were well above the amplitudes observed in normal adult horses, which is in accordance with findings also in the calf (Strain *et al.*, 1989). A gradual decrease of amplitudes was observed during the first three years of life. The higher amplitudes obtained could for example be due to the thinner calvarium in the young individual (resulting in more easily recorded potentials), or closer proximity to the neural generators. Additionally, as the number of neurons in the primary visual cortex has been reported to decrease with increasing age in several species, this may be

another likely explanation for the higher amplitudes in foals (Flood & Coleman, 1988).

In summary, it was concluded that age-matched controls should be used when evaluating foals and young horses, because changes, attributed to the anatomical and physiological development of the skull and central nervous system, will have substantial effects on both peak times and amplitudes in the early life of the horse. Horses over the age of three years can be compared to other adult horses. These were important findings to be used when evaluating clinical patients (study IV).

5.4 Variability and normal reference data (III)

Parameters for estimating the variability, repeatability and test-retest reliability of FVEPs in normal adult horses were determined in study III, to evaluate if this method is sufficiently reliable in horses. Normative data were established for use in the subsequent study on clinical patients (paper IV).

Only some wavelets in the equine FVEP were present in all recordings in all horses, which is similar to what has been described in human studies (Gastaut & Regis, 1964). Intra- and inter-individual coefficients of variation (CVs) were low (5-11 %) for P2, N2 and P4 peak times, which is advantageous for a parameter intended to be used in clinical practice. A substantial intra- and inter-individual variability in amplitudes was observed, which is also in accordance with results from human studies (Andersson *et al.*, 2012; Gastaut & Regis, 1964). A wide range of amplitudes must be considered normal in the FVEP recorded from normal, sedated horses. Hence, even many patients are likely to have amplitudes that fall within the normal range, and only severe abnormalities will cause sufficiently abnormal amplitudes. However, one clinical case diagnosed with optic neuritis, with amplitudes falling below the wide normal range has been described study IV, indicating that amplitudes still may be of use in some conditions.

The variability in peak times between eyes was low, at only 7 % or less. The variation has also been described to be quite similar between eyes within the same subject in humans (Odom *et al.*, 2016). Thus, large differences in peak times between eyes, outside the values determined by the coefficients of repeatability (P2; 5 ms, N2; 18 ms, P4; 18 ms), are likely to indicate abnormal function. Amplitudes were shown to be more variable between eyes. However, the P2N2 and N2P4 amplitudes may provide important information, with coefficients of repeatability (CRs) at 1.7 µV and 2.3 µV, respectively. The estimated parameters for inter-ocular comparison provide important information that can be used in the clinical situation, and enhances evaluation of clinical

patients with suspected unilateral dysfunction, when the fellow eye can serve as control.

The waveforms obtained at separate recording sessions appeared quite similar. The CVs for peak times between sessions were low (3-6%), although higher for amplitudes (24-30%). The mean difference between sessions was low for the P2 peak time, but higher for the N2 and P4 peak times, whereas the mean differences for all amplitude parameters were substantial. Coefficients of repeatability were determined, and differences in peak times between recording sessions falling outside the reported CR values (P2; 5 ms, N2; 16 ms, P4; 39 ms), are likely to indicate either an improvement or deterioration of a condition, which is important information to use when evaluating clinical patients. Coefficients of repeatability for amplitudes were high, again supporting the conclusion that differences in amplitudes between sessions only rarely will provide reliable information regarding the progression of a disease or effect of a treatment.

Some of the variability is likely due to difficulties in establishing the peak or trough of a specific wavelet precisely. Large-amplitude, pointed wavelets are generally easier to pinpoint compared to low-amplitude, or more rounded or elongated wavelets. For example, as P2 was most often a distinct peak that was easy to discriminate, it was not surprising that the P2 peak time showed least variability and highest repeatability. There are also several other factors that are likely to influence variability in equine FVEPs, such as differences in the exact positioning of electrodes in relation to the primary visual cortex, despite the use of bony landmarks as points of reference. Also, the level of sedation should preferably be stable and similar between horses and between sessions. However, it is of course difficult to achieve the identical level of sedation at all time points and in all subjects. External disturbances in the busy clinical environment will affect the level of sedation, and some horses were found to be more sensitive for environmental disturbances than others. Hence, the difference in the level of sedation, is likely to have caused some of the variability seen.

Only one specific breed (Standardbreds) was evaluated in the studies of normal, healthy horses. However, a small number of normal horses from another breed (Icelandic horses, n=5) were also evaluated (unpublished data). The FVEP waveform in these horses was similar to those recorded in Standardbreds. Furthermore, peak times and amplitudes were within the same range and similar variability was seen. Hence, breed differences were not observed, at least not comparing these two breeds.

Differences between females and males in peak times and amplitudes have been described in human subjects (Dotto *et al.*, 2017; Sharma *et al.*, 2015).

However, the number of horses was too few, to study potential gender differences in the equine FVEP.

It was concluded that P2, N2 and P4 peak times should be included in the evaluation of equine, clinical FVEPs. Amplitudes were more variable, which is a limitation for their usefulness in most clinical patients, although as pointed out earlier, they can occasionally provide support to a clinical diagnosis.

5.5 Electrodiagnosic testing in the evaluation of the equine patient with visual impairment (IV)

By using FERGs and FVEPs, abnormalities in the retinothalamocortical pathways were detected, and retinal dysfunction versus post-retinal disease was differentiated in several of the equine cases described. A summary of overall findings in the groups is displayed in table 4.

Typically, FERGs and FVEPs were both normal in horses with cataracts, although abnormal FVEPs were observed in concomitant post-retinal disease and some of the visually deprived young horses. In retinal disorders, FERGs were affected, which also resulted in abnormal FVEPs. In optic neuropathies, FERGs were mostly normal (or mildly affected), whereas FVEPs were profoundly abnormal or non-detectable. In horses with cortical visual impairment, FERGs were mostly normal (although affected in concurrent posterior uveitis), whereas FVEPs showed variable results. Horses that later regained vision had an easily distinguishable waveform. Profoundly affected or extinguished FVEPs were seen in horses with a poor prognosis for recovery.

Table 4. *Overall assessment of groups.*

Group	**FERGs**	**FVEPs**
Cataracts	Normal (supranormal)	Normal (atypical/immature)
Retinal disorders	Abnormal or non-detectable	Abnormal or non-detectable
Optic neuropathies	Normal (mildly affected in glaucoma)	Abnormal or non-detectable
CVIs	Normal (affected if concurrent uveitis)	Abnormal with markedly prolonged P4 wavelet or non-detectable

It was observed that peak times were informative in many of the cases described, and although the normal range for wavelet amplitudes was shown to be wide (paper III), amplitudes were still of interest in some conditions, such as in optic neuritis. Hence, the results showed that both peak times and amplitudes may provide useful information in clinical patients.

Evaluation of gross retinal function with FERG before cataract surgery is recommended in the horse (McMullen & Utter, 2010; McLaughlin *et al.*, 1992). Some patients would also benefit from FVEPs to evaluate post-retinal visual pathways. Normal FVEPs were obtained from several of the cases with cataracts, although some atypical and abnormal findings were observed. In one foal, the FVEP waveform obtained when the cataractous eye was stimulated, was similar to responses seen in normal newborn foals (paper II). However, this was not observed in other foals with similar cataracts and it is difficult to assess whether this represent delayed maturation of visual pathways. Another foal with bilateral cataracts had atypical FVEP waveforms, however not appearing as those seen in newborn foals. The cause of these atypical responses remains unknown. The foal could negotiate the obstacle course well post-cataract surgery, but it was not available for follow-up electrodiagnostic testing.

Results from horses being nearly blind due to total cataracts, but still showing normal FVEPs, highlights the fact that FVEPs only provide an overall assessment of the function of post-retinal pathways, and not a quantitative assessment of visual acuity. To be able to make more precise quantitative assessments, other electrophysiological techniques, such as the PVEP, need to be adapted in the horse.

In horses with retinal disorders and abnormal FERGs, FVEPs were also affected. This implies that an abnormal FVEP, is not always due to dysfunction of the post-retinal pathways per se, as has also previously been discussed by other authors (Lennerstrand, 1982). It is therefore concluded that simultaneous recordings of both FERGs and FVEPs should always be performed, even if lesions only in higher pathways are suspected, to avoid misdiagnosis in clinical patients.

In horses with posterior uveitis, abnormal FERGs were observed. In the human literature, FERGs have been described to aid in the diagnosis and evaluation of human patients with posterior uveitis and to assess retinal involvement (Moschos *et al.*, 2014). Abnormal FERGs were observed in several equine cases with posterior uveitis (chorioretinitis) in paper IV. Visual electrodiagnostics should also be studied further in horses with posterior uveitis, because this may contribute to a better understanding of the pathophysiology in this disease, as well as showing potential to be used diagnostically. A full protocol, including scotopic ERGs and 30 Hz flicker is suggested, as these specifically have been described to be sensitive indicators of disease and can help in assessing treatment strategy in several forms of posterior uveitis in humans (Moschos *et al.*, 2014).

One of the most important applications of FVEPs in human patients is the evaluation of optic neuropathies. It was found that this modality is useful and informative also in the equine patient for this purpose.

In glaucoma, affected eyes had normal, or near-normal FERGs, with only slightly reduced b-wave amplitudes. The FVEP waveform morphologies were profoundly abnormal in all affected eyes, and the only consistently reproducible wavelet had a peak time between 80-128 ms. Although not certain, this wavelet may represent a profoundly delayed P2. The findings are well in line with glaucoma being an optic neuropathy (Plummer *et al.*, 2013). One of the horses with glaucoma was also diagnosed with a large pituitary adenoma (pituitary pars intermedia dysfunction, PPID). Anatomically, pituitary adenomas are in close proximity to the optic chiasm, and have been described to be able to cause damage to the visual pathways in humans (Lachowicz & Lubinski, 2018), either by causing direct compression, resulting in retrograde degeneration of RGCs (Morgan, 2004), or by interfering with regional vascular supply. Compression of neurons in the visual pathways causing blindness has also been described in horses with PPID (Love, 1993). The pituitary tumor may have contributed somewhat to the profoundly abnormal FVEPs in this patient.

In horses with intracranial space-occupying lesions, FERGs were nearly normal but FVEPs were extinguished. This was found to be due to the compression of the optic nerves and subsequent development of optic neuropathy, as has been described in human patients (Hata *et al.*, 2014; Bianchi-Marzoli *et al.*, 1995; Kalenak *et al.*, 1992).

A common cause of optic neuritis and subsequent optic atrophy in the horse is head trauma, causing traumatic damage to the optic nerve (Grahn & Cullen, 2002; Martin *et al.*, 1986). One case, suffering from head trauma, showed signs of optic neuritis in one eye but had regained some, although very limited vision in the same eye. FVEPs confirmed signal transmission through the optic nerve, although with profoundly reduced amplitudes. Optic atrophy had already developed in the fellow, blind eye, and FVEPs were non-detectable. The horse was unfortunately not available for follow-up. In another case, suffering from blindness after acute head trauma and diagnosed with a fracture causing traumatic optic neuropathy OD, the FVEP was non-detectable. Optic atrophy subsequently developed in the affected eye within a few months, and the horse never regained vision in that eye. FVEPs have been described to have a prognostic value in traumatic optic neuropathies in humans (Kumaran *et al.*, 2015; Brecelj, 2014; Holmes & Sires, 2004; Steinsapir, 1999), and an extinguished FVEP is considered to be a poor prognostic indicator for visual recovery. Based on the results from the equine cases described above, it is

suggested that FVEPs may have a potential use in the equine patient as well, as being a prognostic indicator in traumatic optic neuropathy.

In summary, it was concluded that the FVEP is a modality suitable for diagnosing optic nerve dysfunction in the horse, in the same manner as in other species, including humans.

Thalamic and cortical lesions may also cause abnormal FVEPs in different species. Opinions regarding the clinical usefulness of FVEPs for evaluation of cortical visual impairment in human patients diverge. Some authors suggest a diagnostic and prognostic value, for example that an absent FVEP is correlated to poor prognosis for visual recovery (Taylor & McCulloch, 1991), whereas others report that although an absent FVEP is not necessarily a poor prognostic factor, a normal FVEP may be a positive sign (Andre-Obadia *et al.*, 2018; Clarke *et al.*, 1997). Still others maintain that FVEPs are not useful at all for predicting visual recovery (Walsh *et al.*, 2005; Hess *et al.*, 1982).

In veterinary medicine, there are only a few reports on the use of FVEPs in clinical patients with cortical disease. Strain et al. reported cases with scrapie, thiamine-responsive polioencephalomalacia (PEM), suspected listeriosis, and abscessation in thalamus and cerebral cortex in ruminants (Strain *et al.*, 1990a; Strain *et al.*, 1987; Strain *et al.*, 1986a). FERGs were normal in these ruminant patients, whereas FVEPs were usually abnormal.

Within the equine "CVI" group, cases with signs of visual impairment that can be attributed to central, post-chiasmal disease were observed. Occasionally, but not always, these patients were shown to have abnormal FVEPs. Several of the etiologies described to cause cortical visual impairment in humans were represented in our case series (i.e. head trauma, meningitis, hepatic encephalopathy), although only in very limited numbers. Three horses with profoundly abnormal, or extinguished FVEPs, were found to be unresponsive to treatment, and were subsequently euthanized. However, four horses with normal FVEPs, or with normal FVEP morphology and normal amplitudes, but delayed N2 and P4 peak times, recovered and regained vision. These results suggest that FVEPs may provide prognostic information, at least in some conditions causing CVI in the horse. However, due to the limited number of equine patients studied to date, far-reaching conclusions would be premature.

In this series of equine patients with visual impairment, electrodiagnostic testing helped assessing functional impact of diseases affecting the retinothalamocortical pathways. By recording FERGs and FVEPs simultaneously, a subdivision into retinal vs post-retinal dysfunction could be made in many patients, such as horses with optic neuropathies and cortical visual impairment. FVEPs may be of prognostic value in horses with traumatic optic

neuropathy and also possibly in cases with cortical visual impairment, and may thus also be of use in the neurological work-up of a patient.

6 Conclusions

- Equine FVEPs can be readily recorded in the standing, sedated horse in a clinical setting, and the technique is relatively easy to master.
    - The equine FVEP waveform consists of a series of positive (P1-P5) and negative wavelets (N1-N2).
    - Recorded responses are of post-retinal origin.
    - Active electrodes positioned at Pz-30 to Pz-45 will result in easily discriminated wavelets and reproducible waveforms.
    - Averaging ≥100 responses will result in more easily discernible wavelets.

- The overall equine FVEP waveform is similar across the normal life-span of the horse, and the visual system of the foal seems to be relatively well-developed already at birth.
    - There is a decrease in amplitudes and an increase in some peak times with increasing age, and age-matched controls should be used when evaluating foals and young horses.
    - Horses over the age of three years can be compared to other adult horses.
    - The FVEP opens up for objective, functional assessment of the post-retinal visual pathways in this species, and can, together with the FERG, be used to further study the maturation of visual pathways in the young horse.

- Parameters to evaluate variability, repeatability and test-retest reliability were determined.
    - Wavelets P2, N2 and P4 are the most consistent and robust.
    - P2, N2 and P4 peak times should be included in the evaluation of the equine FVEP.

- Amplitudes are more variable, both between eyes and between sessions, but can occasionally provide support to a clinical diagnosis.

- Electrodiagnostic testing provides important information about retinal and post-retinal function in horses with signs of visual impairment.
  - By recording FERGs and FVEPs simultaneously, a subdivision into retinal vs post-retinal dysfunction can be made in clinical patients.
  - FVEPs may be of prognostic value in horses with traumatic optic neuropathy and possibly also in cases with cortical visual impairment, and may thus also be of use in the neurological work-up of a patient.

- Together with the results from clinical assessment and other relevant tests, the use of FERGs and FVEPs in clinical practice will provide a better foundation for adequate treatment plans, assessment of prognosis and well-founded advice to owners, for example regarding animal welfare, handling and safety issues.

7 Future perspectives

Through the work in this thesis, the technique to record FVEPs in horses in a clinical setting has been adapted, and the method has been shown to provide valuable information in the work-up of horses with visual impairment and possibly also in patients with neurological disease. However, several aspects of the equine FVEP remain to be studied further.

- Visual electrodiagnostics should be studied further in horses with visual impairment of different causes (for example posterior uveitis and optic neuropathies) and at different stage of disease, to assess their use in differential diagnostics, ability to evaluate treatment effects and to further explore the prognostic value of these techniques.
- Specifically, the clinical and prognostic value of FVEPs in horses with neurologic signs and cortical visual impairment should be explored in more detail.
- Other electrophysiological methods, such as the PVEP, need to be adapted for use in the horse, so visual acuity can be assessed and quantitative assessments made.
- FVEPs in both normal and visually deprived foals and young horses need to be studied more in detail, to assess the need for further stratification of age-matched data.
- Since variability in the young age-group may differ from adult results, further studies to assess variability parameters in the equine FVEP recorded from the young horse should be performed.
- Variability in FVEPs in clinical patients with visual impairment of different etiologies should also be studied further, since this can differ from normal data.

- A method for obtaining FVEPs at lateral positions should be further explored, because these positions may be important for differing between pre- and post-chiasmal lesions.
- The potential impact of an anesthetic protocol for general anesthesia, a combination of intravenous ketamine/xylazine/diazepam, on the equine FERG and FVEP needs further study.
- Gender differences in FVEPs should be assessed.

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Popular science summary

Vision has evolved during millions of years, and each species has developed specific adaptations to be able to survive in their environment. The horse is a prey species and as such, a "visual-generalist", with good vision under both bright and dim light conditions, which has been beneficial to avoid predators. Horses have good visual acuity, and a huge visual field with only a few blind spots. Although the threat from predators no longer remains today, at least for most horses, vision is still very important. Many horses are expected to perform tasks where adequate vision is needed, when they are used for work, sport and recreation. Sufficient vision is also important for safety reasons, as handling and riding a large animal with impaired vision may pose a risk for the human, as well as for the horse itself. There are a number of diseases that can affect vision in the horse, and thus cause poor performance or behavioural problems. However, it is often difficult to diagnose visual impairment in this species. Traditional techniques available in clinical practice for assessing visual abilities and diagnose a visual impairment, are based on subjective evaluations, and their results are many times difficult to interpret.

In the sophisticated process of vision, light reflected by the environment enters the eye. In the retina, in the back of the eye, the light energy is converted to electrical potentials, and are sent further through the optic nerve and subsequent visual pathways to the visual cortex, for processing and interpretation. Electrodiagnostic methods, can be used to objectively evaluate the function of these visual pathways, and have been used for decades in human patients. A visual stimulus, either flashes of white light or a pattern, is used to stimulate the eye. The retinal potentials generated as a response to the visual stimulus can be measured using electroretinography (ERG). The potentials generated in higher pathways, the visual evoked potentials (VEP), can be recorded from the scalp over the visual cortex, using non-invasive electrodes. The results from recordings are waveforms with wavelets that can be evaluated. Both peak times (the time to each wavelet) and wavelet amplitudes (from peak

to trough) are measured and abnormal function in visual pathways can affect these parameters. Lesions can thereby be detected, and their approximate localization evaluated. Dysfunction in the retina affects the ERG, whereas any anomaly along the visual pathways can affect the VEP, such as retinal and/or optic nerve disorders, compressing lesions such as tumors and cortical disease. When recording VEPs, stimulation using a pattern on a screen (PVEP) is mostly preferred in human medicine, in part because of its lower variability between patients. However, PVEPs require cooperation by the patient (they need to fixate and focus on the pattern), and stimulation through flashes of white light (FVEP) is therefore more often used in uncooperative patients and small children.

In small animal ophthalmology, FERGs are widely used to assess retinal function and disease, both for research purposes and in clinical patients with visual impairment. Techniques for recording FERGs in healthy horses has also been described, and diagnostic FERGs have been reported in clinical patients with retinal disorders. Although not as widely used in veterinary medicine as FERGs, FVEPs have been studied in a large number of our domestic species including the cat, dog, cow, sheep and pig, but FVEPs have not before been evaluated in the horse.

The general aim of this thesis was to establish a technique for recording of FVEPs in horses in clinical practice, and to evaluate whether this technique would aid in objective evaluation of visual impairment in this species. The results showed that FVEPs can be easily recorded in sedated horses in a clinical setting. The recorded waveform consisted of a series of positive (P1-P5) and negative (N1-N2) wavelets. The overall waveform was similar in foals, young and adult horses. An age-related effect on some amplitudes and peak times was seen, and most of the changes occurred early in life. The observed changes are probably due to maturation of visual pathways in the young horse, although the foal seems to have a fairly well-developed visual system already at birth, similar to in other precocious animals such as the lamb and the calf.

Important data on variability and repeatability were reported for the measured parameters in the FVEP waveform. It was shown that P2, N2 and P4 peak times should be included in the evaluation of FVEPs in the horse. Amplitudes were more variable both between horses, and when evaluating the same horse at different sessions, which made them less sensitive as diagnostic markers, but it was shown that they may occasionally help in diagnosing dysfunction.

In a case series of clinical patients, electrodiagnostic testing was shown to be of help in assessing the impact of various diseases affecting the visual input, such as cataracts, retinal disorders, inflammation in the optic nerve, glaucoma, compressing lesions and visual impairment due to cortical disease. By recording FERGs and FVEPs simultaneously, retinal disease could be differentiated from

disease in the higher visual pathway in many patients. It was also shown that FVEPs in horses may provide prognostic information in some diseases, such as in trauma to the optic nerve, and in cases with neurological signs and visual impairment due to cortical disease.

The results from this thesis opens up for the use of FVEPs as an adjunctive, objective method in the clinical evaluation of horses with suspected visual impairment and neurological disease, but also for studies of development and function of the visual pathways in this species. Together with the results from clinical assessment and other relevant tests, the use of FERGs and FVEPs will provide a better foundation for adequate treatment plans, assessment of prognosis and well-founded advice to owners, for example regarding impact on equine welfare, handling and safety issues.

Populärvetenskaplig sammanfattning

Under evolutionen har olika arter utvecklat ett anpassat synsinne för att kunna överleva på bästa sätt i sin miljö. Hästen är ursprungligen ett bytesdjur, och har utvecklats till en ”generalist” vad gäller synförmåga med bra syn i både dagsljus och i mörker, vilket har varit en fördel för att kunna undvika rovdjur. De har också bra synskärpa och ett mycket stort synfält. Även om hotet från rovdjur inte längre är närvarande, åtminstone inte för de flesta hästar, så är synen fortfarande ett viktigt sinne. Många hästar förväntas utföra uppgifter där god syn behövs, till exempel när de används i arbete, för tävling och rekreation. Bevarad synförmåga är också viktigt ur säkerhetssynpunkt, då att rida och hantera en häst med begränsad syn kan innebära en risk för människan, men också för hästen själv.
Det finns många sjukdomar i ögon och synbanor som kan påverka seendet, och därmed orsaka nedsatt prestation och förändrat beteende. Det är emellertid svårt att diagnostisera synnedsättningar hos detta djurslag. Tekniker som idag används i klinisk verksamhet för att utvärdera synförmåga och diagnostisera synnedsättningar baseras på subjektiva utvärderingar, och resultaten från undersökningarna kan ofta vara svåra att tolka.

Synintryck skapas genom att ljus som reflekteras från omgivningen kommer in i ögat och når näthinnans synceller. I näthinnan omvandlas ljusenergin till elektriska potentialer, som sedan skickas därefter vidare genom synnerv och högre synbanor till syncentrum i hjärnan, för vidare bearbetning och tolkning till synintryck. Elektrodiagnostiska metoder är objektiva tester som kan utvärdera synbanornas funktion, och sådana metoder har använts under årtionden inom humanvården. Synstimuli, antingen blixtar av vitt ljus eller ett mönster på en bildskärm, används för att stimulera ögat. De potentialer som skapas i näthinnan kan mätas genom elektroretinografi (ERG). De potentialer som skapas i högre synbanor, visuella retningspotentialer (VEP), kan registreras på skallen över hjärnans syncentrum med hudelektroder. Resultaten från dessa registreringar är vågformer med toppar och dalar som kan utvärderas vidare. Latenstider (tid från stimuli till topp eller dal i kurvan) och amplituder (höjden från topp till dal) kan

mätas, och onormal funktion i synbanorna kan påverka dessa parametrar. Sjukliga förändringar kan påvisas och även lokaliseras. Problem i näthinnans funktion påverkar ERG medan sjukdomar i synbanorna (till exempel störningar synnervsfunktionen, tumörer som komprimerar synbanorna, eller sjukdomar som påverkar hjärnbarkens syncentrum) kan påverka VEP. Inom humanvården används oftast mönsterstimulering vid registrering (pattern-VEP; PVEP), eftersom den metoden bland annat visats variera mindre mellan patienter jämfört med blixt-stimulering (flash-VEP; FVEP). Vid PVEP krävs emellertid att patienten samarbetar och fixerar på mönstret, och till exempel vid undersökning av små barn och patienter i koma kan FVEP användas.

ERG har under många år använts inom smådjursoftalmologin för att utvärdera näthinnefunktion, både hos kliniska patienter och i samband med forskning. Tekniker för att registrera ERG på normala hästar har också beskrivits, och det finns även ett fåtal rapporter från ERG-undersökningar hos hästar med näthinnesjukdom. Även om metoden inte studerats lika ingående som ERG, så har FVEP studerats hos ett stort antal av våra domesticerade arter, såsom katter, hundar, kor, får och grisar, men inte tidigare hos häst.

Huvudsyftet med denna avhandling var att utveckla en teknik för registrering av FVEP hos häst i klinisk verksamhet, och att utvärdera om denna metod kan användas för att objektivt utvärdera synnedsättningar hos detta djurslag. Resultaten visade att FVEP på ett enkelt sätt kan registreras på häst under klinikförhållanden. Vågformen bestod av en serie av positiva (P1-P5) och negativa (N1-N2) toppar och dalar. Vågformens utseende var i huvudsak lika mellan föl, unga samt vuxna hästar. En åldersrelaterad effekt observerades för vissa latenstider och amplituder, främst under de första åren i livet. De skillnader som sågs beror troligen på en mognadsprocess av näthinna och synbanor under de tidiga åren i livet. Fölet verkar emellertid ha ett relativt välutvecklat synsystem redan vid födseln, i likhet med kalvar och lamm.

Metodens variabilitet och repeterbarhet utvärderades, och latenstiderna för P2, N2 och P4 visades vara användbara vid utvärdering av hästens FVEP. Amplituderna var mer variabla, även om de kunde bidra med kliniskt relevant information vid vissa diagnoser.

Resultat från undersökning av kliniska patienter visade att elektrodiagnostiska metoder kunde ge värdefull information vid utvärdering av synnedsättning hos häst. Genom att registrera FERG och FVEP samtidigt, kunde näthinneproblem skiljas från problem i högre synbanor hos många patienter, till exempel vid skada mot synnerven och vid påverkan på hjärnans syncentrum. Resultaten från detta arbete möjliggör användning av FVEP som en objektiv utvärderingsmetod i klinisk verksamhet av hästar med misstänkt synnedsättning och/eller neurologiska problem, men även för studier av synbanornas normala

funktion och utveckling hos detta djurslag. Tillsammans med resultat från övriga, relevanta, kliniska undersökningar, kan ERG och FVEP bidra till bättre underlag för behandlingsplaner, bedömning av prognos, samt mer välgrundade råd till djurägare, till exempel vad gäller påverkan på hästens välfärd, hantering och säkerhetsaspekter.

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