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J. D. Smith

U.S. Department of Agriculture, jdsmith@iastate.edu

J. J. Greenlee

U.S. Department of Agriculture

A. N. Hamir

U.S. Department of Agriculture

M. H. West Greenlee

Iowa State University, mheather@iastate.edu

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Altered electroretinogram b-wave in a Suffolk sheep experimentally infected with scrapie

Abstract

TRANSMISSIBLE spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases in which an abnormal isoform of the cellular prion protein (PrP^{Sc}) accumulates in tissues of the central nervous system. Accumulation of PrP^{Sc} occurs in the retina, a rostral projection of the central nervous system, of both natural and nonnatural host species with TSEs (Foster and others 1999, Spraker and others 2002, Head and others 2003, 2005, Hamir and others 2004, 2005, Kercher and others 2004, Greenlee and others 2006, Hortells and others 2006). In retinas from scrapie-affected sheep, PrP^{Sc} accumulation is primarily observed in the inner and outer plexiform layers, and in the ganglion cell layer (Jeffrey and others 2001, Greenlee and others 2006). Recent studies have reported few (Hortells and others 2006) or no (Greenlee and others 2006) histological lesions in the retinas of sheep affected with scrapie. However, morphological changes in specific retinal cell types have been demonstrated (Smith and others 2008). Despite the morphological consequences of retinal PrP^{Sc} accumulation in sheep (Barnett and Palmer 1971, Smith and others 2008), the functional impact on the retina of these animals is unknown. In the current study, the effect of TSE on retinal function in a scrapie-infected Suffolk sheep using flash electroretinography was investigated.

Disciplines

Comparative and Laboratory Animal Medicine | Veterinary Infectious Diseases | Veterinary Medicine | Veterinary Pathology and Pathobiology

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SHORT COMMUNICATIONS

Altered electroretinogram b-wave in a Suffolk sheep experimentally infected with scrapie

J. D. Smith, J. J. Greenlee, A. N. Hamir,
M. H. West Greenlee

TRANSMISSIBLE spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases in which an abnormal isoform of the cellular prion protein (PrP^{Sc}) accumulates in tissues of the central nervous system. Accumulation of PrP^{Sc} occurs in the retina, a rostral projection of the central nervous system, of both natural and non-natural host species with TSEs (Foster and others 1999, Spraker and others 2002, Head and others 2003, 2005, Hamir and others 2004, 2005, Kercher and others 2004, Greenlee and others 2006, Hortells and others 2006). In retinas from scrapie-affected sheep, PrP^{Sc} accumulation is primarily observed in the inner and outer plexiform layers, and in the ganglion cell layer (Jeffrey and others 2001, Greenlee and others 2006). Recent studies have reported few (Hortells and others 2006) or no (Greenlee and others 2006) histological lesions in the retinas of sheep affected with scrapie. However, morphological changes in specific retinal cell types have been demonstrated (Smith and others 2008). Despite the morphological consequences of retinal PrP^{Sc} accumulation in sheep (Barnett and Palmer 1971, Smith and others 2008), the functional impact on the retina of these animals is unknown. In the current study,

the effect of TSE on retinal function in a scrapie-infected Suffolk sheep using flash electroretinography was investigated.

A Suffolk sheep (number 3742) was inoculated intracerebrally with brain homogenate prepared from a fourth-passage scrapie-affected sheep (Hamir and others 2009). Electroretinography was performed with a visual electrodiagnostic testing system (EPIC 4000; LKC Technologies) 10 months postinoculation (MPI), which was before the onset of clinical signs of disease, and at 12.5 MPI, when the sheep had developed clinical disease characterised by cachexia and severe ataxia, and was euthanased with a barbiturate overdose. Eight age-matched, non-inoculated Suffolk sheep served as a control cohort. All animal procedures were approved by the National Animal Disease Center's animal care and use committee.

Entire globes with a segment of optic nerve were extracted during postmortem examination and histological and immunohistochemical analyses were performed. Immunolabelling for PrP^{Sc} was performed as described by Hamir and others (2004) using primary antisera containing monoclonal antibodies F89/160.5 (O'Rourke and others 1998) and F99/97.6.1 (Spraker and others 2002) each at a concentration of 5 µM/ml. Immunolabelling for glial fibrillary acidic protein (GFAP) was performed as described by Greenlee and others (2006) using a rabbit anti-GFAP polyclonal antibody (Dako) at a 1:7500 dilution.

To evaluate retinal function, flash electroretinography was performed and the amplitude and implicit time of the electroretinogram b-wave were measured for one scotopic (dark-adapted) and one photopic (light-adapted) test condition. The b-wave represents the large positive deflection of the electroretinogram, which occurs under both scotopic and photopic testing conditions. During the preclinical period, the sheep had b-wave amplitude and implicit time values for both the scotopic and

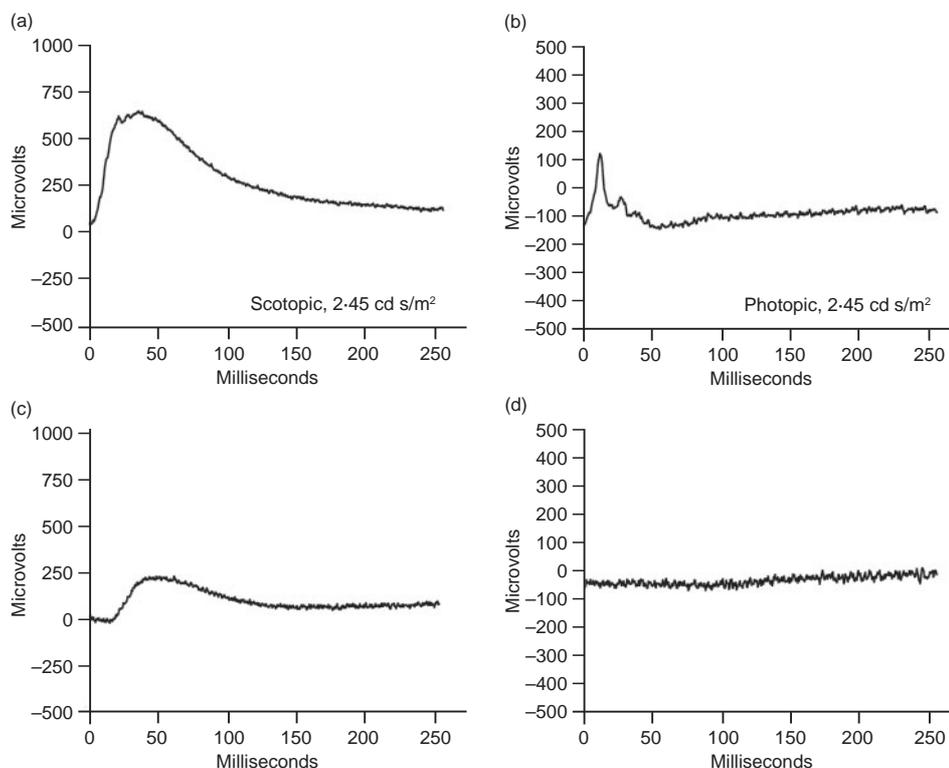


FIG 1: Representative scotopic and photopic electroretinograms (ERGs) from (a, b) a non-inoculated control sheep and (c, d) a sheep during the clinical stage of scrapie. The light intensity for both the scotopic and photopic tests is 2.45 Candela seconds per meter squared (cd s/m²). (c) For the scotopic ERG, the b-wave (large positive deflection) is altered during the clinical stage of disease compared with control sheep. (d) A photopic ERG response could not be elicited from this sheep during the clinical stage of disease

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J. D. Smith, DVM, PhD,
J. J. Greenlee, DVM, PhD, DACVP,
A. N. Hamir, DipAH, BVSc, MSc,
PhD, DiplECVP, MRCVS,
Virus and Prion Diseases of
Livestock Research Unit, National
Animal Disease Center, United
States Department of Agriculture,
Agricultural Research Service, Ames,
IA 50010, USA
M. H. West Greenlee, PhD,
Department of Biomedical Sciences,
College of Veterinary Medicine, Iowa
State University, Ames, IA 50011,
USA

Correspondence to Dr West
Greenlee, e-mail: mheather@
iastate.edu

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FIG 2: (a) Histology and (b) distribution of the abnormal form of the prion protein (PrP^{Sc}) glial fibrillary acidic protein (GFAP) immunoreactivity in the retina of a scrapie-infected sheep. (a) The retina lacked histological signs of degeneration or spongiform change. (b) High levels of PrP^{Sc} immunoreactivity were detected in the inner plexiform layer (IPL) and outer plexiform layer (OPL), with fewer multifocal puncta of immunoreactivity present within the inner nuclear layer (INL) and at the level of the inner limiting membrane. Multifocal cells within the ganglion cell layer (GCL) contained moderate to marked amounts of immunoreactivity within their cytoplasm (arrows). OFL Optic fibre layer, ONL Outer nuclear layer, OS Outer segments. Haematoxylin and eosin. Bars=20 µm

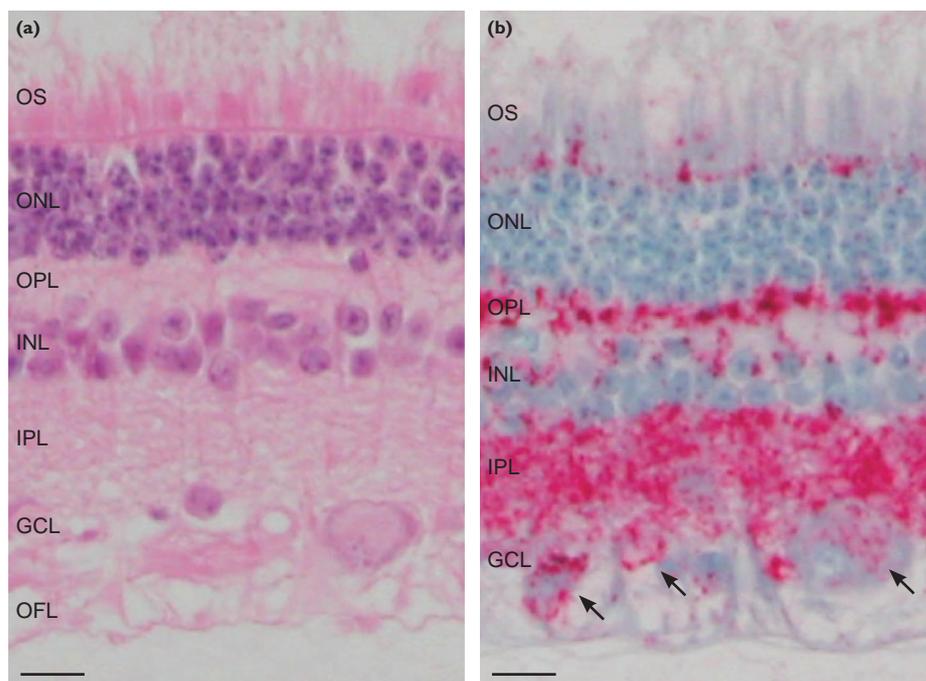
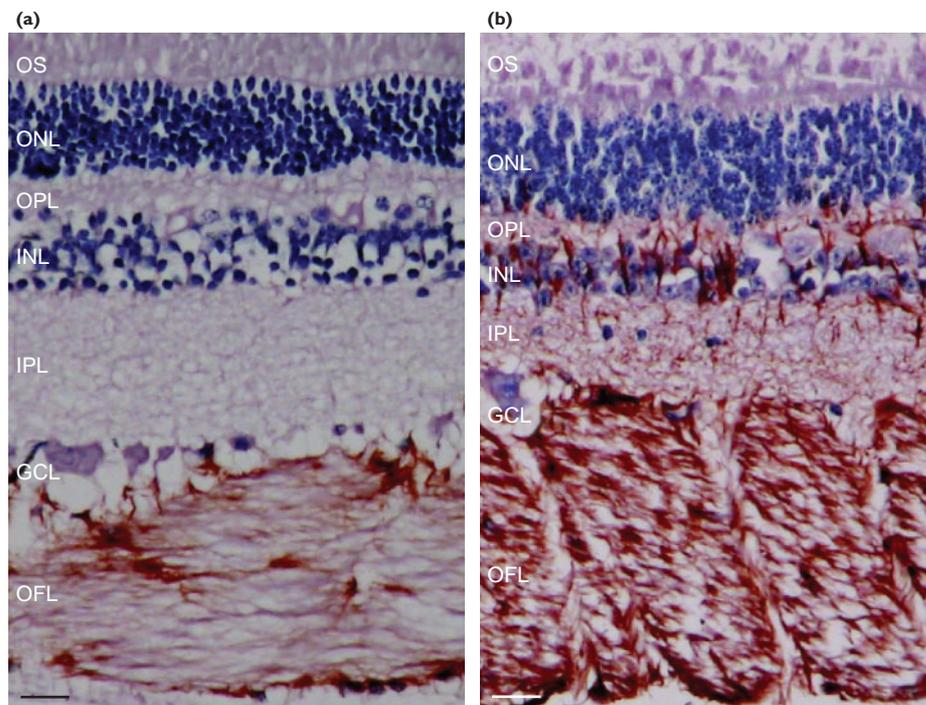


FIG 3: Immunoreactivity for glial fibrillary acid protein (a) is present in the optic fibre layer (OFL) in the retina from a scrapie-free control sheep, (b) is increased in the retina of a scrapie-infected sheep, with positive processes observed with the inner plexiform layer (IPL) and inner nuclear layer (INL). GCL Ganglia cell layer, OPL Outer plexiform layer, ONL Outer nuclear layer, OS Outer segments. Haematoxylin and eosin. Bars=20 µm



photopic tests that were within the range of those obtained for controls (Fig 1a, b), which were consistent with previous reports (Strain and others 1991). The scotopic electroretinogram collected just before the sheep was euthanased due to advanced clinical disease (Fig 1b) revealed decreased b-wave amplitude (54.3 per cent reduction) and prolonged implicit time (86.5 per cent increase) compared with preclinical values (Table 1). A photopic electroretinogram was not detectable (Fig 1d).

Evaluation of haematoxylin and eosin-stained sections from the central retina revealed no significant pathological changes, such as evidence of spongiform change, or pyknotic nuclei (Fig 2a). Immunohistochemistry for PrP^{Sc} (Fig 2b) revealed that the majority of PrP^{Sc} immunoreactivity was observed in the synaptic layers of the retina, with moderate accumulation in the cytoplasm of multifocal retinal ganglion cells. Immunoreactivity for GFAP in the retina of the sheep was increased compared with control retina, with numerous immunoreactive processes present in the inner nuclear layer (Fig 3a, b).

Previous studies have demonstrated PrP^{Sc} accumulation and altered retinal cellular morphology in the retinas of sheep affected with scrapie, but there are no reports regarding the functional impact of scrapie on the ovine retina. Smith and others (2008) have previously demonstrated altered rod bipolar cell and Müller glial cell morphologies in the retinas of scrapie-affected Suffolk sheep, which were lacking overt histological evidence of retinal degeneration. These cell types are responsible for generating the electroretinogram b-wave (Frishman 2006), suggesting that potential alterations in retinal function in scrapie-affected sheep could be detected in the b-wave. The findings in this report confirm this. This report also supports the earlier suggestion that retinal pathology occurs in these animals without overt histological evidence of retinal degeneration. Previous studies, which have clinically evaluated aspects of the visual system in scrapie-affected sheep, have reported an absent menace response and lack of pupillary light reflex in some animals (Healy and others 2003, Vargas and others 2005).

TABLE 1: Scotopic and photopic electroretinogram (ERG) b-wave amplitude and implicit time values for the scrapie-infected sheep during preclinical and clinical stages of disease. Average b-wave values (sd) from eight age-matched, scrapie-free, non-inoculated controls are included for comparison

	Control (sd)	Scrapie-infected sheep	
		Preclinical	Clinical
Scotopic b-wave amplitude (μ V)	405.2 (153.8)	534.7	244.1
Scotopic b-wave implicit time (msec)	26.1 (7.1)	26.0	48.5
Photopic b-wave amplitude (μ V)	178.6 (88.3)	124.5	No recordable ERG
Photopic b-wave implicit time (msec)	12.4 (2.5)	8.0	No recordable ERG

The sheep in this report lacked histological evidence of retinal pathology, despite marked accumulation of PrP^{Sc} and increased expression of GFAP within the retina and evidence of diminished retinal function. A potential mechanism of b-wave alteration in this sheep could be abnormal synaptic function due to accumulation of PrP^{Sc} within the synaptic layers of the retina (inner and outer plexiform layers). The normal cellular form of the prion protein has been implicated in synaptic function (Herms and others 1999, Fournier and others 2000), and within the retina specifically, PrP^{Sc} has been localised to rod photoreceptor and amacrine cell synapses (Gong and others 2007). Additionally, Kovacs and others (2005) have demonstrated colocalisation of PrP^{Sc} and synaptophysin in the cerebral cortices of human beings with sporadic Creutzfeldt-Jakob disease. Accumulation of PrP^{Sc} has also been shown to alter the expression and distribution of synaptic proteins (Ferrer and others 2000, Russelakis-Carneiro and others 2004).

Electroretinography was used to evaluate retinal function in a scrapie-infected Suffolk sheep. The results demonstrate alterations in the electroretinogram b-wave of this sheep when experimentally infected with scrapie. This result, while consistent with previous studies, presents novel information regarding the possible functional consequence of PrP^{Sc} on the retina of sheep affected by scrapie.

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