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Visual abnormalities associated with enhanced optic nerve myelination

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Abstract

Expression of the constitutively active serine/threonine kinase Akt in oligodendrocytes results in enhanced myelination in the CNS. Here, we have examined the effects of this Akt overexpression on optic nerve structure and on optic nerve function, assessed using the visual evoked potential (VEP). Transgenic mice have been generated with the *Plp* promoter driving expression of a modified form of Akt, in which aspartic acids are substituted for Thr308 and Ser473. These *Plp*-Akt-DD (Akt-DD) mice, and littermate controls, were studied at different ages. Optic nerves were examined anatomically at 2 and 6 months of age. At 2 months of age, optic nerves were substantially thicker in Akt-DD mice, reflecting an increase in myelination of optic nerve axons. By electron microscopy, myelin thickness was increased in Akt-DD optic nerve, with extended paranodal domains having excess paranodal loops, and the density of nodes of Ranvier was reduced, relative to control mice. We recorded VEPs in response to strobe flash ganzfeld stimuli presented after overnight dark- and light-adapted conditions at ages ranging from 1 to 10 months. It was possible to record a clear VEP from Akt-DD mice at all ages examined. At 1 month of age, VEP implicit times were somewhat shorter in Akt-DD transgenic mice than in control animals. Beyond 6 months of age, VEP latencies were consistently delayed in Akt-DD transgenic mice. These abnormalities did not reflect an alteration in retinal function as there were no significant differences between ERGs obtained from control or Akt-DD transgenic mice. In young mice, the somewhat faster responses may reflect improved transmission due to increased myelination of

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optic nerve axons. In older mice, where the Akt-DD optic nerve is markedly thicker than control, it is remarkable that optic nerves continue to function.

Keywords

Visual evoked potential; electroretinography; myelination; optic nerve; Akt

1. Introduction

Visual sensation requires the relay of signals generated in the retina to the visual cortex via the optic nerve. Normal optic nerve function requires that individual nerve axons be ensheathed by myelin laid down by oligodendrocytes (Waxman and Bangalore, 2004). As a corollary, demyelination is associated with functional and structural abnormalities of the optic nerve (e.g., Tremain & Ikeda, 1983; Bjartmar & Trapp, 2003; Shindler et al., 2006, 2008; Zandian et al., 2009).

Akt (v-akt murine thymoma viral oncogene homolog) is a serine/threonine kinase, also known as protein kinase B, which we have shown contributes to the regulation of CNS myelination (Flores et al., 2008). This contribution was demonstrated by studying mice in which oligodendrocytes express a transgene encoding a constitutively active form of Akt (Akt-DD), in which the phosphorylation sites used to regulate Akt activity (Thr308, Ser473) have been replaced by aspartic acids (Hutchinson et al., 2001). This constitutively active form of Akt was ideal for these studies, as it was consistently activated, whereas a non-mutated form of the Akt would have been regulated as the endogenous Akt in the oligodendrocyte, and less likely to produce a phenotype. Additionally, this transgene had been used to generate transgenic mice in earlier studies, where it had induced a phenotype, without inducing tumors (Hutchinson et al., 2001), which would be an undesirable outcome for analyzing the impact of Akt regulation of oligodendrocyte function, making such studies uninterpretable. These transgenic mice over-express Akt in oligodendrocytes, at levels that are several fold above normal and that increase with age (cf., Flores et al., 2008). Although Akt-DD mice maintain a normal number of oligodendrocytes, myelination of the optic nerve and CNS white matter is enhanced, and in fact becomes pathologically over produced at older ages and these mice die prematurely, at approximately 14 months of age. The hypermyelination in Akt-DD mice is mediated through increased activation of the mTOR pathway in oligodendrocytes (Narayanan et al., 2009). The increase in myelin thickness has been quantified by X-ray diffraction, and was shown to increase significantly with age, but this could not be quantified from electron micrographs, where fixatives altered the hypermyelinated membrane structure during processing in older animals (Flores et al., 2008). The increased myelin thickness with age is likely a feed-forward regulation, as the Akt is driven by the myelin proteolipid protein (*Plp*) promoter. Thus, as the *Plp* promoter activity increases with myelination, increased Akt is expressed, which in turn increases *Plp* promoter activity to drive increasing levels of Akt indefinitely. As a consequence, the overall thickness of the optic nerve increases with age, although the periodicity of the myelin remains normal, as measured by X ray diffraction (Flores et al., 2008).

The purpose of the present study was to evaluate neural transmission in the presence of hypermyelination. This was accomplished using visual evoked potentials (VEPs) recorded over the visual cortex (Ridder & Nusinowitz, 2006). We report that optic nerve transmission to the visual cortex is maintained in Akt-DD mice, despite a wide array of optic nerve anatomic defects.

2. Results

Figure 1 presents representative electron microscope images of optic nerve cross-sections obtained from non-transgenic (WT) (Fig. 1A, C) or Akt-DD (Fig. 1B, D) mice at 2 months (Fig. 1A, B) or 6 months (Fig. 1C, D) of age. As noted previously (Flores et al., 2008), Akt-DD mice retain a normal number of axons at 2 months of age, but the average thickness of the myelin sheath surrounding each axon is greater than in non-transgenic animals. In comparison to WT, AKT-DD mice continue to generate increasing amounts of myelin (Flores et al., 2008), and in older mice it appears that the excess myelin becomes less tightly associated with compact myelin, although the periodicity of myelin appears normal (Flores et al., 2008), suggesting that there is some compact myelin present. Thus excess myelin is present at 6 months of age, and there is excess myelin membrane, which appears more loosely compacted than in 2-month old Akt-DD mice, when fixed and analyzed by electron microscopy.

The nodal organization of the optic nerve appeared somewhat altered in the Akt-DD mice. Figure 2A presents representative fields of view (FOV) for nodes of Ranvier in 2-month old WT (left) and Akt-DD (right) mice identified using antibodies against sodium channels (green) and caspr (red). Node density appeared decreased in Akt-DD mice, and this was confirmed statistically by quantifying nodes of Ranvier per FOV (Fig. 2B). This reduced density was likely secondary to excess myelination (Fig. 1B), which increased the size of the internode.

In order to assess whether any changes in optic nerve physiology resulted from altered retinal cell function, we tested ERGs of WT and Akt-DD mice, and found no differences at any age examined. Figure 3 presents representative ERGs obtained from 10-month old mice, the oldest age studied. Under both dark- (Fig. 3A) and light-adapted (Fig. 3B) conditions, ERG waveforms of Akt-DD mice were comparable to those of WT littermates. This indicates that hypermyelination does not impair responses of photoreceptors or depolarizing bipolar cells, which provide the primary activity reflected in the a-wave and b-wave, respectively (Robson et al., 2004), and that any abnormality noted in central visual function does not originate in the outer retina. Intensity-response functions for the major ERG components of WT and Akt-DD mice were closely matched at 10-months of age (Fig. 3C, D) and at the younger ages examined (data not shown).

Figure 4A presents representative VEPs obtained from 8-month-old WT and Akt-DD mice under dark- and light-adapted conditions. Under all stimulus conditions, VEPs of WT and Akt-DD mice were similar in waveform and were dominated by a negative polarity component (N1) that peaked 50-80 ms following stimulus presentation (Peachey et al., 1997; Ren et al., 2000; Ridder & Nusinowitz, 2006). Although VEP amplitude changed little

across stimulus intensity, N1 latency shortened with increasing flash intensity (Ren et al., 2000). Figure 4 B-E plots average N1 latency-response functions, for dark-adapted (Fig. 4B, D) and light-adapted (Fig. 4C, E) stimulus conditions. At the ages illustrated (Fig. 4B, C: 1 month; Fig. 4D, E: 8 months), and the other ages examined, N1 implicit time gradually shortened as flash intensity increased. At 1 month of age, the latency functions of Akt-DD mice were shorter than those of WT mice, although the difference was significant for light-adapted (d.f.=1, $F=7.87$, $P=0.010$) but not dark-adapted (d.f.=1, $F=1.002$, $P=0.325$) VEP latency. At 8 months of age, the situation had reversed, and N1 latencies were consistently delayed in Akt-DD mice, as compared to WT littermates (Dark-adapted ERG: d.f.=1, $F=6.93$, $P=0.012$; Light-adapted ERG: d.f.=1, $F=9.53$, $P=0.004$). Figure 4F summarizes how the implicit time difference between Akt-DD and WT mice changed with age. Because the implicit time difference was not intensity dependent, this figure plots the average latency difference across all stimulus intensities between Akt-DD and WT mice as a function of age. At 1 month of age, N1 latency is shorter in Akt-DD mice than in WT littermates. This likely reflects a speeding of the response through the optic nerve due to the additional myelination present at this age (Flores et al. 2008). By 2-4 months of age, this difference had diminished. At later ages, N1 latency was consistently delayed in Akt-DD mice as compared to WT animals.

3. Discussion

The serine/threonine kinase Akt regulates CNS myelination (Flores et al 2008; Meikle et al., 2008; Narayanan et al., 2009), and reduced CNS myelin has been shown to impact motor activities and cognitive functions in different mouse models (Tanaka et al., 2009; Hu et al., 2006). Visual function is clearly dependent on myelination. For example, VEP latencies of *shiverer* mice, which have little or no CNS myelin, are significantly delayed (Lehman & Harrison, 2002; Martin et al., 2006). This pattern matches that seen in patients with optic neuritis (Klistormer et al., 2008; Samsen et al., 2007; Parisi et al., 1999) or multiple sclerosis (Andrade et al., 2007; Watanabe et al., 2009; Papathanasiou et al., 2010; Lascano et al., 2009).

Far less information is available on the impact of enhanced optic nerve myelination on visual function. Our present data indicate that the hypermyelination seen in Akt-DD transgenic mice has a complex influence on transmission through the optic nerve. At younger ages, early in the hypermyelination process, there is a modest decrease in VEP latency, indicative of a faster than normal transmission through the optic nerve. This does not appear to reflect a difference in input into the optic nerve as the function of the outer retina, as reflected in ERG activity, was comparable between WT and Akt-DD mice. Instead, it seems more likely that hypermyelination speeds conduction through the post-retinal visual system.

VEP latency is delayed in older Akt-DD mice. At these ages, the hypermyelination process has advanced and anatomic abnormalities are more severe than in younger mice: the density of nodes of Ranvier remains decreased and the packing of myelin sheaths around the optic nerve is loose. Although the relationship between nodal density and VEP timing has not been defined, it seems likely that this change, along with looser myelin sheath packing, may

act to delay VEP timing. At 2 months of age, VEP latencies of Akt-DD mice were close to normal. At this age, any increase in optic nerve conduction may be compensated for by the decreased density in nodes of Ranvier. Despite the documented anatomical changes in optic nerve structure induced by hypermyelination, a clear albeit delayed signal is still transmitted through the optic nerve up to at least 10 months of age. These results further demonstrate the impact of myelination on VEP, and support the significance of Akt-mediated mechanisms of myelination in remyelination therapy for multiple sclerosis.

4. Experimental procedures

4.1. Mice

The generation and PCR-based identification of Akt-DD transgenic mice studied has been described (Flores et al., 2008). The mice studied here were generated by mating heterozygous Akt-DD transgenic mice with WT animals from the same colony. Transgenic and WT littermates were studied without knowledge of individual genotypes, at 1, 2, 4, 6, 8, and 10 months of age. All experiments involving mice were approved by the Institutional Animal Care and Use Committee of the Cleveland Clinic Foundation.

4.2. Visual Electrophysiology

Initial preparation—After overnight dark adaptation, mice were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg), and their pupils dilated with eyedrops (2.5% phenylephrine HCl; 1% cyclopentolate HCl; 1% mydriacyl). During the recording period, mice were placed on a temperature-regulated heating pad. ERGs and VEPs were recorded in separate sessions, using slightly different procedures.

Recording & Stimulation - ERG—Electroretinograms (ERGs) were used to examine outer retinal function. ERGs were recorded using a stainless steel wire active electrode that contacted the anesthetized (1% proparacaine HCl) corneal surface of the test eye through a layer of 0.8% methylcellulose. Grass platinum needle electrodes placed in the cheek and tail served as reference and ground leads, respectively. Signals were differentially amplified (Gain $\times 10000$, band-pass 1-1,500 Hz), digitized (2,000 Hz), averaged and stored using UTAS-E3000 Electrophysiology System (LKC Technologies, Gaithersburg, MD, USA).

Achromatic strobe flash stimuli were presented in a LKC ganzfeld, under dark-adapted conditions and then superimposed upon a steady adaptation field (23 cd/m²). Under dark-adapted conditions, as stimulus intensity increased from -3.6 to 2.1 log cd s/m², the number of responses averaged decreased (from 10 to 1 at the highest stimulus level), and the interstimulus interval (ISI) increased (from 4 sec to 65 sec). After allowing a 7-min period of light-adaptation, light-adapted ERGs were obtained by averaging the responses to 25 successive flashes presented at 2.1 Hz. In different trials, stimulus intensity ranged from -0.8 to 1.9 log cd s/m².

The amplitude of the a-wave was measured 7 ms after flash onset from the pre-stimulus baseline. The amplitude of the b-wave was measured from the a-wave trough to the peak of the b-wave or, if no a-wave was present, from the pre-stimulus baseline.

Recording & Stimulation - VEP—VEPs were used to evaluate transmission through the visual system and were recorded using three platinum needle electrodes placed subcutaneously. The active electrode was positioned along the midline of the visual cortex; the positions of the reference and ground leads were the same as for ERG recordings and the amplifier ($\times 10K$) band-pass was 1-100 Hz.

VEPs were recorded to achromatic strobe flash stimuli presented in the LKC ganzfeld under both dark- and light-adapted conditions. For dark-adapted VEPs, the ISI ranged from 1.1 to 6 s, increasing with stimulus intensity from -0.2 to 2.1 log cd s/m²; 60 to 20 successive responses were averaged to obtain a VEP waveform. For light-adapted VEPs, a constant ISI of 1.1 s was used and 80 to 70 successive responses were averaged for a series of stimulus intensities (0.0 to 1.9 log cd s/m²) presented superimposed upon the adapting field.

The mouse VEP is dominated by a negative component which is referred to as N1 (Peachey et al., 1997; Ridder & Nusinowitz, 2006). The implicit time of the N1 component was measured at the negative peak.

Response measures obtained from transgenic and non-transgenic mice were analyzed statistically using repeated measures ANOVA. Because the standard error was not constant across all pairs of means, the degrees of freedom were adjusted by multiplying by the degrees of freedom of the Greenhouse-Geisser epsilon, and significance was adjusted accordingly.

4.3. Electron microscopy

Mice were perfused with 2.5% glutaraldehyde/4% paraformaldehyde in 0.08 M Sorenson's buffer. Optic nerves were washed in 0.08 M Sorenson's buffer, postfixed in 1% osmium tetroxide, dehydrated in graded ethanol series, and then embedded in Epon. Ultrathin sections (85 nm) were stained with uranyl acetate and lead citrate. Electron micrographs were taken with a Philips 201 TEM at 60 kV or Philips CM100 TEM at 80 kV.

4.4. Immunocytochemistry

Mice were perfused with 4% paraformaldehyde and the optic nerves were dissected out. Optic nerve cryosections (10 μ m) were washed with PBS and permeabilized using 10% TritonX-100 for 30 min. Blocking was done using 3% normal goat serum/1% BSA in PBS for 1 hour. Cryosections were stained using antibodies that recognize the Na_v1.6 sodium channel (anti-rabbit, Alomone labs, 1:500 dilution) or Contactin associated protein (Caspr; anti-mouse, gift from Dr. Matthew Rasband [Baylor College of Medicine, Houston, TX], 1:1000 dilution.). Sections were incubated at 4°C in primary antibody overnight, washed in PBS and incubated in respective secondary antibodies (1:1000) for 1hr. Samples were then washed and mounted using Vectashield (Vector Laboratories).

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References

- Andrade EP, Sacai PY, Berezovsky A, Salomão SR. Pattern-reversal visual evoked potential abnormalities in patients with defined multiple sclerosis. *Arq Bras Oftalmol.* 2007; 70:943–948. [PubMed: 18235904]
- Bjartmar C, Trapp BD. Axonal degeneration and progressive neurologic disability in multiple sclerosis. *Neurotox Res.* 2003; 5:157–164. [PubMed: 12832230]
- Flores AI, Narayanan SP, Morse EN, Shick HE, Yin X, Kidd G, Avila RL, Kirschner DA, Macklin WB. Constitutively active Akt induces enhanced myelination in the CNS. *J Neurosci.* 2008; 28:7174–7183. [PubMed: 18614687]
- Hu X, Hicks CW, He W, Wong P, Macklin WB, Trapp BD, Yan R. Bace1 modulates myelination in the central and peripheral nervous system. *Nat Neurosci.* 2006; 9:1520–1525. [PubMed: 17099708]
- Hutchinson J, Jin J, Cardiff RD, Woodgett JR, Muller WJ. Activation of Akt (Protein Kinase B) in mammary epithelium provides a critical cell survival signal required for tumor progression. *Mol Cell Biol.* 2001; 21:2203–2212. [PubMed: 11238953]
- Klistorner A, Arvind H, Nguyen T, Garrick R, Paine M, Graham S, O'Day J, Grigg J, Billson F, Yiannikas C. Axonal loss and myelin in early ON loss in postacute optic neuritis. *Ann Neurol.* 2008; 64:325–331. [PubMed: 18825673]
- Lascano AM, Brodbeck V, Lalive PH, Chofflon M, Seeck M, Michel CM. Increasing the diagnostic value of evoked potentials in multiple sclerosis by quantitative topographic analysis of multichannel recordings. *J Clin Neurophysiol.* 2009; 26:316–325. [PubMed: 19752740]
- Lehman DM, Harrison JM. Flash visual evoked potentials in the hypomyelinated mutant mouse shiverer. *Doc Ophthalmol.* 2002; 104:83–95. [PubMed: 11949811]
- Martin M, Hiltner TD, Wood JC, Fraser SE, Jacobs RE, Readhead C. Myelin deficiencies visualized in vivo: visually evoked potentials and T2-weighted magnetic resonance images of shiverer mutant and wild-type mice. *J Neurosci Res.* 2006; 84:1716–1726. [PubMed: 17044038]
- Meikle L, Pollizzi K, Egnor A, Kramvis I, Lane H, Sahin M, Kwiatkowski DJ. Response of a neuronal model of tuberous sclerosis to mammalian target of rapamycin (mTOR) inhibitors: effects on mTORC1 and Akt signaling lead to improved survival and function. *J Neurosci.* 2008; 28:5422–5432. [PubMed: 18495876]
- Narayanan SP, Flores AI, Wang F, Macklin WB. Akt signals through the mammalian target of rapamycin pathway to regulate CNS myelination. *J Neurosci.* 2009; 29:6860–6870. [PubMed: 19474313]
- Papathanasiou ES, Pantzaris M, Myrianthopoulou P, Kkolou E, Papacostas SS. Brainstem lesions may be important in the development of epilepsy in multiple sclerosis patients: An evoked potential study. *Clin Neurophysiol.* 2010 e-pubmed June 11, 2010.
- Parisi V, Manni G, Spadaro M, Colacino G, Restuccia R, Marchi S, Bucci MG, Pierelli F. Correlation between morphological and functional retinal impairment in multiple sclerosis patients. *Invest Ophthalmol Vis Sci.* 1999; 40:2520–2527. [PubMed: 10509645]
- Peachey NS, Roveri L, Messing A, McCall MA. Functional consequences of oncogene-induced horizontal cell degeneration in the retinas of transgenic mice. *Vis Neurosci.* 1997; 14:627–632. [PubMed: 9278992]
- Ren JC, LaVail MM, Peachey NS. Retinal degeneration in the nervous mutant mouse. III. Electrophysiological studies of the visual pathway. *Exp Eye Res.* 2000; 70:467–473. [PubMed: 10865995]
- Ridder WH 3rd, Nusinowitz S. The visual evoked potential in the mouse--origins and response characteristics. *Vision Res.* 2006; 46:902–913. [PubMed: 16242750]
- Robson JG, Maeda H, Saszik SM, Frishman LJ. *In vivo* studies of signaling in rod pathways of the mouse using the electroretinogram. *Vision Res.* 2004; 44:3253–3268. [PubMed: 15535993]
- Samsen P, Chuenkongkaew WL, Masayaanon P, Chirapapaian N, Ruangvaravate N, Loket S. A comparative study of visual evoked potentials in optic neuritis and optic neuritis with multiple sclerosis. *J Med Assoc Thai.* 2007; 90:313–318. [PubMed: 17375637]

- Shindler KS, Guan Y, Ventura E, Bennett J, Rostami A. Retinal ganglion cell loss induced by acute optic neuritis in a relapsing model of multiple sclerosis. *Multiple Sclerosis*. 2006; 12:526–532. [PubMed: 17086896]
- Shindler KS, Kenyon LC, Dutt M, Hingley ST, Das Sarma J. Experimental optic neuritis induced by a demyelinating strain of mouse hepatitis virus. *J Virol*. 2008; 82:8882–8886. [PubMed: 18579591]
- Tanaka H, Ma J, Tanaka KF, Takao K, Komada M, Tanda K, Suzuki A, Ishibashi T, Baba H, Isa T, Shigemoto R, Ono K, Miyakawa T, Ikenaka K. Mice with altered myelin proteolipid protein gene expression display cognitive deficits accompanied by abnormal neuron-glia interactions and decreased conduction velocities. *J Neurosci*. 2009; 29:8363–8371. [PubMed: 19571127]
- Tremain KE, Ikeda H. Physiological deficits in the visual system of mice infected with Semliki Forest virus and their correlation with those seen in patients with demyelinating disease. *Brain*. 1983; 106:879–895. [PubMed: 6317139]
- Watanabe A, Matsushita T, Doi H, Matsuoka T, Shigeto H, Isobe N, Kawano Y, Tobimatsu S, Kira J. Multimodality-evoked potential study of anti-aquaporin-4 antibody-positive and -negative multiple sclerosis patients. *J Neurol Sci*. 2009; 281:34–40. [PubMed: 19339021]
- Waxman, SG.; Bangalore, L. Electrophysiological consequences of myelination. In: Lazzarini, RA., editor. *Myelin biology and disorders*. San Diego, CA: Elsevier Academic Press; 2004. p. 117-141.
- Zandian M, Belisle R, Mott KR, Nusinowitz S, Hofman FM, Ghiasi H. A recombinant HSV-1 expressing murine interleukin-2 induces optic neuritis in different strains of mice. *Invest Ophthalmol Vis Sci*. 2009; 50:3275–3282. [PubMed: 19234357]

Research Highlights

- Expression of serine/threonine kinase Akt in oligodendrocytes results in enhanced myelination
- At 1 month of age, VEP implicit times were shorter in Akt-DD transgenic than in WT mice
- Beyond 6 months of age, VEP latencies were delayed in Akt-DD transgenic than in WT mice
- At 2 months of age, Akt-DD mice showed an increase in myelination for each optic nerve axon
- At 2 months of age, Akt-DD mice showed reduction of density of nodes of Ranvier

Figure 1.

Representative electron micrographs of optic nerve cross-sections obtained from WT (A, C) or Akt-DD (B, D) mice at two (A, B) or 6 months of age (C, D). The myelin sheaths surrounding optic nerve axons are thicker in two-month old Akt-DD mice than in WT animals. The myelination process continues, and the myelin sheaths of six-month old Akt-DD mice are excessive and only loosely organized around some axons. Three animals were studied for each genotype. Magnification: 10,000 $\times$.

Figure 2.

Density of nodes of Ranvier. (A) Representative images of nodes of Ranvier in optic nerve sections of WT (left) or Akt-DD (right) mice aged 2 months. Sections were stained using antibodies against sodium channel $\text{Na}_v1.6$ to label nodes (green) and against Caspr (red) to label paranodes. (B) Quantification of the number of nodes in the optic nerve. Confocal microscope images (40 $\times$) were taken and the number of nodes having paranodes on both sides were quantified manually per field of view (FOV). There was a significant decrease ($p < 0.001$) in the nodal density in Akt-DD mice, relative to WT. A minimum of three sections were measured per animal. Bars indicate average ($\pm$ s.d.) results for three animals for each genotype.

Figure 3.

Representative ERGs obtained from representative 10-month old mice under dark-adapted (A) and light-adapted (B) conditions. Intensity-response functions for dark-adapted a- and b-wave (C) or light-adapted ERG (D). Data points indicate average ($\pm$ s.d.) for 12 WT and 17 Akt-DD mice.

Figure 4.

(A) Representative VEPs obtained from representative 8-month old WT and Akt-DD mice under dark-adapted (left) and light-adapted (right) conditions. (B-E) Intensity-response functions for N1 implicit times obtained under dark-adapted (B, D) or light-adapted (C, E) conditions from 1-month old (B, C) or 8-month old (D, E) mice. In (B, C), data points indicate average ($\pm$ s.d.) for 30 WT and 19 Akt-DD mice tested at 1 month of age. In (D, E), data points indicate average ($\pm$ s.d.) for 19 WT and 20 Akt-DD mice tested at 8 months of age. (F) Summary of age-related N1 implicit time changes in Akt-DD mice. Each data point indicates the difference between the average N1 implicit times for Akt-DD mice relative to that for WT mice. Dark-adapted data are represented by open symbols; light-adapted data are represented by filled symbols. Asterisks indicate ages where have significant differences in implicit times were noted between AKT and WT mice.