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Review

Mechanisms of electrical stimulation in eye diseases: A narrative review

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ABSTRACT

Background: In the last two decades, electrical stimulation (ES) has been tested in patients with various eye diseases and shows great treatment potential in retinitis pigmentosa and optic neuropathy. However, the clinical application of ES in ophthalmology is currently limited. On the one hand, optimization and standardization of ES protocols is still an unmet need. On the other hand, poor understanding of the underlying mechanisms has hindered clinical exploitation.

Main Text: Numerous experimental studies have been conducted to identify the treatment potential of ES in eye diseases and to explore the related cellular and molecular mechanisms. In this review, we summarized the in vitro and in vivo evidence related to cellular and tissue response to ES in eye diseases. We highlighted several pathways that may be utilized by ES to impose its effects on the diseased retina.

Conclusions: Therapeutic effect of ES in retinal degenerative diseases might through preventing neuronal apoptosis, promoting neuronal regeneration, increasing neurotrophic factors production in Müller cells, inhibiting microglial activation, enhancing retinal blood flow, and modulating brain plasticity. Future studies are suggested to analyse changes in specific retinal cells for optimizing the treatment parameters and choosing the best fit ES delivery method in target diseases.

1. Introduction

Electrical stimulation (ES) is a non-pharmacological treatment in which a microcurrent is delivered to the target tissues. ES is suggested to directly impose various biochemical effects on the cells, such as disrupting extracellular structured water, generating electroosmotic fluid flow on the cell surface, modulating cell membrane potential and opening voltage gated channels, imposing mechanical forces on the tension sensitive components, and changing the distribution of membrane components and lipid rafts.[1] These effects facilitate ES to interfere with various pathological processes. For example, ES has been clinically applied in pain mitigation and wound healing. Transcranial ES treatment provided both functional and structural preservations in brain diseases, including depression, autism spectrum disorder, stroke, traumatic brain injury, Alzheimer's disease, and Parkinson's disease.[2] In ophthalmology, ES was firstly applied by Charles LeRoy in 1755 to induce phosphene in a patient with cataract-induced blindness.[3] However, it was in 2002 when the treatment potential of ES was first reported by Morimoto et al. ES was applied at the cutting end of the rat optic nerve and enhanced the survival of the axotomized retinal ganglion cells (RGCs).[4] Two years later, the first clinical trial study by Chow et al. confirmed the protective effect of retina microchip in retinitis pigmentosa (RP) patients.[5] From then on, emerging studies have been conducted to explore the therapeutic potential of ES in eye diseases.

We did a thorough and comprehensive literature search with the ending time in January 2022 and identified 29 experimental studies and 25 clinical trials investigating ES treatment in eye diseases. Our previous systematic review summarized the clinical evidence of ES treatment in blind leading eye diseases and highlighted the effective ES parameters used on RP and optic neuropathy patients.[6] To apply ES-based therapy to cure retinal neurodegenerative diseases and improve biological processes like neuroregeneration, there is an utmost need to unveil the mechanism of how exactly cell behaves in the electrical field. To date, animal studies have generated a great body of evidence indicating the treatment

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effectiveness of ES as well as the mechanisms in both photoreceptor and RGC degeneration (Summarized in Table 1). In this review, we made a summary of both clinical and experimental evidence related to the cellular and molecular changes induced by ES in eye diseases and proposed the major pathways leading to retinal neuroprotection.

2. Methods to induce ES for vision protection

Hitherto, ES has been applied to treat clinical patients with RP, age-related macular degeneration (AMD), optic neuropathy, glaucoma, and retinal artery occlusion (RAO).[6] The parameters of the electric current, such as pulse frequency, current strength, and treatment duration, were proved to be influencing factors of ES therapeutic effects.[6] In addition, the method to deliver the current to the eyeball is suggested to be critical for the treatment outcome by affecting the distribution of the current in the eyeball. For the treatment of clinical patients, electric current was delivered to the eye through transcorneal ES (TcES), transpalpebral ES (TpES), transdermal ES(TdES), and repetitive transorbital alternating current stimulation (rtoACS), defined by the location of the electrodes. TcES was the most used method to treat RP, AMD, glaucoma, and RAO, while rtoACS was commonly used in treating optic neuropathy patients in multiple clinical trials.[6] In animal experiments, ES was used to treat animal models with photoreceptor cell degeneration [Rhodopsin-deficient mice, rd10 mice, P23H-1 rats, *N*-methyl-*N*-nitrosourea (MNU) treated mice, rd/rd mice, rhodopsin P347L transgenic rabbits, light-induced photoreceptor degeneration, mer^{kd} mice, RCS rats], RGC degeneration [DBA/2J mice, acute ocular hypertension (AOH), optic nerve crush/transection (ONC/T), and nonarteritic ischemic optic neuropathy (NAION)] (Table 1). The electric current was delivered to the animal eyes through various routes (Fig. 1) including TcES, TpES, subretinal ES, or direct stimulation on the optic nerve at the proximal injury site. These animal studies provided valuable evidence for the effectiveness of ES in preserving retinal neurons and proposed various underlying mechanisms.

3. ES effects in healthy retina

To understand the underlying mechanisms of ES treatment in eye diseases, a valuable strategy is to observe the effects it imposes on the healthy retinal tissue. In 2011, Willmann et al. analyzed transcriptome changes in healthy rat retina at 4 h post TcES (1 ms/phase, 20 Hz, 200 μA, 60min) treatment.[35] 204 genes were downregulated while 286 were upregulated, which affected cellular processes in tissue development, cell signaling, inflammatory response, cellular growth, proliferation, and cell death mediation. A remarkable change was the downregulation of B-cell lymphoma protein 2 (Bcl-2)-associated X (Bax), a proapoptotic member of the Bcl-2 family. In addition, TcES differentially altered members of the tumor necrosis factor family which has a proapoptotic effect on retinal neurons.[36] These results suggested the potential of TcES in inhibiting apoptosis.

A further study by Kanamoto et al. provided us with more knowledge in ES effects on the retina.[37] The researchers applied TcES to the healthy rat eyes with parameters previously proved to be effective in treating rat eyes with ONC (1 ms/phase, 20 Hz, 30 min).[37,25] Proteomic changes were analyzed at current strength of 50, 100, and 200 μA, at 30 min or 24 h after treatment. 100 and 200 μA TcES induced a relatively greater number of changed proteins than 50 μA, indicating a current strength dependent effect. 15 proteins were increased at 30 min while 20 proteins were increased at 24 h after TcES, suggesting differences existed between acute and chronic ES effects. Out of the total 25 changed proteins, there are 6 physiological factors, 9 cellular signaling molecules, 3 metabolic proteins, 2 immunological proteins, and 5 structural proteins. Specifically, the "after-effect" at 24 h involved neuronal synaptic agents, proteins related to Ca^{2+} regulation, and neuronal regenerative factors, indicating a lasting effect by ES on synaptic transmission, intracellular Ca^{2+} regulation, and retinal regeneration.

4. ES prevent neuronal apoptosis and promote neuronal regeneration

Gene expression analysis of the normal rat eyes treated by TcES revealed its potential to inhibit neuronal apoptosis.[35] This idea was supported by multiple animal studies applying ES to treat photoreceptor degeneration. Bax and Bcl-2 are recognized as a promoter and an inhibitor of apoptosis, respectively.[38] Upregulation of Bcl-2 and downregulation of Bax were induced by TcES in the retina of MNU treated mice and bright blue light exposed rats, both known as photoreceptor cell degeneration models.[10,17] Inhibition of photoreceptor apoptosis by ES was further validated by decreased TUNNEL stained cells.[13,20] Currently, the mechanisms underlying apoptosis inhibition by ES are still poorly understood. Further investigation is warranted to validate whether ES may directly interfere with the apoptosis related pathways in the neurons and/or indirectly affect it through upregulating apoptosis inhibiting factors or downregulating apoptosis promoting factors.

Neurotrophic factors (NTFs) are a group of proteins that act as a promoter of the survival, development, and regeneration of neurons.[39,40] In both the central and peripheral nervous system, NTFs activate multiple pathways to regulate the apoptosis of neurons by binding to p75NTR.[41] Previous studies proved that ES was effective in promoting axonal and neuronal regeneration in peripheral nerve trauma, spinal cord injury, and multiple brain diseases, with increased NTFs as a common feature.[42–45] In fact, NTFs, such as brain-derived neurotrophic factor (BDNF), could be a critical factor contributing to neuronal preservation by ES.[46] Depending on the disease models studied and the methods of electric current delivery, different kinds of NTFs might be involved in the retinal neuroprotection. In the animals with photoreceptor degeneration, TcES upregulated BDNF and ciliary neurotrophic factor (CNTF) in the retina of MNU treated mice[17] and bright blue light exposed rats,[10] TpES upregulated basic fibroblast growth factor (bFGF) signaling in Rhodopsin-deficient mice with photoreceptor degeneration,[20] and sub-retinal ES (SES) elevated bFGF and CNTF in the retina of the mer^{kd} mice and RCS rats.[12,11] Meanwhile, in the rats with ONC induced RGC degeneration, TcES was demonstrated to upregulate insulin-like growth factor 1 (IGF-1) and enhance RGC survival and axonal regeneration.[21,24]

NTFs are known to be produced by glial cells, including astrocyte, microglia, and oligodendrocytes in the injury and disease models.[47] It was reported that in vitro ES at an amplitude of 300 mV and a frequency of 10 Hz for 1 h was able to increase nerve growth factor (NGF) secretion by 6-fold in astrocytes.[48] Similarly, Enayati et al. reported that in vitro ES at a current intensity of 300 μA and a frequency of 20 Hz for 1 h increased gene expression of CNTF by nearly 3-fold in Müller cells, the primary type of glial cells in the retina, and the effect disappeared when L-type voltage-dependent calcium channels were blocked.[49] In vitro ES (20 Hz, 10 mA, 30min) was also reported to upregulate IGF-1 and BDNF in Müller cells by activating L-type voltage-dependent calcium channels.[50,51] In TcES treated rats with ONC, the increased IGF-1 was co-localized with glutamine synthetase, a specific marker of Müller cells.[21,24] These studies indicate Müller cells as a potential source of increased NTFs in ES treatment of eye diseases though more evidence is warranted to validate it.

5. ES inhibit microglial activation

Microglia are the major resident immune cells in the central nervous system (CNS), including the retina. Microglial activation is known to be involved in various brain and retinal diseases, playing either protective or detrimental roles depending on the disease circumstances.[52] In brain diseases such as stroke, traumatic brain injury, Alzheimer's Disease, and Parkinson's Disease, ES was proved to inhibit microglial activation in animal models or clinical patients.[53–56] In the case of eye diseases, amounting evidence has been generated about ES effects on microglial activation in animal models with ON and RGC degeneration.[30,32,33] In Yin et al.'s study, TcES in rat eyes with ONT significantly promoted RGC

Table 1
Summary of animal studies.

References	Animal model	Related disease	ES type	Position of Active/ Reference electrode	Control group	Current parameters	Treatment duration	Evaluation methods	Results
Pardue et al., 2005[7]	RCS rats	RP or AMD	SES	Microphotodiode array implant in the subretinal space	Rats with no surgery; rats with sham surgery; rats with inactive implant;	Subretinal implantation of microphotodiode arrays producing currents from several nA/cm^2 to 1 μA/cm^2, 120 Hz	Microphotodiode array implant at 3 weeks of age	ERG recordings; histology	SES increased b-wave amplitude at 4–6 weeks after surgery but not at 8 weeks; both active and inactive implants increased number of photoreceptors
Morimoto et al., 2007[8]	RCS rats	RP or AMD	TcES	Cornea/NA	Contralateral eyes with sham treatment or no treatment	Biphasic rectangular pulses, 1 ms/phase, 20 Hz, 50 μA or 100 μA	60 min/session, one session per week for 2, 4, and 6 weeks from 3 weeks of age to 5, 7, and 9 weeks of age respectively	ERG; histology	ES at 100 μA increased the thickness of ONL at each time point; ES preserved retinal function at 5 and 7 but not at 9 weeks of age
Schmid et al., 2009[9]	RCS rats	RP or AMD	TRES	Retinal explant on MEA with ganglion cell side up	Healthy retinas; sham	Monophasic, anodic voltage impulses, 500 μs/phase, 20 Hz, variable amplitude at 1 V, 2 V and 3 V (Charge density at 101, 260 and 428 μC/cm^2)	Continuous ES for 1, 2 or 5 days	IHC; TUNEL assay	ES reduced apoptosis of neurons in the INL and decreased microglial activation after 1 day of ES
Ni et al., 2009[10]	light-induced photoreceptor degeneration in SD rats	RP or AMD	TcES	Cornea/Ipsilateral subcutaneous tissue	Healthy rats; sham	Pre-TcES: biphasic rectangular wave pulses, 3 ms/phase, 20–100 Hz, 100–500 μA; post-TcES: biphasic rectangular wave pulses, 3 ms/phase, 20 Hz, 200 μA or 300 μA	Pre-TcES: 90 min/session, one session before exposure to intense light; post-TcES: 60 min/session, one session every 3 days for up to 14 days	ERG; histology; IHC; qRT-PCR; WB	Both pre- and post-TcES increased ONL thickness; photoreceptors rescue by ES was current strength and frequency dependent; post-TcES showed a better and longer-term protective effect than pre-TcES; ES upregulated CNTF, BDNF and Bcl-2, while downregulated Bax
Ciavatta et al., 2009[11]	RCS rats	RP or AMD	SES	Microphotodiode array implant in the subretinal space	Rats with no surgery; rats with sham surgery; rats with inactive implant;	NA	Microphotodiode array implant at 21 days of age	ERG; RT-PCR	ES increased amplitudes of dark- and light-adapted ERG b-waves at 4 weeks post surgery, and promoted FGF2 production at 1 week and 4 weeks post surgery
Mocko et al., 2011[12]	*mer^{kd}* mice	RP or AMD	SES	Microphotodiode array implant in the subretinal space	Contralateral eye; no surgery	Subretinal microphotodiode array producing currents from several nA/cm^2 to 1 μA/cm^2	Microphotodiode array implant at 14 days of age	ERG; histology; RT-PCR	SES failed to change reitnal function and photoreceptor numbers, but increased FGF2 and CNTF expressions at 1 week post surgery
Schatz et al., 2012[13]	light-induced photoreceptor degeneration in SD rats	RP or AMD	TcES	Cornea/NA	Healthy rats; sham	Biphasic rectangular pulses, 2 ms/phase, 20 Hz, 200 μA	60 min/session, ES at 2 h before exposure to bright light	ERG; histology; IHC; TUNEL assay	ES increased luminance function parameter *Vmax* at 1 week, decreased b-wave implicit time for the rod response at 2 weeks, increased ONL thickness, reduced photoreceptor cell death, and preserved outer segment length
Morimoto et al., 2012[14]	rhodopsin P347L transgenic (Tg) rabbits	RP	TcES	Cornea/NA	Sham treated contralateral eyes	Biphasic rectangular current pulses, 10 ms/phase, 20 Hz, 700 μA	60 min/session, one session per week for 6 weeks	ERG; histology; IHC	ES elevated a- and b-wave amplitudes of the photopic ERGs and b-wave amplitudes of the scotopic ERGs, increased ONL thickness, and improved antirhodopsin and peanut agglutinin immunostaining intensities in rod and cone photoreceptors respectively
Cameron et al., 2013[15]	C57BL/6J-Pde6bto^{-2J}/J (rd/rd) mice	RP	TRES	Circular platinum electrode placed behind the	Healthy retinas	For each cell, ES intensity was increased until a clearly	ES was repeated forty times	Whole–cell current and voltage clamp recordings	ES induced activation of both voltage-gated Na+ channels and K+ channels; a large amplitude

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Table 1 (*continued*)

References	Animal model	Related disease	ES type	Position of Active/ Reference electrode	Control group	Current parameters	Treatment duration	Evaluation methods	Results
				photoreceptor layer (wild type) or behind the INL (rd/rd)		measurable response could be repeatedly observed			oscillation existed in the majority of INL cells in rd/rd mice
Rahmani et al., 2013[16]	P23H-1 rats	RP	TcES	Cornea/Between the cheek and gums	Sham	Sine wave current, 5 Hz, 1.5 μA peak to peak,	30 min/session, two sessions per week for 12 weeks	ERG; histology	ES increased b-wave amplitudes and rod sensitivity, but failed to preserve the number or gross structure of rods
Tao et al., 2016[17]	*N*-methyl-*N*-nitrosourea (MNU) treated C57/BL mice	RP	TcES	Cornea/Between the eyes	Heathy mice; sham	Biphasic rectangular pulses, 20 Hz, 100 μA or 200 μA	60 min/session, ES on day 1, 3, 6 post MNU injection	ERG; MEA; histology; IHC; qRT-PCR	Both 100 μA and 200 μA ES increased scotopic and photopic b wave, promoted photoreceptor survival, improved the efficiency of visual signal transmission, downregulated mRNA levels of calpain2 and Bax while upregulated Bcl-2, BDNF and CNTF
Hanif et al., 2016[18]	P23H-1 rats	RP	TcES	Cornea/Between the cheek and gums	Sham	Sine wave current, 5 Hz, 4 μA peak to peak,	30 min/session, two sessions per week for 20 weeks	Optokinetic tracking; ERG; histology; qRT-PCR	ES increased spatial frequency thresholds at all time points, improved inner retinal function at 8 and 12 weeks, increased RGC numbers at 20 weeks, and improved BDNF, caspase 3, FgF2, and glutamine synthetase levels at 1 h but not 24 h post treatment
Agagdaba et al., 2020[19]	rd10 mice	RP	TcES	Cornea/Skin in close proximity to the stimulated eye	Sham	Biphasic square pulses; 2 ms/phase; 2, 10, 20 Hz; 400 μA	30 min/session, one session each day for 5 days	EcOG recordings	ES modulated brain oscillations in a frequency and brain state (awake or anaesthetised)-dependent manner
Yu et al., 2020[20]	Rhodopsin-deficient mice	RP	TpES	Upper and lower eyelid/Abdomen	Contralateral eyes receiving no treatment (naive) or a sham procedure (sham)	Positive monophasic rectangular pulse trains followed by negative monophasic rectangular pulse trains; increasing frequencies from 2 pulls per second (PPS) to 200 PPS; 100 μA	4 spots on the eyelids, 40 s per spot every session, one session each day for 7 consecutive days	ERG recordings; IHC	ES improved retinal function temporarily and the effect could be prolonged by additional ES sessions; ES promoted photorecetor cell survival, induced Müller cell proliferation and migration toward the ONL and transdifferentiation into photoreceptor cells, and upregulated FGF2 signaling
Morimoto et al., 2002[4]	ONT in wistar rats	ONI/ Glaucoma	ES of transected optic nerve	On the end of optic nerve stump	Healthy rats; sham	Monophasic pulses, 0.05 ms/phase, 20 Hz, 0, 20, 30, 50 or 70 μA	2 h/session; ES within 10 min after ONT	RGC counting	ES at 30, 50 and 70 μA enhanced the survival of axotomized RGCs on day 7 after ONT
Morimoto et al., 2005[21]	ONT in wistar rats	ONI/ Glaucoma	TcES	Cornea/Nil	Healthy rats; sham	Biphasic rectangular pulses, 0.5, 1, or 3 ms/phase, 20 Hz, 100 μA	60 min/session; ES immediately after ONT	Histology; IHC; qRT-PCR; WB	ES enhanced the survival of axotomized RGCs in a pulse duration dependent way on day 7 after ONT; ES increased IGF-1 expression in Müller cells
Miyake et al., 2007[22]	ONC in Long-Evans rats	ONI/ Glaucoma	TcES	Cornea/Nil	Healthy rats; sham	Biphasic square pulses, 50 μs/phase, 20 Hz, 500 μA	TcES immediately after ONC for 6 h (5 animals)	Histology; ERG; VEP recording	ES increased VEP amplitude, which was preserved for 1 week, and enhanced the number of retinal axons projected centrally beyond the crushed region
Okazaki et al., 2008[23]	ONT in wistar rats	ONI/ Glaucoma	ES of transected optic nerve	On the proximal stump of the optic nerve	Healthy rats; sham	Pre-ES: monophasic square pulses, 50 μs/phase, 20 Hz, 300 μA, 60 min/session; Post-	Pre-ES: ES at 3 h or right before ONT; Post-ES: ES	Histology	ES for 30 min immediately after ON transection was sufficient to promote RGC survival; 20 Hz but

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Table 1 (*continued*)

References	Animal model	Related disease	ES type	Position of Active/ Reference electrode	Control group	Current parameters	Treatment duration	Evaluation methods	Results
						ES: monophasic square pulses, 50 μs/phase, 10, 20, and 50 Hz, 50 μA, 10, 30, 60, and 120 min/session	Immediately or 3 h after ONT		not 10 Hz and 50 Hz was effective to promote RGC survival; ES at 1 h before or immediately after ONC was effective to promote RGC survival, indicating a short time-window
Tagami et al., 2009[24]	ONC in wistar rats	ONI/ Glaucoma	TcES	Cornea/NA	Healthy rats; sham	Biphasic rectangular pulses, 1 ms/phase, 20 Hz, 100 μA,	60 min/session, 4 protocols were used: single application on immediately after ONC (day 0); two applications on days 0 and 7; four applications on days 0, 4, 7, 10; daily applications on days 0–12	Axonal Growth quantification; RGC counting; histology; IHC	ES applied daily increased number of regenerating axons, promoted IGF-1 production, and enhanced RGCs survival at 12 days after ONC
Morimoto et al., 2010[25]	ONT in wistar rats	ONI/ Glaucoma	TcES	Cornea/NA	Healthy rats; sham	Biphasic rectangular pulses, various parameters were tested: pulse durations of 0.5,1, 2, 3, and 5 ms/phase, 20 Hz, 100 μA, 60 min; current intensities of 50,100, 200, 300 and 500 μA, 1 ms/phase, 20 Hz, 60 min; frequencies of 0.5, 1, 5, 20, 50, and 100 Hz at 100 μA, 1 ms/phase, 60 min; stimulation duration of 15, 30, and 60 min at 100 μA, 1 ms/ phase, 20 Hz; waveform changed from symmetrical, asymmetrical, and symmetrical with an inter-pulse interval of 0.5 ms or 1 ms at 100 μA, 1 ms/phase, 20 Hz, 60 min	single session immediately after ONT (day 0) or repeated sessions on days 0, 4, 7, and 10 after ONT	RGC counting	Optimal neuroprotection was observed at pulse duration of 1 and 2 ms/phase, current intensity of 100 and 200 μA, and stimulation frequency of 1, 5, and 20 Hz; more than 30 min of ES was required to have a neuroprotective effect; Symmetric pulses without an inter-pulse interval were most effective; Repeated ES was more neuroprotective than a single ES
Wang et al., 2011[26]	Retinal ischemia in SD rats	AOH	TcES	Cornea/Ipsilateral forehead	Healthy rats; sham	Biphasic rectangular pulses, 3 ms/phase, 20 Hz, 300 μA	60 min/session, one session immediately after ischemic insults and thereafter one session every 2 days until day 14	ERG; histology; IHC; WB	ES increased RGC density at day 7 and 14, increased the thickness of the inner limiting membrane to outer limiting membrane, IPL and ONL, improved the amplitude of scotopic b-wave, and elevated glutamine synthetase expressions
Sergeeva et al., 2012[27]	ONC in rats	ONI/ Glaucoma	rtcACS	Cornea/Ear	Healthy rats	Biphasic square pulses, 1 ms/ phase, stimulation trains of 30 s were delivered at different frequencies in the following order: 10, 12, 9, 11, 8, 10, 9, 12 Hz with 10 s breaks between each, followed by a 2-min break and another series in the same order, 100 μA	12 min/session, two sessions at one week after ONC	EEG	ES significantly increased theta power with a parallel shift of the dominating peak to higher frequency in the normal rats but not in the ONC rats
Henrich-Noack et al., 2013A[28]	ONC in Lister Hooded Rat	ONI/ Glaucoma	rtcACS	Cornea/Ear	Healthy rats; sham	Biphasic square pulses, 1 ms/ phase, stimulation trains of 30 s were delivered at different frequencies in the following	ES immediately after ONC and on days 3, 7, 11, 15, 19, 23 post ONC	Brightness discrimination; in vivo confocal neuroimaging; EEG	ES promoted neuronal survival on day 28 post ONC, but failed to change brightness

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Table 1 (*continued*)

References	Animal model	Related disease	ES type	Position of Active/ Reference electrode	Control group	Current parameters	Treatment duration	Evaluation methods	Results
						order: 10, 12, 9, 11, 8, 10, 9, 12 Hz with 5 s breaks between each, followed by a 2-min break and another series in the same order, 100 μA		recordings; RGC counting	discrimination and EEG power spectra
Henrich-Noack et al., 2013B[29]	ONC in Lister Hooded Rats	ONI/ Glaucoma	TcES	Cornea/Ear	Healthy rats; sham	Biphasic square pulses, 1 ms/ phase, 20 Hz, 100 μA	60 min/session; ES immediately after ONC and on day 11 post ONC	In vivo confocal neuroimaging	ES promoted RGC survival on day 3 but not on day 7 and 15, and reduced ONC-associated neuronal swelling and shrinkage especially in RGCs
Yin et al., 2016[30]	ONT in SD rats	ONI/ Glaucoma	TcES	Cornea/Ipsilateral forehead	Healthy rats; sham	Biphasic rectangular pulses, 2 ms/phase, 20 Hz, 200 μA	60 min/session, ES immediately after ONT and on day 4, 7, 10 after ONT	Histology; IHC; WB	ES promoted RGC survival, reduced Iba-1+ microglial number on day 7 but not day 14, and decreased TNF-α production on day 7 and 14 after ONT
Henrich-Noack et al., 2017[31]	ONC in Lister Hooded Rats and B6.Cg-Tg(Thy1-YFP)HJrs/J transgenic mice	ONI/ Glaucoma	rtcACS	Rats: Cornea/Tail. Mice: Cornea/ Contralateral ear	Healthy rats, mice; sham	Rat: biphasic square pulses, 10 ms/phase,varing frequencies (5 Hz ± 3 Hz), 200 μA. Mice: biphasic square wave, 1 ms/phase, varing frequencies (10 Hz ± 2 Hz), 100 μA	Rats: 23 min/session; ES immediately post ONC and on day 4. Mice:24 min/ session; ES immediately post ONC and on day 3, 6, 9, 12	In vivo Confocal Neuroimaging; Visual Evoked Potentials	ES promoted RGC survival, and induced dendritic pruning and abolished cell signaling in surviving neurons
Fu et al., 2018[32]	Ocular ischemia on mongolian gerbils,	AOH	TcES	Cornea/NA	Healthy gerbils; sham	Bipolar rectangular pulses; 1 ms/phase; 20 Hz; 100 μA	60 min/session; one session immediately after IOP elevation (day 1) and one session at day 4, followed by 2 sessions each week for 1 month	ERG recordings; IHC; WB; qRT-PCR	ES increased scotopic b wave and photopic PhNR amplitude, promoted RGC survival, decreased Iba-1+ microglial cell number, increased IL-10 expression while reduced IL-6 and COX-2 expression as well as NF-κB phosphorylation
Jassim et al., 2021[33]	DBA/2J mice	Secondary glaucoma	TcES	Cornea/Back of the neck	Young age DBA/ 2J mice; age matched non-stimulated DBA/ 2J mice	Biphasic square pulses; 1 ms/ phase; 20 Hz; 100 μA	10 min/session, one session every 3 days for 8 weeks from 10 to 12 months of age	C-fos activation analysis; Anterograde transport analysis; IHC; RGC and axon quantification; WB	ES did not change IOP; ES promoted axon but not RGC survival, decreased $CD3^+$ T cells and Iba-1+ microglial cell number in the retina, decreased pAMPK/AMPK ratio and p75NTR.
Osako et al., 2013[34]	NAION in SD rats	NAION	TcES	Cornea/Oral cavity	Healthy rats; sham	Biphasic square pulses, 1 ms/ phase, 20 Hz, 100 μA	60 min/session, ES at 3 h after NAION induction and on day 1, 4, 7, 14, and 28	OCT; ERG; RGC counting	ES preserved STR amplitude at day 28 but not day 14, and promoted RGC survival at both day 14 and 28

AMD, age-related macular degeneration; AOH, acute ocular hypertension; BDNF, brain-derived neurotrophic factor; CNTF, ciliary neurotrophic factor; EcOG, electrocorticogram; ERG, electroretinography; ES, electrical stimulation; FGF2, basic fibroblast growth factor; IGF-1, insulin-like growth factor 1; IHC, immunohistochemistry; INL, inner nuclear layer; IOP, intraocular pressure; NA, not applicable; NAION, Nonarteritic ischemic optic neuropathy; ONC, optic nerve crush; ONI, optic nerve injury; ONL, outer nuclear layer; ONT, optic nerve transection; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RGC, retinal ganglion cell; RP, retinitis pigmentosa; rtcACS, Repetitive transcorneal alternating current stimulation; SES, subretinal electrical stimulation; TcES, transcorneal electrical stimulation; TpES, transpalpebral Electrical Stimulation; TRES, transretinal electrical stimulation; VEP, visual evoked potential; WB, western blot.

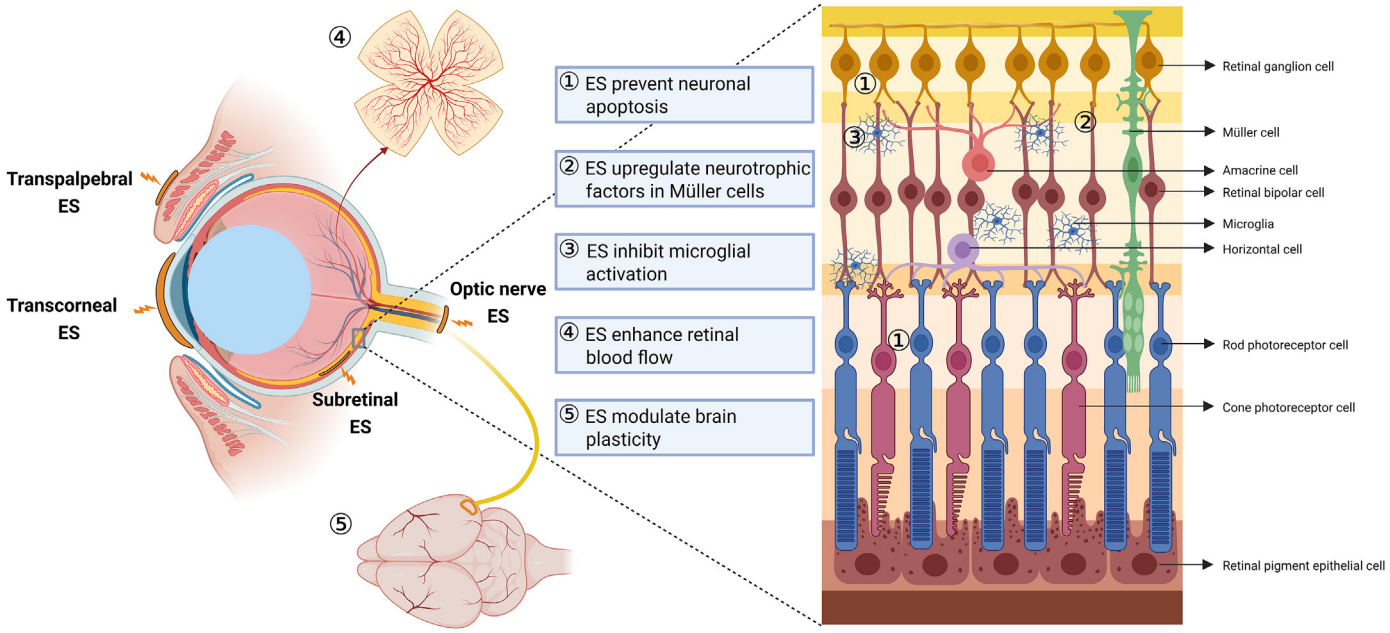

Fig. 1. A schematic diagram illustrates the electrode position and the major mechanisms induced by ES treatment in retinal diseases. Electric current was delivered to the rodent eye by placing the electrode on various location. Such as on the eyelid (transpalpebral ES), on the cornea (transcorneal ES), beneath the retina (subretinal ES), or at the proximal injury site of the optic nerve (optic nerve ES). ES is proposed to protect the diseased retina through: (1) preventing neuronal apoptosis; (2) upregulating neurotrophic factors in Müller cells; (3) inhibiting microglial activation; (4) enhancing retinal blood flow; and (5) modulating brain plasticity (Diagram is created in BioRender.com).

survival at both 1 and 2 weeks after injury, decreased TNF-α expression, and inhibited microglial activation at 1 week but not 2 weeks after injury.[30] In the retina of gerbil eyes with retinal ischemia, TcES increased RGC survival might related to decreased Iba-1 positive microglial cell number, reduced IL-6 and COX-2 expression as well as NF-κB phosphorylation, and increased IL-10 level.[32] Moreover, microglial inhibition by TcES was observed in DBA/2J mice, a genetic secondary glaucoma model, accompanied by RGC axonal protection.[33]

Despite of these findings, it is still poorly understood how ES modulates microglial function. A previous report found that transcranial direct current stimulation (tDCS) could directly impose effects on microglial physiological properties in the brain of normal mice through modulating voltage sensitive microglial channels.[57] Nevertheless, in addition to microglia, ES could also induce changes in morphological shape and molecular expression of certain proteins in neurons and astrocytes.[58] Considering that neurons and glial cells interact closely with each other to maintain the homeostasis of the microenvironment in CNS, microglial cell changes in ES treatment could also be contributed by ES effects on neurons and other glial cells.

6. ES enhance retinal blood flow

Retinal or choroidal vascular dysfunction are proved to be a contributor of neuronal death or even a primary risk factor in the major blind leading eye diseases, including RP, AMD, glaucoma, and diabetic retinopathy.[59–61] Thus, modulation of the reduced blood flow in the retina could be a promising therapeutic strategy for related diseases. ES has been previously validated to improve tissue blood flow in the treatment of brain, spinal cord, muscle, and autonomic nervous system.[62–66] In terms of eyes, ES could increase chorioretinal blood flow in normal human subjects.[67] Recently, ES was indicated to modulate retinal blood flow in RP patients.[68,69] Bittner, et al. reported that weekly TcES of RP patients was able to increase the central retinal artery mean flow velocity after 2 weeks of treatment and improve retinal blood flow in the macular vessels after one week of treatment.[68] In addition, TcES could increase mean oxygen saturation in the retinal arterioles and decrease mean oxygen saturation in retinal venules of the RP patients, though it had no effect on the diameters in the arterioles and venules.[69] Nevertheless, more evidence is needed to demonstrate the potential of ES in modulating retinal blood flow and its relationship with treatment outcome in eye diseases. Moreover, future studies should try to explain how ES imposes its effects on retinal blood flow. Several hints could be found in studies applying ES in the brain, in which perivascular nerves, the endothelial lining, astrocytes, and neurons of neurovascular units could be modulated by ES current and contribute to the vascular phenomena changes.[66]

7. ES modulate brain plasticity

The early studies about ES application to patients provided evidence that the neuronal electric signal elicited by ES in the retina could reach the visual cortex and induce phosphene.[3,70] This is further validated by the observation of increased intrinsic signals and evoked field potentials in visual cortex by TcES in cats.[71] This phenomenon raised the possibility that ES of the eye can impose effects on the brain function which is usually deteriorated in eye diseases. The hypothesis was supported by clinical studies from Fedorov and Sabel's group, which found that rtoACS of the optic neuropathy patients led to improvements in visual acuity (VA), visual field (VF), and EEG power spectra. Intriguingly, VA and VF improvements were associated with increased EEG alpha power.[72,73] Improvements in EEG power spectra by rtoACS in optic neuropathy patients were further confirmed by the following studies by Schmidt, et al.[74] and Gall, et al..[75] The clinical evidence indicated the ability of rtoACS to enhance cortex plasticity at the minimum, in patients with optic neuropathy. Nevertheless, repetitive transcorneal alternating current stimulation (rtcACS) in rats with ONC failed to induce EEG changes.[28,27] Researchers proposed that a minimal level of brain activation was required to ensure ES effects on cortical plasticity, as they observed increased EEG theta power by rtcACS in normal but not ONC rats.[27]

Besides optic neuropathy, modulation of brain oscillations by ES of the eyes may also exist in other diseases, such as photoreceptor degeneration. Effects on the brain oscillations by TcES were suggested to be current frequency and brain state dependent. Agadagba et al. applied daily TcES to rd mice, a mouse model of photoreceptor degeneration, for 5 days at various frequencies (2, 10, and 20 Hz). They found increased power of theta, alpha, and beta oscillations in the contralateral visual cortex at 10 Hz stimulation in the awake but not anaesthetised mice at the end of treatment.[19] Other parameters of electric current, such as current intensity and pulse widths, could also influence the strength of intrinsic signals in the visual cortex evoked by TcES.[71] Though the connection between increased brain plasticity and retinal preservation in ES treated eyes is poorly understood, EEG function might be used as an indicator to optimize the ES parameters.

8. Concluding remarks and future directions

ES-based treatment holds great therapeutic potential due to its capability to non-invasively and non-pharmacologically affect cellular activities. Clinical studies demonstrated promising ES therapeutic effects on RP and optic neuropathy. While optimization and standardization of ES protocols are still an unmet need, basic science study to explore the related cellular and molecular mechanisms would facilitate the clinical application of ES. In this review, we summarized the in vitro and in vivo evidence related to cellular and tissue response to ES in eye diseases. These include ES prevents neuronal apoptosis, promotes neuronal regeneration, increases neurotrophic factors production in Müller cells, inhibits retinal microglial activation, enhances retinal blood flow, and modulates brain plasticity (Fig. 1). Other protective factors may also exist. For example, TcES increased glutamine synthetase could protect against glutamate excitotoxicity by catalyzing the amidation of glutamate to glutamine.[26] TcES also decreased $CD3^+$ T cells in the retina DBA/2J mice, which might contribute to the preservation of the degenerating axons.[33]

Most of the aforementioned mechanisms are not specific in the eye. ES in the other tissues, such as muscle, peripheral nervous system, spinal cord, and brain, has provided multiple hints for understanding ES effects in ophthalmology. As recently reviewed by Zhao et al. [1], the introduced electric current has direct effects on biomolecules and cells, such as altering the distribution and flow of the charged ions, membrane-bound proteins, cytoskeleton, and other cellular components. These effects are supposed to vary depending on the parameters of the current, including waveforms, pulse duration, frequency, current strength, treatment duration, as well as the distribution of the current determined by the delivery method. In the future, optimization and standardization of ES therapeutic protocols in treating different retinal diseases with sensitive detection methods for clinical efficacy evaluation are necessary to promote its clinical utility.

Study Approval

Not Applicable for a review paper.

Author Contributions

KC and JL: conceptualization. JL and AKM: literature search and data extraction. JL and AKM: writing original draft. KFS, VWL and KC: manuscript review and editing. All authors read and approved the final manuscript.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviations

AMD	Age-related macular degeneration
AOH	Acute ocular hypertension
Bax	B-cell lymphoma protein 2-associated X
Bcl-2	B-cell lymphoma protein 2
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
EcOG	Electrocorticogram
EEG	Electroencephalogram
ERG	Electroretinogram
ES	Electrical stimulation
IGF-1	Insulin-like growth factor 1
IHC	Immunohistochemistry
INL	Inner nuclear layer
IOP	Intraocular pressure
MNU	N-methyl-N-nitrosourea
NA	Not applicable
NAION	Nonarteritic ischemic optic neuropathy
NGF	Nerve growth factor
NTFs	Neurotrophic factors
ONC	Optic nerve crush
ONI	Optic nerve injury
ONL	Outer nuclear layer
ONT	Optic nerve transection
qRT-PCR	quantitative reverse transcription polymerase chain reaction
RAO	Retinal artery occlusion
RGC	Retinal ganglion cell
RP	Retinitis pigmentosa
rtcACS	repetitive transcorneal alternating current stimulation
rtoACS	repetitive transorbital alternating current stimulation
SES	Subretinal electrical stimulation
TcES	Transcorneal electrical stimulation
tDCS	Transcranial direct current stimulation
TdES	Transdermal electrical stimulation
TpES	Transpalpebral electrical stimulation
TRES	Transretinal electrical stimulation
VA	Visual acuity
VEP	Visual evoked potential
VF	Visual field
WB	Western blot

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