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Research Report

The effects of electrical stimulation on neurite outgrowth of goldfish retinal explants

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ABSTRACT

The central nervous system (CNS) in adult mammals loses the ability to regenerate after injury. Although electrical stimulation (ES) has been shown to promote neural regeneration, the underlying mechanisms by which ES enhances CNS regeneration remain elusive. The aim of the present study was to investigate the effect of ES on neurite outgrowth of goldfish retinal explants. The optic nerve of adult goldfish was intraorbitally crushed 7–14 days before retinal explants preparation. The explants were cultured in a laminin-coated indium tin oxide (ITO, transparent conducting oxide) device for ES application. Various strengths and waveforms of ES were applied to examine their effect on neural outgrowth. When the retinal explants were stimulated for 1 h with intermittent pulses on day 1 (20 Hz; 10 pulses every 2 s), the regenerated neurite length was significantly increased compared to explants that were stimulated with continuous square waves or continuous pulses over the same time course. It was also found that increased ES strength and repeated ES each day can enhance neurite outgrowth significantly. These results suggest that intermittent pulse ES is able to promote neurite regeneration most effectively in goldfish retinal explants, and that this electrically stimulated neurite outgrowth using ITO conductive electrodes may provide a useful platform for investigating cellular mechanisms of CNS regeneration.

1. Introduction

It is well known that neurons in the mammalian central nervous system (CNS) rarely regenerate and typically die soon after injury (Goldberg and Barres, 2000; Liu et al., 2011). In contrast, the peripheral nervous system (PNS) and developing mammalian CNS can regenerate their neurites after injury (Chen et al., 2007). Numerous studies have revealed potential strategies for neural regeneration in adult mammalian CNS, including providing a permissive environment (David and Aguayo, 1981), removing inhibitors in myelin such as Nogo, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein (Kim et al., 2003), and changing the intrinsic state of neurons (Cai et al., 1999; Dergham et al., 2002; Qiu et al., 2002;

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Song et al., 1998). All of these are being actively investigated in order to seek therapeutic approaches to promoting neurite outgrowth after injury.

It is known that electrical activity plays an important role in early neural development (Spitzer, 2006), and the endogenous electric field at injured sites stimulates and directs nerve regeneration and wound healing *in vivo* (Song et al., 2004). Attempts have been made to reconstruct the natural environment where neurite outgrowth is promoted by electrical stimulation (ES). For example, it has been shown that ES can influence the orientation of regenerating neurites (Patel and Poo, 1982, 1984), induce PC 12 cells differentiation without NGF (Kimura et al., 1998), effectively accelerate regeneration of motor neurons (Al-Majed et al., 2000b), and promote regeneration of both sensory neurons and peripheral axons of the dorsal root ganglion (DRG; Geremia et al., 2007; Udina et al., 2008). In the visual system, it has been demonstrated that surviving retinal ganglion cells (RGCs) extend their axons when stimulated with physiological levels of ES (Goldberg et al., 2002). Similarly, applying monophasic square pulses of ES to a transected rat optic nerve can markedly increase the RGC density (Okazaki et al., 2008). Furthermore, noninvasive transcorneal electrical stimulation (TES) has been shown to significantly increase the regenerated length of crushed optic nerve and the survival rate of axotomized RGCs in rats *in vivo* (Miyake et al., 2007; Tagami et al., 2009). In clinical trials, the visual function of patients with nonarteritic ischemic optic neuropathy or traumatic optic neuropathy has been improved by TES with seemingly little drawbacks (Fujikado et al., 2006).

However, these effects of electrical activity on neural regeneration are double edged. For example, when applying different patterns of electrical activity to DRG sensory neurons, it has been demonstrated that the filopodia and lamellipodia of the growth cones are retracted immediately following ES, and that the growth rate of neurites is influenced by the frequency and pattern of the ES (Fields et al., 1990). Similarly, it has also been reported that loss of electrical activity might be a pivotal signal that triggers axon outgrowth after a peripheral lesion (Enes et al., 2010). These findings suggest that the effectiveness of ES on neural regeneration depends on appropriate electrical activity being present (Fields et al., 1997; Morimoto et al., 2010).

The goldfish visual system is one of the most studied CNS in vertebrates. Retinal explants of the goldfish form an effective experimental model that allows the investigation of the molecular mechanisms of optic nerve regeneration (Bates and Meyer, 1996; Challacombe and Elam, 1995; Heacock and Agranoff, 1977; Matsukawa et al., 2004; Nusetti et al., 2005). In the present study, our aim was to examine the effect of ES on neurite outgrowth of goldfish retinal explants after optic nerve crush (Fig. 1). By applying various waveforms of electrical stimulation (Fig. 2) to goldfish retinal explants using the indium tin oxide (ITO) electrodes, we systematically characterized the effects of the various ES patterns on neurite outgrowth. This approach of using transparent ITO conductive device in examining the ES effect on neurite outgrowth of goldfish retinal explants provides a potential opportunity to explore cellular mechanisms of CNS regeneration upon electrical stimulation.

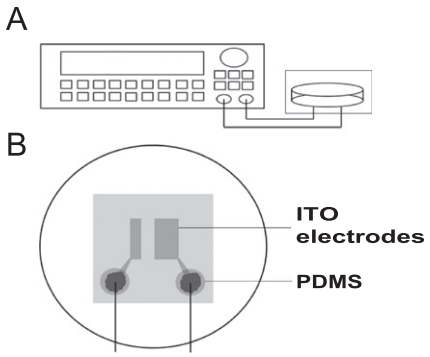

Fig. 1 – Experimental setup and electrical stimulation device. (A) The ITO conductive device was connected to a function generator in order to provide electrical stimulation. (B) The rectangular ITO conductive electrodes, which are 2 mm apart, were coated on a borosilicate glass. The copper wires connected to the function generator were attached to the ITO electrodes via silver paint covered by biocompatible PDMS.

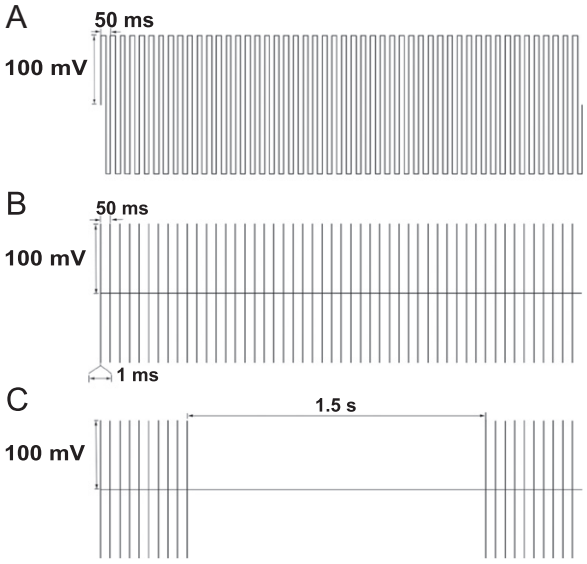

Fig. 2 – Patterns of electrical stimulation used in the present study. (A) Continuous biphasic square wave. The frequency was 20 Hz and various amplitudes (100 mV/mm, 200 mV/mm, 500 mV/mm, and 1000 mV/mm) were applied. (B) Continuous pulse electrical stimulation. The duration of each pulse (100 mV/mm) was 1 ms and the frequency was 20 Hz. (C) Intermittent pulse electrical stimulation. Ten repeated pulses (100 mV/mm) were generated every 2 s, and each pulse duration was 1 ms (100 mV/mm) and the frequency was 20 Hz.

2. Results

2.1. *Neurite outgrowth in goldfish retinal explants was normal in ITO conductive devices*

To examine whether the ITO electrodes influence neurite outgrowth, we cultured retinal explants on glass coverslips, ITO coated chips, and ITO conductive devices. As shown in Fig. 3, there was no significant difference between the neurite length of retinal explants cultured on glass coverslips and ITO coated chips. There was also no significant difference in