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Research article

The effect of intravitreal injection of vehicle solutions on form deprivation myopia in tree shrews

Alexander H. Ward [a, *], John T. Siegwart Jr. [b], Michael R. Frost [b], Thomas T. Norton [b]

[a] Genetics, Genomics and Bioinformatics Theme, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[b] Department of Vision Sciences, School of Optometry, University of Alabama at Birmingham, Birmingham, AL 35294, USA

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ABSTRACT

Intravitreal injection of substances dissolved in a vehicle solution is a common tool used to assess retinal function. We examined the effect of injection procedures (three groups) and vehicle solutions (four groups) on the development of form deprivation myopia (FDM) in juvenile tree shrews, mammals closely related to primates, starting at 24 days of visual experience (about 45 days of age). In seven groups ($n = 7$ per group), the myopia produced by monocular form deprivation (FD) was measured daily for 12 days during an 11-day treatment period. The FD eye was randomly selected; the contralateral eye served as an untreated control. The refractive state of both eyes was measured daily, starting just before FD began (day 1); axial component dimensions were measured on day 1 and after eleven days of treatment (day 12). Procedure groups: the myopia (treated eye − control eye refraction) in the FD group was the reference. The sham group only underwent brief daily anesthesia and opening of the conjunctiva to expose the sclera. The puncture group, in addition, had a pipette inserted daily into the vitreous. In four vehicle groups, 5 μL of vehicle was injected daily. The NaCl group received 0.85% NaCl. In the NaCl + ascorbic acid group, 1 mg/mL of ascorbic acid was added. The water group received sterile water. The water + ascorbic acid group received water with ascorbic acid (1 mg/mL). We found that the procedures associated with intravitreal injections (anesthesia, opening of the conjunctiva, and puncture of the sclera) did not significantly affect the development of FDM. However, injecting 5 μL of any of the four vehicle solutions slowed the development of FDM. NaCl had a small effect; myopia development in the last 6 days (−0.15 ± 0.08 D/day) was significantly less than in the FD group (−0.55 ± 0.06 D/day). NaCl + Ascorbic acid further slowed the development of FDM on several treatment days. H_2O (−0.09 ± 0.05 D/day) and H_2O + ascorbic acid (−0.08 ± 0.05 D/day) both almost completely blocked myopia development. The treated eye vitreous chamber elongation, compared with the control eye, in all groups was consistent with the amount of myopia. When FD continued (days 12–16) without injections in the water and water + ascorbic acid groups, the rate of myopia development quickly increased. Thus, it appears the vehicles affected retinal signaling rather than causing damage. The effect of water and water + ascorbic acid may be due to reduced osmolality or ionic concentration near the tip of the injection pipette. The effect of ascorbic acid, compared to NaCl alone, may be due to its reported dopaminergic activity.

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1. Introduction

Intravitreal injection, in which a substance, dissolved in a vehicle solution, is placed into the vitreous chamber, is a frequently used tool in both clinical and basic research studies (Avery et al., 2006; Brown et al., 2006; Feldkaemper et al., 2009; Ganesan and Wildsoet, 2010; Haritoglou et al., 2006; Iturralde et al., 2006; Norton et al., 1994; Pickett-Seltner and Stell, 1995; Rohrer et al., 1995; Rohrer et al., 1993; Stone et al., 1989; Zhu and Wallman, 2009). This approach is often used to deliver neurotransmitter agonists and antagonists to the vicinity of the retina so as to observe their impact on retinal function. From the vitreous, these substances, typically small molecules, are presumably moved by diffusion across the inner limiting membrane into the retina (Araie

* Corresponding author. 302 Worrell Building, University of Alabama at Birmingham, Birmingham, AL 35294-4390, USA.
E-mail address: alexward@uab.edu (A.H. Ward).

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and Maurice, 1991; Park et al., 2005) where they come into contact with the target receptors.

In isolated retinal preparations, known concentrations of neurotransmitter analogs can be maintained in the fluid bath and at the retinal surface. However, connections to central brain structures are disrupted in these preparations and visual behaviors cannot occur. When intravitreal injections into intact eyes are used, there is less control over the precise initial concentration and dissipation over time of the injected substances. Nonetheless, intravitreal injection is a useful approach because administration is simple and because the substances that disperse in the vitreous are localized near the retina and are carried through it. In addition, except for anesthesia during the intravitreal injection, animals can be awake with potentially normal retinal signaling and visual behaviors. Alternative approaches, such as sub-conjunctival or peribulbar injections are more indirect than intravitreal administration; the substances must pass through the sclera, choroid, and retinal pigment epithelium to reach the retina. In addition, an unknown proportion of the administered substance remains outside the eye and diffuses away, making it more difficult to know the dose that actually reaches the retina; these substances may also affect the choroid, the sclera, and/or extraocular structures.

The choice of the vehicle solution into which the substances of interest are dissolved is a potentially important factor because the vehicle itself might affect retinal signaling. Previous studies (when the vehicle has been specified) have used a variety of solutions, including normal saline, phosphate buffered saline (PBS), and water. Sometimes ascorbic acid (typically 1 mg/mL) has been added to these vehicles as an anti-oxidant (Rohrer et al., 1993; Schaeffel et al., 1995; Schaeffel et al., 1994; Schmid and Wildsoet, 2004). The vehicle solution used has been reported to affect the response to form deprivation in some (Rohrer et al., 1993; Schmid et al., 2013), but not all cases (F. Schaeffel, personal communication, 2013). We examined two vehicles, 0.85% NaCl and water, both alone and supplemented with 1 mg/mL ascorbic acid. We avoided phosphate buffered saline (PBS) because some substances we planned to investigate in subsequent studies would not dissolve at the needed concentrations in this vehicle.

The impact of these vehicle solutions was assessed in tree shrews, a well-established model of myopia development that, like most mammals, lack the inner cartilaginous scleral layer present in chicks and many other vertebrates. The dependent variables in the present study were the amount of monocular form-deprivation myopia (FDM) and the rate of myopia development (slope in diopters [D]/day) that occurred over an 11-day treatment period, compared with the untreated fellow eye. When a translucent diffuser is held in front of an eye early in postnatal development, the retina detects the form deprivation (FD) and generates what have been described as "GO" signals (He et al., 2014; Norton, 1999; Rohrer and Stell, 1994; Schaeffel and Howland, 1991). Retinally-generated signals are not only sent to central visual targets, but also pass through a direct retino-scleral pathway comprised of the retinal pigment epithelium (RPE) and choroid to reach the sclera where they produce scleral remodeling (Gao et al., 2011; Guo et al., 2014; McBrien et al., 2001; Moring et al., 2007; Norton et al., 1994; Norton and Rada, 1995; Siegwart and Norton, 1999) that results in ocular (vitreous chamber) elongation (Marsh-Tootle and Norton, 1989). As measured in choroid and sclera, nearly identical GO signals are produced by FD and another myopiagenic stimulus, a negative-power lens, and these GO signals are distinct from the STOP signals that occur during recovery from induced myopia (Guo et al., 2014; He et al., 2014). Thus, the GO signals produced by FD are not merely the absence of STOP signals. As FD continues, the elongation of the deprived eye moves the retina behind the focal plane so that the eye becomes myopic (McFadden et al., 2004; Shen and Sivak, 2007; Sherman et al., 1977; Wallman et al., 1978; Wiesel and Raviola, 1977). In the present study, FD was selected rather than negative-lens wear because the myopia produced by a negative lens is limited by the dioptric power of the lens. With FD there is no limitation since it is an open loop condition; eyes continue to elongate for many days as long as the diffuser remains in place (McBrien and Norton, 1992; Smith et al., 1999; Troilo and Judge, 1993; Wallman and Adams, 1987).

The development of both FDM and lens-induced myopia (LIM) is dependent on the presence of nearly continuous FD or lens wear. If FD or lens wear is discontinued for short daily periods (30 min–1 h), the amount of myopia that develops is greatly reduced (Kee et al., 2007; Napper et al., 1995; Schmid and Wildsoet, 1996; Shaikh et al., 1999). Thus, FDM is a sensitive system in which to evaluate the possible impact of injected vehicles.

Ideally, the injection procedures (daily anesthesia and scleral puncture) also should not affect retinal signaling so the development of FDM should be unaffected. However, repeated anesthesia and re-opening of a scleral puncture potentially could affect myopia development by reducing intraocular pressure (IOP), by producing tissue responses, or by interrupting retinal GO signaling. To assess this issue, two relevant procedure groups were assessed in addition to four groups that received vehicle solutions.

2. Materials and methods

2.1. Subjects and experimental groups

As in previous studies from this laboratory (Guo et al., 2013; He et al., 2014; McBrien and Norton, 1992), juvenile tree shrews (*Tupaia glis belangeri*) were raised in our breeding colony by their mothers on a 14 h light/10 h dark cycle. Tree shrews are small mammals (dichromats) that are closely related to primates with excellent vision for their size (2–4 cyc/deg) (Norton et al., 2003; Petry et al., 1984). All procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research and were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. The first day both eyes are open, which occurs about three weeks after birth, is considered to be the first day of visual experience. Experimental groups were balanced to include both males and females and avoided multiple pups from the same parents. Which eye was treated with FD (and injections) was balanced between left and right eyes in each group.

The seven groups in this study ($n = 7$ per group) are summarized in Table 1. Animals in all groups received monocular FD for 11 days with a translucent diffuser held in a goggle frame. The groups are divided into two classes: three procedure groups and four vehicle groups. Procedure groups included the FD, sham, and puncture groups. The FD group was our reference group; these animals received only monocular form deprivation with daily measures of refractive state. Sham group: on day 1 of treatment, after pre-treatment refractive and axial component measures were made, the animals were anesthetized with 5% isoflurane. After instilling a drop of topical anesthetic in the to-be treated eye, an opening was made in the temporal conjunctiva that was re-opened on subsequent days. After application of a drop of artificial tears to both eyes, the goggle containing the diffuser was attached; the animal was weighed and allowed to recover from anesthesia in a nesting box in the lab for at least 5–10 min before it was returned to its cage in the animal colony. This procedure, including brief (approximately 5 min) removal of the diffuser, was repeated for 10 more days. The removal of the diffuser mimicked the amount of time taken to perform the intravitreal injections. The final refractive and axial measures were made on day 12, which was 24 h after the 11th treatment.

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