ARTICLE

The Most Engaging Neurophysiology Lab Exercise Ever Performed in an Undergraduate Laboratory Course

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*Key words: nerve growth factor (NGF); retinal ganglion cell (RGC); retina; growth cone; anatomy;* Aplysia californica*; long term potentiation (LTP); physiology*

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**MATERIALS AND METHODS**

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## RESULTS

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## DISCUSSION

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**Testing the effects of altered retinal neurotrophins on RGC axonal arborization in the tectum**

Control-, anti-BDNF, or BDNF-treated green fluorescent microspheres were injected into the retina of stage 43 tadpoles. RGC axons were labeled anterogradely by EYFP lipofection or DiI microinjection. RGC axon arbor morphology was visualized at 0 and 24 hours in the live,anesthetized tadpole by confocal microscopy.

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**Rdritic rborization is temporally sensitive to increased retinal BDNF** **levels**. To determine if RGCs were sensitive to enhanced retinal BDNF in a stage-dependent fashion, *Xenopus* retinae were treated with control or BDNF coupled microspheres from stage 38 to 45 or stage 42 to 45. All morphological measures of dendritic arborization revealed a stage-dependent response to retinal BNDF. Earlier exposure to exogenous BDNF (stages 38-45) inhibited dendritic arborization more dramatically than later exposure to BDNF (stages 42-45). Primary dendrites and secondary branching were decreased by treatment starting at 38, whereas altering BDNF levels from 42 onward only affected secondary branching. This may be due to the fact that primary dendritogenesis was well underway by stage 42.

**Retinal BDNF inhibits RGC dendritic arborization in a dose-dependent fashion** To determine if RGCs are sensitive to the concentration of exogenous retinal BDNF, *Xenopus* retinae were microinjected with 1-100 ng/ml BDNF or control treated microspheres at the onset of dendritic arborization. All morphological measures of dendritic arborization revealed a dose-dependent response to retinal BNDF. The highest concentration of BDNF most dramatically inhibited dendritic arborization.

**Tectal BDNF retrogradely enhances RGC dendritic arborization** To determine if tectal BDNF influences RGC dendritic arborization within the retina, tadpoles received tectal injections of microspheres treated with control, BDNF, or anti-BDNF function-blocking antibodies. Microsphere-containing neurons labeled with rhodamine dextran were analyzed morphologically. All morphological measures of dendritic arborization revealed that increasing tectal BDNF enhances RGC dendritic arborization. Correspondingly, tectal applications of ant-BDNF limits RGC dendritic arborization.

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**RGC axon arborization dynamics are unaffected by retinal BNDF levels**

Our previous studies showed that increasing tectal BDNF levels promotes RGC axon arborization. To determine if retinal BDNF influences RGC axon arborization at a distance, tadpoles were intraocularly injected with control, BDNF, or anti-BDNF treated microspheres. Altering retinal BDNF levels had no significant effects (p>0.05) on RGC axon arbor complexity as exemplified by the increase in total branch number and total arbor length 24 hours after treatment. Error bars = SEM.

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