

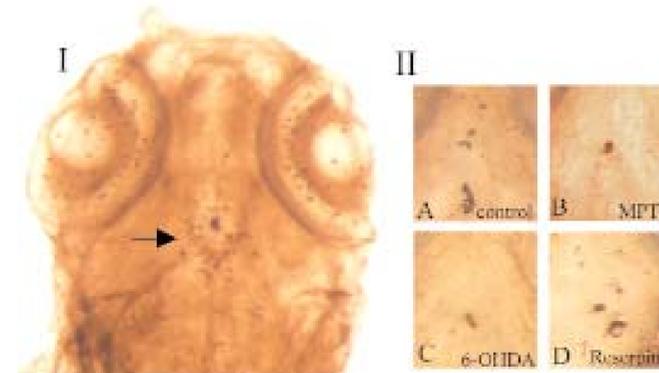
Zebrafish Neural Bioassays

INNER EAR HAIR CELLS



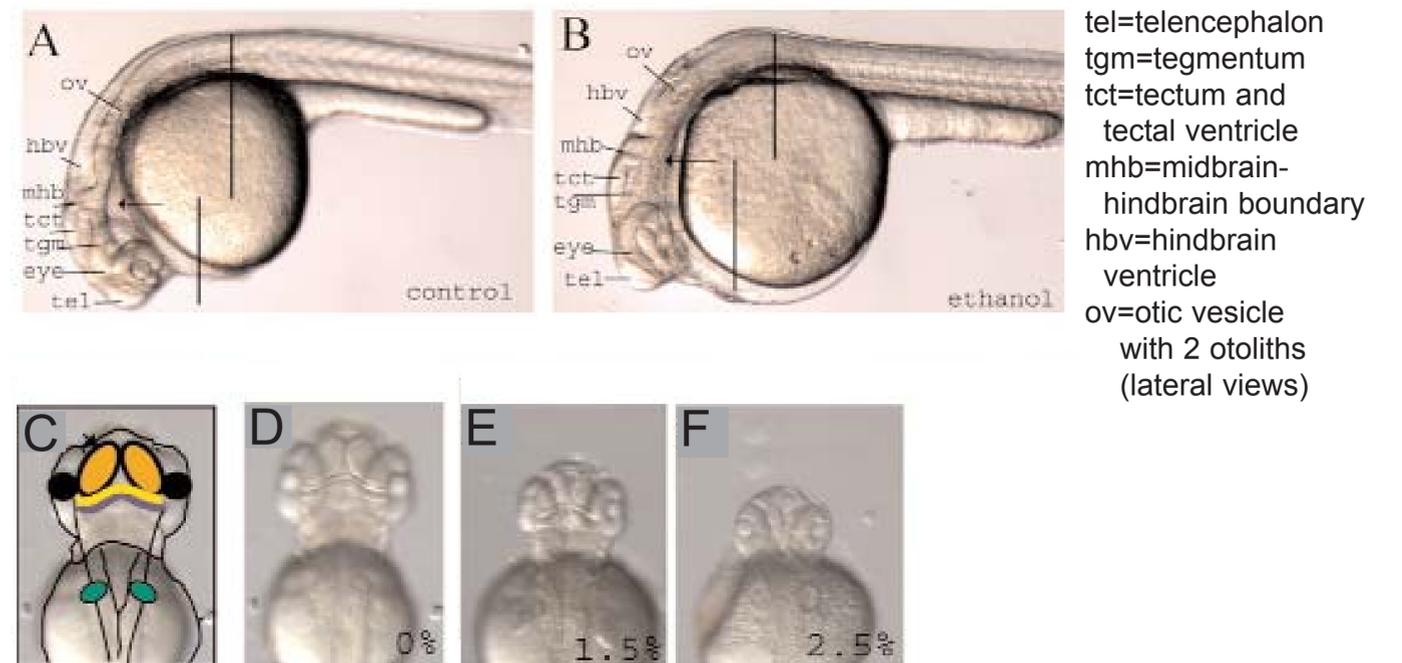
Hair cell loss after drug treatment

DOPAMINERGIC NEURONS



All the tyrosine hydroxylase-positive neurons in zebrafish diencephalons have been shown to be dopaminergic neurons. Drug effects can be assessed by antibody staining (I). Drugs, such as MPTP (B), 6-OHDA (C), and Reserpine (D), which cause dopaminergic neuron loss in mammals, also cause loss in zebrafish.

DEVELOPMENTAL NEUROTOXICITY



tel=telencephalon
tgm=tegmentum
tct=tectum and tectal ventricle
mhb=midbrain-hindbrain boundary
hbv=hindbrain ventricle
ov=otic vesicle with 2 otoliths (lateral views)

Drug effects on the developing brain can be visually assessed. Untreated animals were used as controls (A)(C)(D). Zebrafish at the early gastrulation stage were incubated with or without 1% ethanol for 3 hours, then incubated with normal growth water. At 30 hpf, a smaller hindbrain ventricle and a larger midbrain were observed in embryos exposed to 1% ethanol (B). At 48 hpf, normal embryos showed tightly organized tectum and midbrain-hindbrain boundary (C,D). In contrast, an increase in ethanol concentration decreased head size and delayed development of the brain in a dose-dependent manner after exposure to 1.5% (E) and 2.5% (F) ethanol.

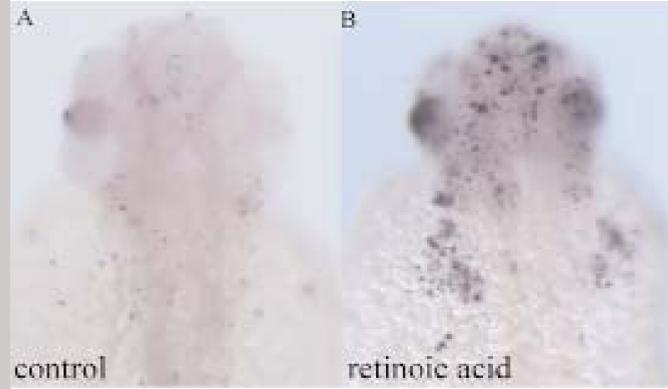
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*Methods of screening agents for activity using teleosts are covered by US patents: 6,299,858 and 6,656,449 owned by Phylonix.

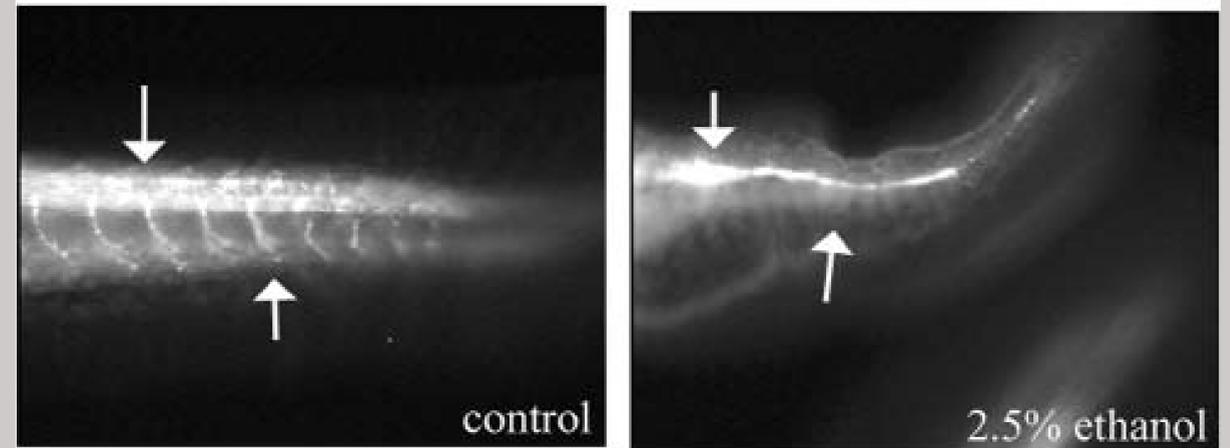


NEUROTOXICITY



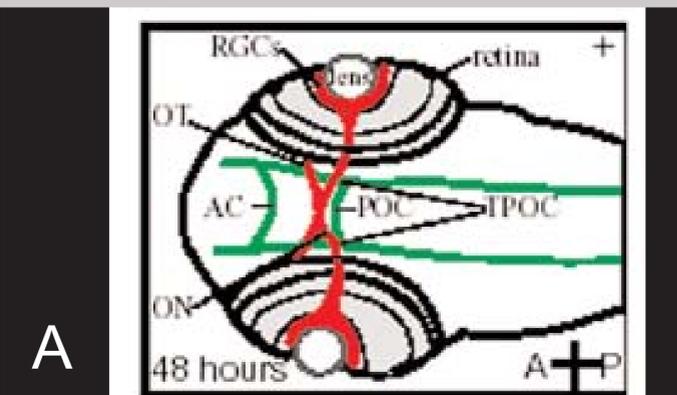
TUNEL staining can be used to detect apoptosis. Compared to control (A), retinoic acid induced apoptosis in the brain region (B). In addition, TUNEL can be used with other antibody staining methods to detect cell type specific apoptosis.

MOTOR NEURONS

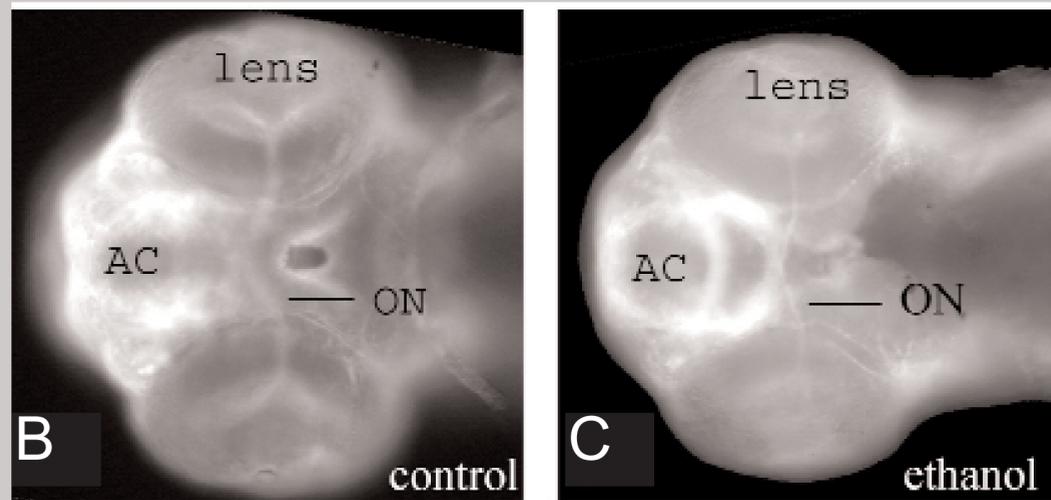


Motor neuron morphology can be assessed using antibody staining. After ethanol exposure, axon loss on peripheral motor neurons was observed.

OPTIC NERVE

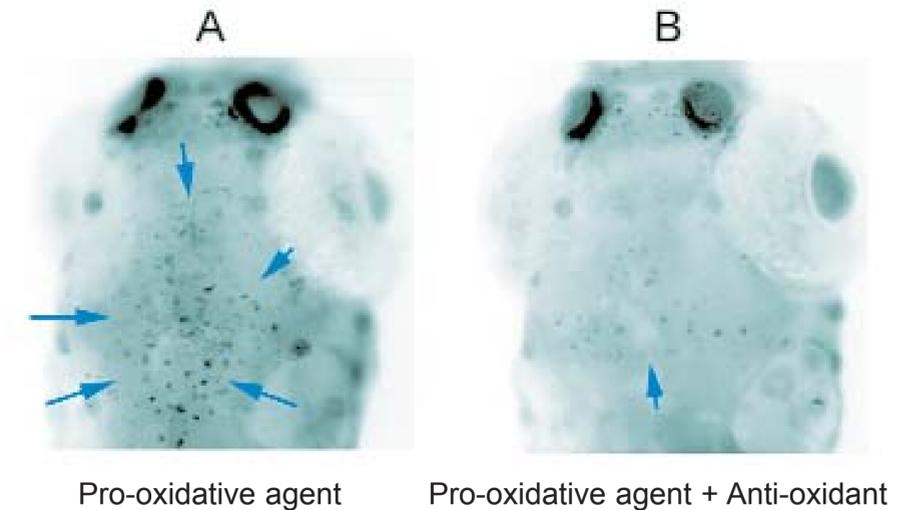


Organization of the nervous system can be examined by antibody staining. (A) is a schematic drawing of the optic nerve, optic track and commissure in zebrafish. Compared to the untreated control (B), reduced staining of the optic nerve (ON) was observed after exposing 48 hour post fertilization (hpf) embryos to 1% ethanol (C).



ON=optic nerve
 OT=optic tract
 AC=anterior commissure
 POC= postoptic commissure
 RGCs=retinal ganglion cells
 P=posterior
 A=anterior
 TPOC=tract postoptic commissure

NEUROPROTECTIVE AGENTS



Conditions for neuronal injury, such as oxidation, demyelination, and axonal loss can be mimicked in zebrafish and drug screening for neuroprotective agents can be performed. Injection of a pro-oxidative agent induced neuronal apoptosis in the brain (A). An anti-oxidant exhibited protective effects on neuronal death and reduced apoptosis in the brain (B). The number of dots decreased after anti-oxidant treatment.