**Supplemental Movie Legends: Karlstrom and Bergan R24 Application: An Expression Atlas of the Adult Zebrafish Brain**

**01.Oxytocin in the hypothalamus:*Tg(oxt:GFP).*avi**

Hypothalamic projections of oxytocin expressing neuroendocrine cells as revealed by the *Tg(oxt:GFP)* line(Gutnick et al., 2011)**.**GFP-expressing cells shown in cyan, autofluorescence (red) illuminates the brain and blood vessels.  Projections can be seen from the presumptive fish-equivalent of the paraventricular nucleus through the lateral hypothalamus to converge at the ventral midline of the hypothalamus where the pituitary stalk attaches.  These projections would continue into the pituitary, which was removed during dissection. Line obtained from Engert lab.

**02. Aligned\_Images:*Tg(oxt:GFP)(g)&Tg(her5:GFP)(r*). avi**

Separate images of the *Tg(oxt:GFP)* line (Gutnick et al., 2011)(here shown in green) and *Tg(her5PAC:EGFP)ne1939* line (Chapouton et al., 2006) (here in red) were aligned with the 6-month reference brain image.  Small isolated groups of *her5* expression cells are seen in neurogenic zones of the midbrain, while *oxt* expressing neuroendocrine cells are again seen to project from the dorsal hypothalamus to the pituitary stalk region.  The pituitary was removed during dissection.

**03.SonicHedeghogSource:*Tg(shh:GFP).rot.mov***

3-D rotation showing Sonic Hedgehog (Shh) expressing cells in the adult brain as revealed by the *Tg(shh:GFP)* line (Neumann and Nuesslein-Volhard, 2000). Radial glia of the floor plate (hindbrain/spinal cord), as well as in proliferative cells in the hypothalamus (Karlstrom lab data not shown) express Shh throughout life.  A previously unidentified neuronal pathway can be seen from the hypothalamus to the dorsal telencephalon.

**04.SonicHedeghogSource:*Tg(shh:GFP).Zstack.mov***

Z-stack movie of Sonic Hedgehog (Shh) expressing cells in the adult brain as revealed by the *Tg(shh:GFP)* line (Neumann and Nuesslein-Volhard, 2000). Radial glia of the floor plate (hindbrain/spinal cord), as well as in proliferative cells in the hypothalamus (Karlstrom lab data not shown) express Shh throughout life.  A previously unidentified neuronal pathway can be seen from the hypothalamus to the dorsal telencephalon.

**05.SonicHedeghogResponse:*Tg(GBSptch2:GFP).rot.mov***

The *Tg(GBSptch2:GFP)*line from our lab (Shen et al., 2013) reveals sonic hedgehog responsive cells in the adult brain.

**06.GlutamatergicNeurons.*Tg(slc17a6b:DsRed).*Rot.avi**

Glutamatergic neurons revealed in the *Tg(slc17a6b:DsRed)* [nee *Tg(vglut2a:dsRed*)]line. (deCarvalho et al., 2014)

**07.GlutamatergicNeurons.*Tg(slc17a6b:DsRed)*.ZStack.mov**

Transverse Z-stacks of the *Tg(slc17a6b:DsRed)* [nee *Tg(vglut2a:dsRed*)]line, starting posteriorly in the hindbrain and moving anteriorly into the telencephalon.

**08.DopaminergicNeurons.*Tg(slc6a3:EGFP)*.rot**

Dopaminergic neurons revealed in the ***Tg(slc6a3:EGFP)***(Dopamine transporter) line (Xi et al., 2011).

**09.AdultBrainDissections**

Movie showing zebrafish brain dissection technique.  Zebrafish are euthanized in MS-222 then decapitated caudal to the gills.  After removing the skull cap, eyes, and jaws (1/2 way through this movie) the dissection is stopped and the fish head is placed in an eppendorf of cold 4% PFA overnight.  The next day the head is placed in a dish of cold PBS and dissection is continued.  This 2-step dissection helps reduce damage to the tissue and preserves brain shape.

**10.3Mo,2yr&3yrFishInBeakers.mov**

3 month old fish on left, 2 year old fish middle, 3 year old fish on right.  Zebrafish can live well beyond 3 years, but most fish facilities euthanize fish by the age of 1 to 1.5 years to keep healthy breeding stocks.  We have begun to raise fish through 3 years of age in order to build the life-course gene expression atlas.

**References**

Chapouton, P., Adolf, B., Leucht, C., Tannhauser, B., Ryu, S., et al., 2006. her5 expression reveals a pool of neural stem cells in the adult zebrafish midbrain. Development 133, 4293-4303.

deCarvalho, T.N., Subedi, A., Rock, J., Harfe, B.D., Thisse, C., et al., 2014. Neurotransmitter map of the asymmetric dorsal habenular nuclei of zebrafish. Genesis 52, 636-655.

Gutnick, A., Blechman, J., Kaslin, J., Herwig, L., Belting, H.G., et al., 2011. The hypothalamic neuropeptide oxytocin is required for formation of the neurovascular interface of the pituitary. Dev Cell 21, 642-654.

Neumann, C.J., Nuesslein-Volhard, C., 2000. Patterning of the zebrafish retina by a wave of sonic hedgehog activity. Science 289, 2137-2139.

Shen, M.C., Ozacar, A.T., Osgood, M., Boeras, C., Pink, J., et al., 2013. Heat-shock-mediated conditional regulation of hedgehog/gli signaling in zebrafish. Developmental dynamics : an official publication of the American Association of Anatomists 242, 539-549.

Xi, Y., Yu, M., Godoy, R., Hatch, G., Poitras, L., et al., 2011. Transgenic zebrafish expressing green fluorescent protein in dopaminergic neurons of the ventral diencephalon. Dev Dyn 240, 2539-2547.