caused a significant increase in the presence of radial glial cell bodies in the ventricular zone and floor plate of the neural tube. Kitl1, also known as Eg5, is a plus-end directed motor protein responsible for establishing the forces required to stabilize and separate the bipolar mitotic spindle. Our analysis confirms that eg5 expression occurs in a Gfap+ radial glial population throughout the ventricular zone and floor plate. Furthermore, labeling for anti-phosphohistone H3 and α-tubulin in kitl1 mutants has shown monastral spindles characteristic of mitotic arrest. We have confirmed this phenotype by treatment with S-trityl-l-cysteine, a specific Eg5 inhibitor previously reported to cause mitotic arrest through monastral spindle formation. Lastly, by labeling for sensory, motor, and specific interneuron populations, we show that Eg5 mediated division is required for proper neuronal development within the neural tube. Our findings support a model in which Eg5 is an important mediator of neural stem cell division and neuronal patterning during embryonic CNS development. Currently, we are counting the overall nuclei in the neural tube as well as conducting cell death assays and characterizing other glial populations to test whether the increase in Gfap+ cell bodies following Eg5 inhibition is a compensatory mechanism in response to a decrease found in specific neuron populations.

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Program/Abstract #378
Genetic dissection of sonic hedgehog/Gli signaling in adult neurogenesis
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Sonic hedgehog (Shh), a key regulator of embryonic neurogenesis, signals directly to GFAP-expressing neural stem cells (NSCs) in the subventricular zone (SVZ) of the adult forebrain. The specific mechanism by which Shh/Gli signaling regulates SVZ stem and/or progenitor maintenance, however, is not well understood. We are interested in uncovering the roles of Gli2 and Gli3 activator (A) and repressor (R) functions during adult mouse neurogenesis. Using Gli knock-in reporter alleles, we found Gli2,3.2 to be expressed in adult SVZ NSCs but not in committed neuroblasts or mature neurons, suggesting that Shh signaling is downregulated upon cell differentiation. To determine the requirement for Gli2 and Gli3 in SVZ NSCs, we used a transgenic mouse GFAP-Cre line (mGFAP-Cre) that recombines postnatally. As expected, Smo receptor inactivation causes a significant reduction in SVZ proliferation and a decrease in SVZ slow-cycling NSCs. Preliminary analysis of mGFAP-Cre;Gli2lox/lox and Gli3lox/lox conditional knockout (CKO) brains indicates a small decrease in proliferation when Gli2 is inactivated, whereas ablation of Gli3 results in a slight increase in proliferation, consistent with loss of the dominant Gli-A and Gli-R, respectively. Most importantly, removal of Gli3 in Smo CKOs appears to partially rescue the neurogenesis defects, indicating that unattenuated levels of Gli3-R underlie the phenotype in Smo CKOs. We are currently analyzing the olfactory bulb interneuron populations in Shh/Gli signaling mutants to test whether there is a differential requirement for Shh in interneuron production.

Program/Abstract #379
Response of glial precursors to embryonic brain injury
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Perinatal brain injury involves glial precursors, but the neural mechanisms controlling astrocyte ontogeny after injury remain incompletely understood, partly due to a lack of appropriate markers and animal models. We analyzed astrocyte precursor response to injury at the beginning (E11) and peak (E15) of gliogenesis in an avian tectal model of penetrating embryonic brain trauma. At both ages, lateral ventricular dilatation, necrotic foci, periventricular cysts and intraventricular hemorrhages were observed distal to stab wounds, two days after a unilateral stab injury to optic tecta. Neuronal (TUBB3) and oligodendrocyte precursor (PLP) markers were down-regulated, even far-removed from the wound site; while the mature astrocyte marker, GFAP, was up-regulated at the wound site, around necrotic areas and cysts, plus in usual areas of GFAP expression. Increased inflammatory response and apoptotic cell death were also confirmed in the injured tecta. Increased expression of nestin, aggrecan, NPA, SOX9 and GLAST at the wound site and in the ventricular zone (VZ) of the injured tecta indicated an astrogial precursor response. Furthermore, increased levels of Notch receptor 1 and Delta 1 in the ventricular zone of the injured side, 24 h after injury, might indicate an early differentiation response. However, cell division increased in the VZ only in early (E11) injury, but not later (E15), indicating that in late injury the astrogliogenesis occurring after acute injury is predominantly due to precursor differentiation rather than precursor proliferation. The inability to replenish the glial precursor pool during the critical period of vulnerability to injury may be an important cause of subsequent developmental abnormalities.

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Program/Abstract #380
Post-traumatic neural regeneration in sea cucumbers (Echinodermata: Holothuroidea)
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Echinoderms are invertebrate deuterostomes closely related to chordates. They are well known for their extraordinary regenerative capacities, which include complete recovery from injuries to the nervous system. Studies of neural regeneration in echinoderms will contribute to our knowledge of the evolution of neural plasticity and better understanding of neurogenesis in higher taxa. The echinoderm nervous system possesses a major non-neural cell type, which shows typical characteristics of radial glia. This cell type plays a key role in neural regeneration. Transection of the radial nerve cords triggers dedifferentiation of the glial cells in the injured tissues. The dedifferentiating cells remain connected to each other and form tubular outgrowths on either side of the wound. These glial scaffolds grow towards each other and are thought to support neuronal migration and re-growth of nerve processes. This growth phase is accompanied by a 10-fold burst in glial cell division, whereas proliferation of non-glial cells remains low. At least some glial cells are capable of giving rise to neurons as evidenced by co-expression of glial and neuronal markers. As to the molecular mechanisms underlying neuronal recovery, the response to the injury involves remarkable changes in gene expression, including up-regulation of Wnt9 and TCTP, which are known to be involved in patterning of the neural ectoderm and protection of cells against stress conditions and apoptosis, respectively. Moreover, among the most significantly up-regulated transcripts are retrotransposon-like elements, which are highly expressed by the cells at the wound site shortly after transection, as well as by the glial cells of the growing tubular regenerates.

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