Visual Activity Regulates Neural Progenitor Cells in Developing Xenopus CNS through Musashi1

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Abstract

Regulation of progenitor cell fate determines the numbers of neurons in the developing brain. While proliferation of neural progenitors predominates during early CNS development, progenitor cell fate shifts toward differentiation as CNS circuits develop, suggesting that signals from developing circuits may regulate proliferation and differentiation. We tested whether activity regulates neurogenesis in vivo in the developing visual system of Xenopus tadpoles. Both cell proliferation and the number of musashi1-immunoreactive progenitors in the optic tectum decrease as visual system connections become stronger. Visual deprivation for 2 days increased proliferation of musashi1-immunoreactive radial glial progenitors while visual experience increased neuronal differentiation. Morpholino-mediated knockdown and over-expression of musashi1 indicate that musashi1 is necessary and sufficient for neural progenitor proliferation in the CNS. These data demonstrate a novel mechanism by which increased brain activity in developing circuits decreases cell proliferation and increases neuronal differentiation through the down-regulation of musashi1 in response to circuit activity.

Keywords: neurogenesis, visual experience, Xenopus, musashi1, radial glial cells, BrdU, progenitor pool, differentiation, cell cycle, N-β-tubulin, nrp1

Introduction

The number of neurons in the brain is largely determined by the regulation of neural progenitor cell proliferation and the survival and differentiation of their progeny. The pool of neural progenitor cells can be expanded by symmetric divisions that give rise to two neural progenitor cells, maintained by asymmetric divisions that result in one of the progeny remaining a neural progenitor cell while the other differentiates, or depleted by terminal differentiation (Butt et al., 2005; Gotz and Huttner, 2005; Huttner and Kosodo, 2005; Kriegstein and Alvarez-Buylla, 2009; Noctor et al., 2007). While expansion and maintenance of neural progenitor cells is favored over differentiation during early CNS development, differentiation progressively dominates progenitor cell fate leading to depletion of the pool of progenitors...
as CNS circuits develop (Kriegstein and Alvarez-Buylla, 2009), suggesting that signals from developing circuits may shift the fate of neural progenitors and their progeny. Indeed, recent studies suggest that neuron-derived trophic factors may induce progenitors to stop dividing and differentiate (Botia et al., 2007; Kriegstein and Alvarez-Buylla, 2009), however there is little evidence of endogenous activity-dependent regulation of progenitor cells in intact animals. Therefore, a fundamental open question in the regulation of neurogenesis is whether feedback mechanisms from developing neuronal circuits regulate progenitor fate to expand, maintain or deplete the pool of neural progenitor cells in the developing CNS in vivo.

To address this question we tested whether visual activity affects the rate of ongoing cell proliferation in the developing visual system of Xenopus laevis tadpoles, where cell proliferation in the optic tectum continues over an extended period of development while the visual circuitry is both functional and still in the process of development (Cline, 2001; Peunova et al., 2001; Straznicky and Gaze, 1972). We find that cell proliferation in the tectum, detected by BrdU incorporation, decreased as visual circuitry matured between stages 46 and 49. Over the same period, immunoreactivity for MCM7, a marker of cells with proliferative potential (Crevel et al., 2007; Facoetti et al., 2006; Khalili et al., 2003), and musashi1, an RNA binding protein that is essential for maintenance of the neural progenitor population (Glazer et al., 2008; Kaneko et al., 2000; Okano et al., 2005) decreased, correlating with the developmental decrease in proliferation. These data are consistent with the idea that visual activity in the more mature circuit could negatively regulate cell proliferation. Indeed, brief visual deprivation for 2 days increased cell proliferation in the optic tectum compared to animals with visual experience, suggesting that feedback from the developing visual circuit shifts the fate of neural progenitors. We used sequential exposure to two differentially halogenated thymidine analogs (IdU and CldU, referred to collectively as XdUs) to reveal the division history of proliferating cells (Encinas and Enikolopov, 2008; Vega and Peterson, 2005) and found that a larger fraction of cells in animals with brief visual deprivation remain in the cell cycle, whereas more cells exit the cell cycle and differentiate into neurons in animals with visual experience. Interestingly, visually-deprived animals have more musashi1-immunoreactive radial glial progenitors than animals with visual experience. Morpholino-mediated knockdown and rescue experiments show that musashi1 is required for the increased proliferation seen with visual-deprivation. Finally, exogenous expression of musashi1 in stage 49 radial glial cells, which have little detectable endogenous musashi1-immunoreactiviy and low proliferative activity, increases their proliferation. Our study suggests that sensory experience plays a role in neurogenesis in the developing CNS in vivo by regulating the fate of progenitors and their progeny.

**Results**

**Cell proliferation in the optic tectum decreases with visual system development**

In the visual system of Xenopus laevis tadpoles retinal ganglion cells project axons to the contralateral optic tectum where they form synapses with tectal neurons (Fig. 1A-C). Between stages 39 and 49, a period of 6–7 days, the visual system of Xenopus tadpoles develops rapidly to accommodate the behavioral needs of the animal. Retinal ganglion cells first innervate and transmit visual information to the optic tectum at stage 39 (Holt and Harris, 1983) when the majority of cells in the tectum have radial glial morphology and neurons have very simple dendritic arbors (Wu et al., 1999). An initial topographic retinotectal map is established by stage 45 (O'Rourke and Fraser, 1996) and between stages 46 and 49 visual experience drives many aspects of visual circuit development pertaining to the detection and
processing of visual inputs (Bestman and Cline, 2008; Chiu et al., 2008; Cline and Haas, 2008; Engert et al., 2002; Pratt and Aizenman, 2007, 2009; Pratt et al., 2008; Tao and Poo, 2005) even as ventricular layer cells with radial glial morphology persist in the tectum (Tremblay et al., 2009). Although it is well known that tectal ventricular layer cells proliferate throughout tadpole stages of development and generate neurons within the tectum (Peunova et al., 2001; Straznicky and Gaze, 1972), a potential relation between development of the functional visual circuit and cell proliferation has not been explored.

To test whether rates of cell proliferation in the optic tectum change over this period of visual system development, we exposed tadpoles at stage 46, 48 and 49 to 2 hr of XdU and either processed the brains immediately or allowed the animals to develop in normal rearing solution for another 22 hr before processing the brains as wholemounts for XdU immunodetection. We delivered XdU by exposing tadpoles to rearing solution containing 10 mM XdU for 2 hr. This method efficiently labels proliferative cells in the brain and allows greater control over XdU exposure time than standard injection methods (Peunova et al., 2001). Brains were processed to detect XdU with antibodies and a complete confocal Z-series of images was collected through the midbrain of wholemount brains or cryostat sections. As previously reported, proliferating cells, identified by exposure to 3H-thymidine (Straznicky and Gaze, 1972) or BrdU-labeling (Peunova et al., 2001) with a short survival time, line the ventricle (Figs 1D,F and 3A,B). We counted XdU-labeled cells in the ventricular layer at the tectal midline using the dissector method and indexed cell counts to an estimated volume of 20,000 µm³ (see Fig 1D and methods). Stage 46 tadpoles have significantly more XdU-labeled cells (99.6 ± 2.4 cells/20,000 µm³, n=8) than either stage 48 (36.1 ± 2.3 cells/20,000 µm³, n=8; p<0.05) or stage 49 (8.9 ± 0.8 cells/20,000 µm³, n=7; p<0.05) tadpoles (Fig 1H, Supplementary Movies S1, S2 and S3). The XdU-labeled cells continue to divide over the next 22 hr to approximately double the number of XdU-labeled cells (Fig 1I). Stage 46: 180.4±4.9 cells/20,000 µm³, n=8; Stage 48: 71.2±3.9 cells/20,000 µm³, n=8; Stage 49: 20.3±0.9 cells/20,000 µm³, n=7). These data suggest that proliferation gradually decreases between stages 46 and 49, a time interval during which the visual circuit matures.

Cells expressing MCM7 or Musashi decrease with development

We next tested whether MCM7 and Musashi1, which have been characterized as markers of proliferative cells in other experimental systems, change expression over the developmental period when XdU incorporation decreased. MCM7 is a part of minichromosome maintenance complex (MCM) of proteins and is expressed in cells with proliferative potential (Crevel et al., 2007; Facoetti et al., 2006; Khalili et al., 2003). Proteins in the MCM complex are down-regulated when cells become quiescent, differentiated, or senescent (Facoetti et al., 2006; Padmanabhan et al., 2004). Antibodies to MCM7 are thought to label the population of cells that is not yet differentiated and is capable of proliferation (Blow and Dutta, 2005). In the optic tectum, we find that MCM7-immunoreactive cells are present in the cell layers lining the ventricle (Fig. 2 A1,A2 and B1, B2) and partially overlap with the distribution of cells detected by incorporation of XdU following 2h exposure. MCM7 expression was down-regulated between stage 46 and 49 (Fig. 2 A1,A2 and B1, B2), consistent with the decrease in XdU-incorporation over this time period.

The Musashi proteins are highly conserved RNA binding proteins (Good et al., 1993; Nakamura et al., 1994; Sakakibara et al., 2001), whose founding member, Musashi1, was originally discovered in Xenopus and named nrp1 (Richter et al., 1990). In vertebrates, there are 2 genes, musashi1 and musashi2, the latter of which is homologous to xrp1 in Xenopus. We focused on Musashi1 because it is expressed
exclusively in the CNS and is enriched in CNS progenitor cells across phyla (Amato et al., 2005; Good et al., 1993; Kaneko et al., 2000; Nakamura et al., 1994; Sakakibara et al., 1996; Sakakibara and Okano, 1997) and because it is required for maintenance of neural stem cells (Okano et al., 2002; Okano et al., 2005; Sakakibara et al., 2001; Sakakibara et al., 2002), whereas musashi2/xrp1 is widely expressed throughout the body. Musashi1 protein expression is down-regulated in differentiated neurons (Kaneko et al., 2000; Nakamura et al., 1994; Sakakibara et al., 1996; Sakakibara and Okano, 1997). We find that musashi1-immunoreactive cells are present in the proliferative layer lining the ventricle of the optic tectum (Fig 2 C1, C2 and D1,D2) and overlap with the distribution of MCM7-immunolabeled cells. Musashi1-immunoreactive cells decrease in number between stages 46 and 49 from 158.3±8.2 cells/20,000 µm to 53±4.3 cells/20,000 µm (p< 0.05, n=4, 4), consistent with the decrease in XdU- and MCM7- labeled cells. These data suggest that the pool of progenitor cells gradually decreases between stages 46 and 49.

Musashi-expressing cells are radial glial progenitor cells

To test whether the musashi1-immunoreactive cells are neural progenitors in the tadpole CNS, we exposed stage 48 tadpoles to XdU for 2 hr and fixed half of them immediately to analyze the distribution of XdU labeling and musashi1-immunoreactive cells. The remaining tadpoles survived for an additional 72 hours in the absence of XdU before fixation at stage 49. The majority of the cells labeled with a 2 hr exposure to XdU are located in the ventricular layer and label with antibodies to musashi1 (Fig 3 A1-4), indicating that musashi1-immunoreactive cells are progenitors. When XdU-labeled animals survived for an additional 72 hr (in the absence of further XdU exposure) the number of XdU-labeled cells increased (Fig 3 B1-4), indicating that the XdU-labeled neural progenitor cells continue to proliferate. Furthermore, after 72 hr, the XdU-labeled cells were distributed within the tectal cell body layer where mature tectal neurons are located (Fig 3B4).

Studies in mammalian cortex indicate that radial glia are neural progenitors (Kriegstein and Alvarez-Buylla, 2009), however the potential role of radial glial cells as neural progenitors in midbrain subcortical structures has not been established. We find that musashi1-immunoreactive cells in the tadpole optic tectum extend a radial process to the pia, indicating that they have radial glial morphology (Fig 3 C1 and C3). To further characterize the musashi1-immunoreactive cells, we labeled radial glial cells in stage 47 tadpoles by bulk electroporation of a CMV::eGFP expression plasmid into ventricular layer cells (Haas et al., 2002). The day after electroporation, eGFP is expressed in cells with radial glial morphology lining the ventricle (Haas et al., 2002; Tremblay et al., 2009). The majority of eGFP-expressing radial glial cells in stage 47 optic tectum are musashi1-immunoreactive (Fig 3D,E). These results show that a 2 hr exposure to XdU in vivo labels musashi1-immunoreactive neural progenitors and that musashi1-immunoreactive cells are radial glia.

Visual deprivation increases cell proliferation in the optic tectum

The developmental decrease in progenitor cell proliferation correlates with the maturation of the functional visual system in Xenopus tadpoles and suggests that visual circuit function may negatively regulate progenitor cell activity. To test whether visual experience regulates cell proliferation in the tadpole optic tectum, animals were reared in a 12 hr light/12 dark cycle until stage 46 when rates of cell proliferation are still relatively high (Fig 1). Tadpoles were separated into 3 groups: One group continued under the normal 12 hr light/12 dark cycle, called ‘ambient light’. The second group was provided with enhanced visual stimulation (from an array of LEDs flashing on and off at 1Hz (Sin et al., 2002)) during
the 12 hr light period of the light/dark cycle and the third group was deprived of visual stimulation (by keeping them in the dark, see diagram in Fig 4A). All tadpoles were kept in the dark during the 12 hr dark period of light/dark cycle.

We determined the division history of proliferating cells in the optic tectum using an assay that is based on a double-label protocol in which animals were exposed to two deoxyuridine analogs, ClDu and Idu, at an interval longer than the cell cycle to identify cells which remain proliferative (Encinas and Enikolopov, 2008; Vega and Peterson, 2005) as shown in Fig 4B. ClDu and Idu can be identified by immunostaining in Xenopus optic tectum without significant cross-reactivity or labeling bias (Supplemental Figure S1). We refer to ClDu and Idu generically as XdU. We provided a 2-hour exposure to the first deoxyuridine analog X1dU (either ClDu or Idu; green in Fig 4B) and allowed tadpoles to grow in the absence of X1dU label for 24 hr, followed by a 2 hr exposure to the second deoxyuridine analog X2dU (either ClDu or Idu; red in Fig 4B). We used a 24h interval in between the XdU and XdU exposures based on the observation that the number of XdU-labeled cells approximately doubled over 24 hr (Fig 1I). The optic tectum was then analyzed for the presence of X1dU and X2dU labeling in 30 µm cryostat sections. Three labeling combinations are predicted: 1. cells with only X1dU (green in Fig 4B) 2. cells with only X2dU label (red in Fig 4B) and 3. cells labeled with both XdU's (yellow in Fig 4B). The number of cells labeled with X1dU (green) relative to the total number of labeled cells is the fraction that were in S phase at the first XdU exposure, but were not in S phase at the time of exposure to X2dU. This value gives an estimate of the cells that exit the cell cycle between the times of exposure to X1dU and X2dU. The number of cells labeled with X2dU (red) relative to the total number of labeled cells is the fraction that was in S phase only at the second XdU exposure but not during exposure to X1dU. This value would represent a potential recruitment of quiescent progenitors to proliferate. The number of cells labeled with both XdUs (yellow) relative to the total number of labeled cells is the fraction of cells that were in S phase during both XdU exposure periods and therefore provides an estimate of the population of progenitors that remained proliferative during the observation window. Although this protocol does not identify all cells that are proliferating or all cells that remain in the cell cycle over the 24 hr interval (Encinas and Enikolopov, 2008; Vega and Peterson, 2005), it allows us to compare cell proliferation and cell cycle parameters in tadpoles reared in a normal 12 hr light/12 dark cycle (referred to as ambient light) with animals provided with either enhanced visual stimulation or deprived of visual stimulation.

We counted XdU-labeled cells along the midline ventricular layer in sections through the optic tectum and found that tadpoles subjected to reduced visual experience had more XdU-labeled cells in the same volume (79 ± 7.6 cells/20,000µm³, n=5) compared to animals that were exposed to either ambient light (48.7 ± 5.0 cells/20,000µm³, n=5; p<0.05) or enhanced visual stimulation (44 ± 7.3 cells/20,000µm³, n=5; p<0.05; Fig 4C, D and E). Similar indices of XdU-labeled cells were seen in the optic tecta of tadpoles exposed to either enhanced visual activity or ambient light. These data suggest that visual deprivation for 2 days increases cell proliferation in the optic tectum. They further suggest that the amount of visual activity provided by ambient light is sufficient to reduce cell proliferation in the tectum.

Next, we determined the fraction of X1dU-labeled cells that also incorporated X2dU, as an estimate of the cell population that continued to proliferate over the 24h period. Animals deprived of visual experience have a significantly larger fraction of X1dU and X2dU double-labeled cells (82.1 ± 1.9%) compared to tadpoles exposed to either ambient light (62.4 ± 1.6%, p<0.05) or enhanced visual stimulation (70.9 ± 3.5%; p<0.05; Fig 4F). A smaller proportion of double-labeled cells in animals with visual experience indicates that relatively fewer X1dU-labeled cells were in S phase of the cell cycle at the time of exposure.
to the second label, X$_2$dU. Furthermore, visual experience results in a higher fraction of cells labeled with only X$_1$dU compared to visual deprivation (Fig 4G) consistent the idea that visual experience changes the fate of tectal progenitors so they exit the cell cycle or become quiescent. Together, these data indicate that visual system activity decreases proliferative activity, while reduced visual experience maintains cells in a proliferative state by decreasing cell cycle exit and maintaining cells in the progenitor pool at the expense of differentiation.

**Visual experience increases neuronal differentiation**

The data presented above suggest that visual experience may change the fate of the progeny of tectal progenitor cells so they differentiate into neurons. To test whether visual experience promotes neuronal differentiation of newly generated cells, animals were reared in their normal 12h light/12h dark conditions until stage 47 and exposed to XdU for 2h in rearing solution. Animals were then either deprived of visual experience for 48h or exposed to enhanced visual stimulation as described in Fig 5C. We used N-β-tubulin antibodies to label differentiated neurons (Moody et al., 1996). Visual experience increases the proportion of XdU-labeled cells that differentiated into N-β-tubulin-labeled neurons over the intervening 48h compared to that seen in visually-deprived animals (61.7 ± 1.5% vs 40.2 ± 1.1%, p<0.05, n=11,11 animals, respectively). These results indicate that visual experience changes the fate of progeny to exit the cell cycle and differentiate into neurons.

**Visual deprivation expands the neural progenitor cell population by increasing Musashi1 expression**

The reduced proliferation seen in animals with visual experience could result from two types of mechanisms in relation to neural progenitor cells. The number of neural progenitor cells could remain constant while the cell divisions occur less frequently or the size of neural progenitor pool could decrease with visual system activity. Either mechanism would result in a decreased fraction of X$_1$dU and X$_2$dU double-labeled cells and a reciprocal increase in the fraction of cells that stop dividing after X$_1$dU exposure. To differentiate between these two possibilities we tested whether the musashi1-expressing neural progenitor cell population is affected by visual experience. Animals were reared under normal conditions to stage 47 when they were either deprived of visual experience or provided with visual experience, as shown in Figure 5C. The number of musashi1-expressing cells was significantly lower in tecta of animals with visual experience (267.2 ± 15.3 cells/20,000µm$^2$, n=7) compared to tecta of visually-deprived animals (426.9 ± 32.8 cells/20,000µm$^2$, n=7; p<0.05) (Fig 6 A, B and C). These results are consistent with a model in which the decrease in cell proliferation seen with visual stimulation results from a reduction in number of musashi1-expressing neural progenitor cells, whereas visual deprivation increases the pool of musashi1-expressing neural progenitor cells.

The experiments described above indicate that musashi1 expression correlates with the proliferative activity of tectal neural progenitor cells. To test whether musashi1 is required for the increase in cell proliferation in visually-deprived animals, shown in Figure 4, we knocked down nrp1B, the Xenopus homolog of musashi1 using morpholino antisense oligonucleotides to nrp1B. To test the efficacy of knockdown, morpholinos against nrp1B or control scrambled morpholinos were electroporated into the right tectal lobe. After 48 hours, we compared the intensity of musashi1 immunoreactivity in ventricular layer cells of the right and left optic tecta. Morpholinos against nrp1B reduced the ratio of musashi1 immunoreactivity in the electroporated right tectum to the unelectroporated left tectum to 57.2 ± 0.03% (p<0.05), whereas control morpholinos did not show any significant difference in musashi1 immunoreactivity between right and left tecta after unilateral electroporation (Supplementary Data).
We then electroporated stage 46 tadpoles in both tectal lobes with nrp1B morpholinos or control morpholinos and subjected animals to reduced visual stimulation for 60 hrs followed by 2hr exposure to XdU. Morpholinos against nrp1B significantly reduced the number of XdU-labeled cells (36.93 ± 1.94 cells/20,000µm³, n= 14; Fig 6 E,G;) compared to control morpholinos (52.8 ± 1.7 cells, n= 16; p<0.05; Fig 6 D,G). Furthermore, coelectroporation of plasmid containing mouse musashi1 cDNA with morpholinos directed against Xenopus nrp1B mRNA completely rescued the morpholino-induced reduction in proliferation (62.5 ± 4.0 cells/20,000µm³, n= 11; p<0.05; Fig 6 F, G). These data indicate that musashi1 protein levels are negatively regulated by visual activity in the optic tectum and that musashi1 is necessary for the increased cell proliferation seen in visually-deprived animals. Together with data presented in Figure 5, the data show that progenitors increase musashi expression, increase proliferative activity and expand the progenitor pool in the absence of visual input but that visual experience triggers two changes in the system: 1. a decrease in musashi expression and a decrease in proliferative activity in radial glial cells and 2. an increase in the rate at which newly generated progeny differentiate into neurons.

To test whether musashi1 is sufficient to increase cell proliferation in the CNS we returned to stage 49 tadpoles, where musashi1 expression is relatively low in ventricular layer cells (Figure 2). Electroporation of ventricular layer cells in stage 49 tadpoles with a dual promoter plasmid co-expressing mouse musashi1 and eGFP or eGFP alone labeled radial glial cells (Figure 7A,B). In vivo time-lapse images of eGFP+ cells co-expressing mouse musashi1 show a greater increase in eGFP-expressing cells over 6 days than seen in control animals, suggesting that exogenous expression of musashi1 increases proliferation of radial glia. Furthermore, we tested whether musashi1 expression in stage 49 tadpoles increases XdU incorporation. Stage 49 tadpoles were electroporated with a dual promoter plasmid co-expressing either eGFP alone or mouse musashi1 and eGFP and after 60 hr, animals were exposed to XdU for 2 hr immediately before sacrifice. Mouse musashi1 expression doubled the number of XdU-labeled cells compared to expression of eGFP alone (Musashi1: 21.3±1.3 cells/20,000µm³, n=17, eGFP: 10.9±0.9 cells/20,000µm³, n=18; p< 0.05).

**Discussion**

Rates of neurogenesis decrease in the developing CNS as neuronal circuits become functional, suggesting that neuronal activity generated by developing circuits may regulate the addition of new cells to the circuit. We have used quantitative analysis of cell proliferation in the developing visual system of tadpoles to show that rates of cell proliferation decrease as the visual circuit matures. These results demonstrate a temporal correlation between the establishment of functional circuits and a decrease in cell proliferation and suggest the presence of a negative feedback mechanism from the developing circuit to neural progenitors to limit their proliferation. Consistent with this idea, we demonstrate that sensory input activity decreases cell proliferation in the optic tectum and increases the rate at which newly generated cells differentiate into neurons. We provide evidence that immunoreactivity to musashi1, a highly conserved mRNA binding protein which is required to maintain progenitor activity across phyla (Okano et al., 2005), is down-regulated during the developmental period when cell proliferation decreases and the normal developmental down regulation does not occur when animals are deprived of visual experience for 2 days. We show that knockdown of nrp1B, the Xenopus homolog of musashi1 prevents the visual-deprivation induced increase in cell proliferation. Finally, we report that expression of mouse musashi1 increases rates of cell proliferation when endogenous protein has been knocked down by morpholinos or has been developmentally down-regulated. These data indicate that the control of neural
progenitor proliferation and cell fate during CNS development is regulated by neuronal activity such that increased activity from developing circuits changes the fate of newly generated cells so they exit the cell cycle and differentiate into neurons. The data further suggest that musashi1 expression is regulated by activity in the intact CNS and that activity-dependent downregulation of musashi1 expression decreases cell proliferation and increases neuronal differentiation in the developing CNS. Finally, the data indicate that neural progenitor proliferative activity can be dynamically regulated by increasing or decreasing musashi1 expression, either by modulating brain activity or by more direct manipulation of musashi1 expression levels.

Tectal neural progenitor cells are radial glia

We show that musashi1-expressing neural progenitor cells in tadpole optic tectum are radial glial cells and that they generate tectal neurons. Neural progenitor cells with radial glial morphology have been reported in cortical regions of many vertebrates, suggesting that radial glial neural progenitors are highly conserved morphologically and functionally in cortical development (Kriegstein and Alvarez-Buylla, 2009), however the role of radial glial cells as neural progenitors in subcortical brain regions has not been clearly demonstrated. The decrease in cell proliferation and musashi1 immunoreactivity between stages 46 and 49 and with enhanced visual experience suggest that the progenitor pool is depleted in response to activity-dependent signals. It is interesting to note that electroporation of ventricular layer cells in stage 49 tadpoles with a CMV::eGFP expression plasmid labels radial glial cells, showing that radial glial cells persist at these stages, but they have lower proliferative activity and low musashi1 expression. Expression of mouse musashi1 in radial glial cells of stage 49 tadpoles increases XdU incorporation and increased the number of eGFP-expressing cells over a 6 day period of in vivo imaging. This experiment suggests that the normal experience-dependent developmental decrease in expression of musashi1 in radial glial cells decreases their proliferative activity, but that mechanisms that increase musashi expression in radial glia, either through a decrease in sensory input or by exogenous expression of musashi1, can increase proliferation in these relatively quiescent progenitors.

The increased XdU incorporation seen in stage 46 animals compared to stage 49 animals could indicate that S phase of the cell cycle is longer in younger animals. Similarly, the differences in XdU-incorporation in animals in which musashi expression is decreased by knockdown or increased by exogenous expression of mouse musashi, could arise from corresponding changes in the length of S phase. To attempt to address this possibility, we labeled tecta with antibodies to phospho-histone 3 (PH3) to determine the mitotic index. We found that PH3 labels 2-6 cells/20,000µm3 along the tectal midline, but the labeling is too sparse to test for statistically significant differences across stages (data not shown). Although we cannot strictly conclude that the mitotic index does not change with developmental stage and musashi1 expression, our data nevertheless provide evidence for regulation of neurogenesis by visual experience and musashi1 expression in radial glial progenitors.

Control of neurogenesis and brain size by an interplay of ‘intrinsic’ cell autonomous and ‘extrinsic’ non-cell autonomous mechanisms

Regulation of neurogenesis establishes the number, type and distribution of cells in the CNS by regulation of progenitor maintenance and proliferation, and the subsequent survival and differentiation of progeny into neurons or glia. Controlling the size of the progenitor pool by regulating the fate of progenitors and their progeny to either die, differentiate or continue to proliferate is a key regulatory event in brain development and ultimately in the establishment of functional circuits and behavior. For instance,
maintaining progenitors in an actively proliferative state enlarges the progenitor pool and can enlarge the cerebral cortex of mice (Chenn and Walsh, 2002) by setting an upper bound on the potential number of neurons generated (Lehmann et al., 2005). Conversely, premature transition of progenitors from an active proliferative state to differentiated cells can deplete the progenitor pool, while increasing or decreasing progenitor survival controls cortical size (Depaepe et al., 2005; Putz et al., 2005). A variety of extrinsic factors can affect the fate of neural progenitor progeny by either maintaining progenitor fate or promoting differentiation (Ninkovic and Gotz, 2007), including neurotransmitters (Gandhi et al., 2008; LoTurco et al., 1995; Sadikot et al., 1998; Spitzer, 2006), secreted peptides/proteins (Botia et al., 2007; Suh et al., 2001; Wexler et al., 2009), and transmembrane or GPI-linked proteins (Depaepe et al., 2005; Putz et al., 2005; Qiu et al., 2008). Despite the plethora of possible extrinsic signals, whether single signals or combinations of signals operate downstream of visual system activity to decrease rates of cell proliferation during CNS development is not clear.

Extrinsic signals must be read and interpreted by intrinsic cell autonomous mechanisms to culminate in cell fate regulation. Our data indicate that sensory input activity depletes the neural progenitor population by down-regulating musashi, suggesting that musashi is a prominent ‘intrinsic’ maintenance/differentiation switch which can respond to non-cell autonomous signals from developing brain circuits to mediate feedback from the developing circuit and control cell proliferation. Musashi is thought to function as a translational repressor. Using genomic and proteomic approaches, musashi was found to affect the expression of a large number of targets involved in the cell cycle, apoptosis, cell proliferation and cell differentiation (de Sousa Abreu et al., 2009). Musashi may promote maintenance of neural progenitor cells by blocking translation of Numb, a repressor of Notch signaling (Imai et al., 2001). Activation of the Notch signaling pathway in turn promotes the maintenance of neural progenitor cells (Hitoshi et al., 2002; Tokunaga et al., 2004). In addition, musashi represses expression of p21WAF-1, a cyclin-dependent kinase inhibitor, which causes exit from the cell cycle (Battelli et al., 2006). A third target of musashi repression is doublecortin, a microtubule associated protein that is expressed at early stages of neuronal differentiation and is important for neuronal migration, differentiation and plasticity (Horisawa et al., 2009). Although considerable evidence suggests that musashi maintains neural progenitors in a proliferative state by repressing translation of proteins required for cells to exit the cell cycle and differentiate, it is not clear whether a single gate keeper integrates multiple extrinsic signals to control cell fate (Suh et al., 2009). In addition to musashi, other proteins including musashi2, Sox2 and PTEN promote neuronal progenitor cell self-renewal and decrease differentiation (Graham et al., 2003; Groszer et al., 2006; Ohtsuka et al., 2001; Sakakibara et al., 2002). Activity-dependent regulation of musashi1, musashi2, Sox2 or PTEN has not been reported and would be an interesting topic to pursue.

How signals from developing circuits regulate intracellular signaling pathways controlling cell fate of radial glial progenitors is not clear. Calcium transients downstream of neurotransmitter receptor activity (LoTurco et al., 1995) or bioactive peptides (Botia et al., 2007) may cause progenitors to exit the cell cycle and differentiate. A recent study demonstrated that Xenopus tadpole radial glial cell bodies exhibit slow calcium transients in response to visual stimulation (Tremblay et al., 2009). The same study used time-lapse in vivo imaging to show that radial glial processes near the pia are dynamic and that visual stimulation increases radial glial structural dynamics by a mechanism that includes NMDA receptor-mediated NO signaling. This is interesting because previous studies in Xenopus tadpoles indicated that NOS-expressing neurons are positioned immediately adjacent to the radial glial cell bodies, and that decreasing NO signaling increases cell proliferation in the tadpole CNS and decreases cell motility (Peunova et al., 2001; Peunova et al., 2007), suggesting that cells temporarily stop exploratory structural
rearrangements when they devote their cytoskeletal resources to divide. Together these studies suggest that visual system activity negatively regulates radial glial cell proliferation in the tadpole optic tectum by a signaling pathway that includes an NMDA receptor-mediated increase in NO, which decreases proliferation in tectal progenitors.

**Role of activity in neurogenesis in the developing and adult CNS**

It is widely recognized that neurogenesis continues in the adult brain and that exposing animals to enriched environments or exercise increases adult neurogenesis, presumably through activity-induced increases in trophic factors or neurotransmitters (Brown et al., 2003; Rochefort et al., 2002; Whitman and Greer, 2009; Zhao et al., 2008). Further investigations to distinguish whether the increased neurogenesis in adult CNS is the outcome of increased cell proliferation, differentiation or survival of differentiated progeny suggest that, even though exercise may increase rates of proliferation in the hippocampal dentate gyrus (Brown et al., 2003), it appears that the more prevalent effect of increased brain activity in adult animals is to increase the survival of differentiated neurons in target brain regions (Whitman and Greer, 2009; Zhao et al., 2008). By contrast, we find that visual experience reduces rates of cell proliferation, increases cell cycle exit and increases neuronal differentiation of newly generated cells, but tUNeL staining suggests that cell death is not a significant factor in regulating neurogenesis in the developing optic tectum (Peunova et al., 2001), at least over the time-course of our studies. These data indicate that visual experience limits self-renewal of neural progenitor cells, thereby depleting the progenitor pool, and shifts the fate of progeny to terminal neuronal differentiation. These differences likely reflect distinct functions of neurogenesis in the adult and developing CNS. During CNS development the function of neurogenesis is to generate a large population of neurons in a relatively finite time period so that functional neural circuits can be assembled de novo. By contrast, adult-generated neurons integrate into pre-existing neural circuits where they are thought to modulate learning and memory (Whitman and Greer, 2009). Therefore, different responses to activity-dependent signals in neural progenitor cells in the developing and adult CNS likely reflect the requirements of the CNS for the generation and maintenance of new neurons at different stages of life.

**Material and Methods**

**Animals**

Albino Xenopus laevis tadpoles, obtained from our lab colony or commercial sources (Nasco, Fort Atkinson, WI), were reared in a 12 hr light/12 hr dark cycle incubator at 24°C. Animals were staged according to Nieuwkoop and Faber (Nieuwkoop and Faber, 1956).

**Reduced and enhanced visual stimulation protocol**

Tadpoles were deprived of visual experience by placing them in a black plastic box. Enhanced visual stimulation was applied by placing tadpoles in a box containing an array of light emitting diodes (LEDs) flashing on and off at 1 Hz to create a simulated motion stimulus, as described (Sin et al., 2002). In all the experimental conditions tadpoles were reared in a 24°C incubator. The time-course of the reduced or enhanced visual experience protocol is shown in Figure 4A.

**Immunohistochemistry**

Xenopus tadpoles were anesthetized with 0.02% MS222 (3-aminobenzoic acid ethyl ester), immersed in 4% paraformaldehyde (Electron Microscopy Sciences, Fort Washington, PA) in PBS and microwaved for
8 sec (750 W GE microwave) followed by 2h in fixative at room temperature (Li et al., 2010). After rinsing the animals, their brains were dissected and either analyzed in wholemount, were cryoprotected in 30% sucrose and cut into 30 µm horizontal sections with a cryostat, or embedded in gelatin and cut into 30 µm horizontal sections with a vibratome. Sections were blocked in 5% goat serum and 0.3% Tween 20 in PBS for 1 hr before incubating overnight at 4°C with anti-MCM7 (1:250; Abcam, Cambridge, MA), anti-musashi1 (1:250; Abcam, Cambridge, MA) or anti-β-tubulin I+II (1:200; Sigma-Aldrich, St. Louis, MO) antibodies. Detection was performed using appropriate fluorophore-tagged secondary antibodies (Molecular Probes/Invitrogen, Eugene, OR). Sections or brains were mounted in ProLong™ Gold (Molecular Probes/Invitrogen, Eugene, OR) and imaged with a Zeiss LSM 510 Meta confocal microscope (Carl Zeiss, Jena, Germany). When levels of labeling were compared across samples, all samples were prepared, photographed and analyzed in parallel using the same acquisition and analysis settings.

Labeling with XdU’s

Tadpoles were exposed to the halogenated thymidine analogs, BrdU, IdU or CldU, referred to collectively as XdU’s (MP Biomedicals, Solon, OH) at 10 mM in Steinberg’s solution (60 mM NaCl, 0.7 mM KCl, 0.8 mM MgSO$_4$, 0.3 mM Ca(NO$_3$)$_2$ and 1.4 mM Tris, pH 7.4) for 2 hr. We found that delivery of XdU by injection into the pericardial cavity or by 2hr exposure of tadpoles to XdU in rearing solution resulted in comparable labeling of proliferative cells. We used exposure in rearing solution for XdU delivery in our experiments because this method allows greater control of XdU delivery.

Analysis of history of cell division

Sequential exposure to the differentially halogenated thymidine analogs CldU and IdU was used to determine the history of proliferative activity, as described (Encinas and Enikolopov, 2008; Vega and Peterson, 2005). Tadpoles were exposed to IdU in Steinberg’s solution for 2hr and transferred to fresh Steinberg’s solution. After 24 hrs they were exposed to CldU in Steinberg’s solution for 2 hr, rinsed in fresh Steinberg’s solution, anesthetized with 0.02% MS222 and fixed as described above. For wholemount analysis, dissected brains were treated with −20°C methanol for 1 hr followed by proteinase K treatment as described (Peunova et al., 2001). For detection of IdU and CldU, wholemount brains or sections were treated with 2N HCl for 1 hr at 37°C, rinsed in PBS and incubated in 5% normal goat serum and 0.3% Tween 20 in PBS for 1 hr before incubating overnight at 4°C with 1:400 IdU specific antibody (BD Biosciences mouse anti-BrdU Cat # 347580) and 1:400 CldU-specific antibody (Accurate rat anti-BrdU Cat# OBT0030G). The IdU-specific antibody was detected using Alexa 488 labeled goat anti-mouse secondary antibody (Molecular Probes/Invitrogen, Eugene, OR). The CldU-specific antibody signal was enhanced using biotin tagged goat anti-Rat secondary antibody followed by detection with Cy5-streptavidin (Jackson ImmunoResearch). To reduce crossreactivity, highly cross-adsorbed secondary antibodies were used. Wholemounts and sections were mounted in ProLong™ Gold (Molecular Probes/Invitrogen, Eugene, OR) and imaged with a Zeiss LSM 510 Meta confocal microscope (Carl Zeiss, Jena, Germany). Images were collected using a Zeiss Fluo 20X 0.75 NA or CAPochromat 40X 1.2 NA water objective. No significant crossreactivity was observed for IdU and CldU (Supplementary Fig 1). To test whether there is a labeling bias in our method for detecting IdU and CldU (i.e. whether we can detect a cell in S-phase by IdU or CldU with equal probability) we exposed tadpoles to equimolar CldU and IdU simultaneously for 2 hr. All the cells were double-labeled, indicating that we can detect S-phase cells in tadpoles using IdU or CldU with equal probability (Supplementary Fig 1). We used CldU and IdU interchangeably and obtained the similar results.
Data Analysis

Image analysis and cell counting were performed using Metamorph (Universal Imaging Corporation/Molecular Devices, Downingtown, PA) image processing software. Images were background subtracted and cells were counted using the dissector method with the “manually count object” feature of Metamorph. In wholemounts of brains, cells in the ventricular layer at the midline of the optic tectum in an area 100 µm along the ventricular layer X 20µm lateral from the ventricular layer were counted in two non-neighboring optical sections. The first optical section was selected where the two tectal lobes meet at the dorsal midline and the second optical section was 8-10 µm ventral from the first. The average diameter of cell nuclei is 5 µm and does not change with the different stages or treatments analyzed. The rostral boundary for counting cells within the tectal ventricular layer was defined by the anterior dorsal commissure and the caudal boundary was identified as the start of the curvature to the caudolateral edge of the tectum, as shown in Figure 1. Cell counts from the 2 sections were added and presented as cell number per 20,000 µm³. We used cryostat sections for high-resolution 40X analysis to determine the presence of IdU only, CldU only or both IdU and CldU in cells. Two horizontal sections from each brain corresponding to about 60-90 µm and 120-150 µm from the dorsal side respectively were used for analysis. Cells were counted in the entire z stack through the section collected with 2 µm z-step size. The rostral and caudal boundaries for counting cells were the same as for wholemounts.

Knockdown and over-expression of Musashi1

Lissamine-tagged morpholino antisense oligonucleotides against nrp-1B, the musashi1 homologue in Xenopus laevis (GeneTools,Philomath, OR) with the sequence GCGCTTCTGTCTCCATTCGGTCTCT, or the five basepair mismatched oligonucleotide with the sequence GCCCTTGTGTGTCCAATCCGTGTCT, were electroporated into the optic tectum of stage 46 tadpoles as described (Bestman and Cline, 2008; Chiu et al., 2008). The morpholino against nrp-1B does not recognize the xrp1, the Xenopus homologue of musashiz, according to prediction algorithms by GeneTools (GeneTools,Philomath, OR). The tadpoles were provided with visual experience or deprived of visual experience as described above. At specified times after electroporation of morpholinos, tadpoles were exposed to XdU for 2hr in Steinberg's solution, fixed and analyzed in wholemounts as described above.

Mouse musashii cDNA was generously provided by Dr. Imai and Dr. Okano as pcDNA-Flag-Musashi1 (Imai et al., 2001). Mouse musashii was subcloned into a dual CMV promoter vector (Bestman and Cline, 2008) to co-express eGFP and musashii. Control eGFP expression was from the dual promoter construct with no construct in the second site. Plasmids were electroporated into the optic tectum of anesthetized tadpoles as described (Bestman and Cline, 2008; Chiu et al., 2008).

In vivo time-lapse imaging

Animals were screened for eGFP expression on a fluorescence microscope and imaged on a spinning disk confocal microscope (Perkin Elmer) with a 20X water immersion lens (0.9 NA), as described (Ruthazer and Cline, 2004).

Statistical tests

Data were tested with the non-parametric Mann–Whitney–Wilcoxon test to compare between groups unless stated otherwise. Data are represented as mean ± SEM.

Supplementary Material
Acknowledgements

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Footnotes

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Developmental decrease in proliferative cells in the Xenopus tadpole optic tectum. A-C. The Xenopus optic tectum includes a functional visual circuit and proliferative ventricular layer cells. A. Phase contrast image of an albino *Xenopus* tadpole head. Retinal ganglion cells axons project via the optic nerve (in red) to the contralateral optic tectum. B. Midbrain of *Xenopus laevis* showing the optic tectal lobes (ot). C. Cartoon of the cell types in *Xenopus* optic tectum. Afferent axons from retinal ganglion cells (red) form synapses with dendrites of tectal neurons (green) in the lateral neuropil. Tectal cell bodies are located medially from the retinotectal neuropil. Radial glial cells line the ventricle (blue).

D-G. Proliferation in the optic tectum. Stage 46 (D and E) and 49 (F and G) tadpoles were labeled with a 2-hr exposure to XdU and fixed immediately thereafter. D, F. Images of 3-D merge of a z series of horizontal confocal sections through the midbrain. E, G. Images of a series of single optical sections every 10 μm from dorsal to ventral tectum. The proliferating cells labeled by XdU incorporation line the ventricle and decrease significantly between stage 46 and stage 49. XdU-labeled cells were counted in the region marked by the bracket in D. H, I. Quantitative analysis of numbers of XdU-labeled cells in the midline ventricular layer of stage 46, 48 and 49 tadpoles (n=8, 8 and 7 animals respectively) which were exposed to XdU for 2 hr, then fixed and analyzed either immediately (H) or after further rearing in the absence of XdU for 22 hr (I) as shown in the schematic on top of each graph. XdU incorporation decreases significantly from stage 46 to stage 49. Scale bar = 50 μm in F, also applies to D. *** p<0.0001.

**Figure 2**
Developmental decrease in MCM7 and Musashi1 immunoreactivity. Confocal images of 30 µm cryostat sections through the optic tectum of stages 46 (A, C) and 49 (B, D) tadpoles labeled with anti-MCM7 antibody (green; A1, B1) or anti-musashi1 antibody (C1, D1) and a nuclear stain SytoxO (blue; A2-D2). Cells located along the ventricular layer are highly immunoreactive for MCM7 and musashi1. E. Quantitative analysis of the decrease in number of Musashi1 immunoreactive cells between stages 46 and 49. Scale bar = 20 µm. *p<0.05.

Figure 3
Lineage and identity of proliferating neural progenitor cells in optic tectum. A-B. Stage 48 tadpoles were exposed to XdU for 2h in rearing solution and were either fixed immediately (A) or after 72 hrs of further development in the absence of XdU (B). Confocal images of 30 µm cryostat sections through the optic tectum of tadpoles labeled with antibodies to musashi1 (msi1; green) and XdU (red). Nuclei were stained with SytoxO (blue). Musashi1-immunoreactive cells line the ventricle and are labeled by incorporation of XdU with a 2h survival time (A). The progeny of XdU-labeled neural progenitor cells are distributed throughout the cell body layer of the optic tectum after a 72 hr survival time (B). Note that the images of musashi1-immunoreactivity in B1 were taken with longer exposure periods than the images in A1, according to the lower levels of musashi1-immunoreactivity in ventricular layer cells at stage 49 (see Fig 2C,D). C. Musashi1-immunoreactive cells have radial processes extending to the pia. D,E. Radial glial cells, visualized by expression of eGFP, are immunoreactive for musashi1. Stage 47 tadpoles were electroporated with an eGFP expression plasmid, fixed after 24 hrs and analyzed for Musashi1 immunoreactivity in 30 µm vibratome sections. D1-D3. 3-D merge of a z series containing a complete eGFP-expressing cell with radial glial morphology (green; D1,D3) and Musashi1 immunolabeling (red; D2, D3). E1-E3. A single optical section through the ventricular cell region showing eGFP (E1, E3) colocalization with Musashi1 immunolabeling (E2, E3). Scale bar in D3 = 10 µm and E3 = 5 µm.

Figure 4
Visual deprivation increases cell proliferation in the optic tectum. A. Timeline of the experimental protocol. The 12 dark/12h light rearing conditions are shown as shaded and white bars. During the 12h ‘light’ period animals were either exposed to normal ambient light, enhanced visual stimulation or reduced visual stimulation (see methods). Animals were exposed to X\textsubscript{dU} for 2h and allowed to develop in the absence of X\textsubscript{dU} for 24 hours, after which they were exposed to X\textsubscript{dU} at the times marked by the green and red arrows, respectively. This protocol provides an estimate of the number of proliferating cells (labeled with X\textsubscript{dU} and/or X\textsubscript{dU}), the fraction of X\textsubscript{dU}-labeled cells that remain in the cell cycle (double labeled with X\textsubscript{dU} and X\textsubscript{dU}), and the fraction of X\textsubscript{dU}-labeled cells that exit the cell cycle (X\textsubscript{dU} only). B. A cartoon of representative results of the assay to detect the division history of proliferating cells as labeled in A. Cells labeled in green incorporated X\textsubscript{dU} only. Cells labeled in red incorporated X\textsubscript{dU} only. Cells labeled in yellow were in S phase during exposure to X\textsubscript{dU} and again during exposure to X\textsubscript{dU}. C, D. Images of the dorsal midline ventricular layer of the optic tectum of tadpoles with enhanced visual experience (C1-C4) or reduced visual experience (D1-D4) labeled with anti-X\textsubscript{dU} (green; C2, D2) and anti-X\textsubscript{dU} (red; C3,D3). C1, D1. Images of the X\textsubscript{dU} and X\textsubscript{dU}-labeled cells in merged optical sections of the complete z-series through the depth of the 30 µm cryostat section. C2-C4 and D2-D4. Magnified single optical sections from the z-series in C and G showing a fraction of cells labeled with X\textsubscript{dU} only, X\textsubscript{dU} only and both X\textsubscript{dU} and X\textsubscript{dU}. Scale bar = 10 µm for C2-4 and D2-4. Scale bar in D4 corresponds to 50 µm in C1 and D1. E-G. Graphs of total numbers of X\textsubscript{dU}-labeled cells (E), fraction of X\textsubscript{dU}- and X\textsubscript{dU}-labeled cells (F) fraction of X\textsubscript{dU}-only labeled cells (G). * p<0.05, ** p < 0.001 compared to animals with reduced visual stimulation.
Visual experience increases neuronal differentiation. Stage 47 tadpoles were exposed to XdU for 2hr and allowed to develop in absence of XdU for 48 hrs while subjected to either enhanced visual stimulation or reduced visual stimulation as described in panel C. A, B. Images of single optical sections of N-β-tubulin (green; A1, A3, B1 and B3) and XdU (red; A2, A3, B2 and B3) immunoreactivity in 30 µm vibratome sections from animals subjected to enhanced visual stimulation (A) or reduced visual stimulation (B). C. Timeline of the experimental protocol. D. Percent of XdU+ cells that are double-labeled with N- β-tubulin antibody. Enhanced visual stimulation increases the proportion of newly generated cells that differentiate into neurons. Scale bar in A3 = 10 µm. **p<0.0001
Musashi1 is required for visual experience-dependent regulation of cell proliferation. A-C. Visual deprivation for 48h increases the number of Musashi1 (Msi1) immunoreactive neural progenitor cells compared to animals with enhanced visual experience. Images of Musashi1 immunoreactivity in 30 µm cryostat sections (A1, B1; green in A3, B3) and SytoxO (A2, B2; blue in A3, B3) and merged images (A3, B3). C. Visual deprivation significantly increases the number of cells with detectable musashi1-immunoreactivity compared to visual stimulation (n=5 each). D-G. Morpholino-mediated knockdown of nrp1B, the Xenopus homolog of musashi1, decreases cell proliferation in the optic tectum while expression of mouse Musashi1 rescues cell proliferation in animals with nrp1B knockdown. Both tectal lobes of stage 46 tadpoles were co-electroporated with expression constructs and morpholinos. Animals were deprived of visual stimulation for the next 60h and then were exposed to XdU for 2h immediately before fixation. D-F. Optical sections showing XdU-labeled cells in animals coelectroporated with the control eGFP expression plasmid (ContVec) and control scrambled morpholino (ContMO) (D), the control plasmid and morpholinos to nrp1B (Msi1MO) (E), or a plasmid expressing mouse musashi1 (mMsi1) and morpholinos to nrp1B (F). Morpholinos against nrp1B significantly decreased the number of XdU-labeled cells in the ventricular layer compared to control morpholinos (D,E). The nrp1B morpholino-mediated decrease in proliferation was completely rescued by simultaneous expression of mouse musashi1 (F). G. Quantification of numbers of XdU-labeled cells in animals treated as in D-F. Scale bar in B3 = 10 µm, E= 20 µm.*** p<0.0001.
Musashi1 expression is sufficient to increase cell proliferation. A.B. In vivo images of eGFP-expressing cells collected over 6 days after electroporation of stage 49 tadpoles with a dual promoter plasmid expressing eGFP alone (A) or eGFP and mouse Musashi1 (mMsi1; B). Each image is a 3-D merge of a z-series through the optic tectum taken 2 days (d2, A1, B1), 4 days (d4, A2, B2) and 6 days (d6, A3, B3) after electroporation. C,D. Musashi1 expression in stage 49 tadpoles increases XdU incorporation. Animals were electroporated with plasmid expressing eGFP (C) or eGFP/ mMsi1 (D) and 2 days later were exposed to XdU for 2hrs. Whole mount brains were analysed for XdU immunolabeling. C,D shows single optical sections of XdU labeled cells in eGFP- (C) and eGFP/ mMsi1-expressing animals (D). E. Quantitative analysis of XdU-labeled cells with or without exogenous expression of mMsi1. Exogenous expression of mMsi1 at stage 49 increases proliferation compared to control. Scale bar in B3 and D = 20 µm, ***p<0.0001