Neuronal regeneration in the goldfish telencephalon following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) insult

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Abstract

The constitutive regenerative ability of the goldfish central nervous system makes them an excellent model organism to study neurogenesis. Intraperitoneal injection of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was used to deplete tyrosine hydroxylase-positive neurons in the adult goldfish telencephalon. We report novel information on the ability of the goldfish to regenerate (∼3–4 d post-MPTP insult) damaged neurons in telencephalic tissue by observing the rapid incorporation of bromodeoxyuridine into newly generated cells, which precedes the recovery of motor function in MPTP-treated animals. Specifically, the telencephalon area telencephali pars dorsalis in female goldfish, which is associated with fish motor activity, regenerates following MPTP toxicity. The remarkable ability of goldfish to rapidly regenerate damaged neurons provides insight into their use as model organisms to study neuroregenerative abilities within a few days following injury. We provide evidence that goldfish are able to regenerate neurons in ∼3–4 d to both replenish and recover baseline catecholaminergic levels, thus enabling the fish to reestablish basic activities such as swimming. The study of neuron regeneration in the damaged goldfish brain will increase our understanding of vertebrate neurogenesis and regeneration processes following central nervous system injury.

Key words: cellular regeneration, goldfish, MPTP, BrdU, telencephalon, tyrosine hydroxylase

Introduction

Goldfish (*Carassius auratus* (Linnaeus, 1758)) and other teleosts are amenable model organisms to study neurogenesis (Stevenson and Yoon 1978; Stuermer et al. 1992; Sullivan et al. 1997) because they exhibit remarkably higher neurogenic rates than mammals and, thus, a greater ability to regenerate damaged axons and tissues following central nervous system (CNS) injury (Levine 1983; Stuermer 1986). It has been estimated that the rate of neurogenesis in the CNS of teleosts is approximately one order of magnitude higher than that observed in mammals (Zupanc and Sîrbulescu 2011). For example, ∼6000 cells or 0.06% of the total zebrafish (*Danio rerio* (Hamilton, 1822)) brain cell population are newly generated within any 30 min period (Hinsch and Zupanc 2007; Zupanc and Sîrbulescu 2011). Newly generated neurons originate from radial glial cells (RGCs), which are known as one of the major progenitor cell populations (Pellegrini et al. 2007; Ganz et al. 2010; Chapouton et al. 2011; Rothenaigner et al. 2011; Xing et al. 2014). There are up to 16 proliferative zones in the teleost brain and the telencephalon exhibits numerous cellular pools and is a major source of RGCs (Zupanc and Horschke 1995; Zupanc et al. 2005;

Kaslin et al. 2009; März et al. 2010; Pellegrini et al. 2016). In this study, we provide evidence that female goldfish can regenerate neurons ~3–4 d post central nervous system injury leading to the recovery of cathecolaminergic baseline levels to re-establish normal basic functions such as swimming activities. Only female goldfish were used in this study as differences in cellular proliferation and neurogenesis in adult fish exists between sexes (Zikopoulos et al. 2000; Ampatzis and Dermon 2007).

The neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), can destroy dopaminergic neurons in the brain of teleosts and induce dopamine (DA) depletion and Parkinson-like symptoms (Poli et al. 1990; Pollard et al. 1992; Youdim et al. 1992; Lucchi et al. 1998; Lam et al. 2005; McKinley et al. 2005). The MPTP models of Parkinson's disease (PD) experience many of the symptoms exhibited by human patients. This includes neuronal cell body destruction in the CNS motor axis as well as decreased tyrosine hydroxylase (TH) activity and DA content in the forebrain, midbrain, cerebellum, and vagal lobes of fish (Poli et al. 1990; Pollard et al. 1996), mice (Hallman et al. 1985), rat (Chiueh et al. 1984), guinea pigs (Chiueh et al. 1984), and primates (Burns et al. 1983). The goldfish ventral telencephalon, in particular the area telencephali pars dorsalis (Vd), plays a role in motor control in teleosts and is a region negatively impacted by MPTP toxicity (Goping et al. 1995; Wullimann and Mueller 2004; Sallinen et al. 2009). The ability of fish to regenerate damaged cells and tissues and reestablish normal functions following injury has been extensively studied in the spinal cord (Bernstein 1964; Sîrbulescu et al. 2009), cerebellum (Adolf et al. 2006; Zupanc and Sîrbulescu 2011), and retina (Stuermer et al. 1992; Becker and Becker 2007, 2008). Previous studies have reported reversible changes in forebrain DA levels following MPTP treatment where DA returned to baseline levels ~8 d post-injection (dpi) (Pollard et al. 1992; Adeyemo et al. 1993; Popesku 2009).

The ability of fish to incorporate bromodeoxyuridine (BrdU) into mitotically active cells following injury has been reported by previous studies (Zupanc and Horschke 1995; Ampatzis and Dermon 2007; Zupanc and Sîrbulescu 2011). For example, actively dividing cells were visualized 1–10 d following a lesion in the cerebellum of the weakly electric fish *Apteronotus leptorhynchus* (Ellis in Eigenmann, 1912) (Zupanc and Ott 1999). In this animal model, newly generated cells were able to migrate to specific target areas, differentiate into neurons, and integrate into existing neural networks (Stroh and Zupanc 1996; Zupanc and Ott 1999). Zupanc and Horschke (1995) showed that BrdU is available for DNA synthesis 4 h after intraperitoneal injection in *A. leptorhynchus* and another electric fish, a species of *Eigenmannia* (Jordan and Evermann, 1896), and is able to incorporate into new cells and mark the occurrence of neurogenesis.

Teleost species remove damaged or compromised cells almost exclusively via apoptosis following CNS injury in comparison with necrotic cell death observed in mammalian models (Beattie et al. 2000; Vajda 2002; Liou et al. 2003). There is also activation of RGCs in the goldfish hypothalamus and telencephalon following MPTP administration marked by the observed increase of glial fibrillary acidic protein (GFAP) (Xing et al. 2017). The role of glial activation following MPTP administration in teleosts is not well understood but could potentially contribute to the generation of newly synthesized neurons in the goldfish brain.

In this study, we report on the ability of the goldfish to rapidly regenerate (~3–4 dpi) damaged DAergic neurons in the area Vd by observing the rapid incorporation of BrdU into newly generated cells, which precedes recovery of motor function in MPTP-treated animals. The tyrosine hydroxylase (TH) immunoreactivity was used to determine if new catecholaminergic neurons are generated after MPTP administration. The rapid neuroregenerative ability observed in the goldfish telencephalon provides an explanation for the reported recovery of motor function and catecholaminergic levels in the goldfish brain following CNS injury.

Materials and methods

Animal maintenance

Adult female goldfish (*Carassius auratus* (Linnaeus, 1758)) were purchased from a commercial supplier (Mt. Parnell Fisheries Inc., Mercersburg, Pennsylvania, USA) and maintained at 18 °C under a natural-simulated photoperiod on standard flaked goldfish food. Fish were kept in 70 L tanks (15–18 fish per tank). All procedures were performed according to the guidelines of the Canadian Council on Animal Care and were approved by the University of Ottawa animal care committee. Goldfish were anesthetized using 3-aminobenzoic acid ethylester (MS-222; 0.05% in water, Sigma-Aldrich, Oakville, Ontario, Canada) for all handling and sampling procedures.

Experimental and sampling procedures

The neurotoxin MPTP (Sigma-Aldrich M0896, Oakville, Ontario, Canada) was dissolved in 0.6% saline to give a dose of 50 µg/g body weight of fish at 2 µL/g. Sexually recrudescent female goldfish (September 2014 experiment; $\sim$33 g $\pm$ 1.6; gonadosomatic index $\sim$2.8 $\pm$ 0.5) either received a single intraperitoneal injection of MPTP or a control 0.6% saline injection at time 0 similar to the treatment schedule used by Pollard's group (Pollard et al. 1992; Goping et al. 1995). All female goldfish also received a single intra-peritoneal injection of BrdU (Life Techonologies B23151, Burlington, Ontario, Canada) dissolved in 0.6% saline at a dose of 150 µg/g body weight at 3 dpi of MPTP prior to sampling at 4 and 7 dpi. Female goldfish were randomly sampled at 4 and 7 dpi when they were perfused intracardially with 0.6% saline ($\sim$10 mL) and then 4% paraformaldehyde (PFA, Sigma-Aldrich, P6148, Oakville, Ontario, Canada) freshly depolymerized in phosphate-buffered saline (PBS; pH = 7.4). Following perfusion, the brains, whole pituitary, and gonads were carefully dissected from the goldfish. The gonadosomatic index was calculated by dividing the gonad weight by body weight of the goldfish $\times$ 100.

Behavioural analysis

Female goldfish (December 2014 experiment; 32 g $\pm$ 1.4; gonadosomatic index 1 $\pm$ 0.1; $n = 10$) received either a single intraperitoneal injection of MPTP (50 µg/g body weight) or a control 0.6% saline injection at time 0. The mobility measurements were performed once per day at 0, 1, 3, and 7 dpi in the late morning where time 0 was measured immediately after the administration of MPTP. Fish movement and swimming motions were recorded using an Olympus Stylus TG-850 (Olympus, Center Valley, Pennsylvania, USA) camera in individual tanks. The fish were placed in 4 cm of water in a 13 cm $\times$ 30 cm transparent aquarium similar to the experiment performed by Weinreb and Youdim (2007). The fish were allowed to acclimatize for 30 min prior to recordings. The total movement of a single fish, which includes the total distance of swimming and time of rest, was measured over a period of 5 min using Logger Pro 3 (Vernier Software & Technology, Beaverton, Oregon, USA). Statistical analysis was performed using SPSS Statistics (version 21, IBM, New York, New York, USA). The data for total distance traveled were normally distributed and compared using one-way analysis of variance (ANOVA) followed by Tukey's test to compare the effects of MPTP treatment. The resting time data were not normally distributed and were therefore compared using the Kruskal–Wallis test followed by Dunn's multiple comparison test to determine the effects of MPTP treatment. p-values <0.05 were considered statistically significant.

Immunostaining

Goldfish brains were dissected and collected after intracardial perfusion and postfixed with 4% PFA overnight at 4 °C. The following day, the brains were washed in a series of solutions (1$\times$ PBS (2$\times$ 30 min); 0.85% NaCl (1$\times$ 40 min); Ethanol (EtOH) 70%/NaCl 0.85% 1:1 (1$\times$ 30 min); EtOH 70% 2$\times$ 20 min; EtOH 85% (1$\times$ 40 min); EtOH 95% (1$\times$ 40 min); EtOH 100% (2$\times$ 30 min)) at 4 °C

in preparation for paraffin embedding (toluene (2× 45 min); melted paraffin (3× 60 min)). All the brains were embedded in paraffin prior to sectioning. Six-micrometre sections were obtained for staining using a motorized microtome (HM 350, Microm, Heidelberg, Germany) and the sections were placed on Superfrost plus microscopic glass slides (Fisher Scientific 12-550-15, Toronto, Ontario, Canada). To visualize incorporated BrdU and TH, the paraffin sections were deparaffinized by washes in Xilol (2× 20 min) and serial dilutions of ethanol (2 min per wash) (2× EtOH 100%; EtOH 95%; EtOH 85%; EtOH 70%; EtOH 50%; EtOH 30%). The sections were then washed with 1× PBS (2× 10 min) prior to incubation with 50% formamide/50% 2× saline–sodium citrate (SSC) for 3 h in a 65 °C water bath. Following the incubation, the sections were washed with 2× SSC (20× SSC stock solution: 3 mol/L NaCl, 0.3 mol/L Sodium citrate, pH 7.0) (2× 5 min) prior to DNA denaturation by incubating the sections in 2 N HCl for 30 min at 37 °C. This was followed by washing in sodium borate buffer (0.1 mol/L, pH 8.5) for 2× 5 min at room temperature (RT) and then with 1× PBS with 0.2% TritonX-100 (3× 5 min). Nonspecific protein binding sites were blocked using 1% nonfat milk with 0.2% Triton X-100 in 1× PBS for 45 min at RT. Sections were then incubated with rat anti-BrdU (dilution 1:300; Abcam ab6326, Cambridge, Massachusetts, USA) and rabbit anti-TH antibodies (dilution 1:400; Millipore Ab152, Toronto, Ontario, Canada) in 1% milk with 0.2% Triton X-100 in 1× PBS at 4 °C overnight in a moist chamber. After 1× PBS with 0.2% TritonX-100 rinses of 3× 10 min on a shaker, the sections were incubated in Alexa Fluor 594 goat anti-rat immunoglobulin G (IgG) (dilution 1:300; Invitrogen A11007, Burlington, Ontario, Canada) and Alexa Fluor 488 goat anti-rabbit IgG (dilution 1:300; Life Technologies A11008, Burlington, Ontario, Canada) secondary antibodies in 1× PBS with 0.2% TritonX-100 for 1.5 h at RT. The sections were then washed three times in 1× PBS with 0.2% Triton X-100 for 5 min and mounted with anti-fading medium Vectashield with 4,6-diamino-2-phenylindole (DAPI) (Vector Laboratories, Burlingame, California, USA) and kept in the dark until visualization of slides with Nikon A1RsiMP confocal (Nikon, New York, New York, USA) microscope.

Immunocytochemistry for GFAP was used to identify RGCs (Forlano et al. 2001; Xing et al. 2015) in relation to TH-immunoreactivity in the ventral telencephalon. The paraffin sections were deparaffinized as described above and then washed with 1× PBS (2× 10 min). Nonspecific protein binding sites were blocked with 1% milk with 0.2% Triton X-100 in 1× PBS for 45 min at RT. Sections were then covered with mouse anti-pig GFAP antibody (1:500; Chemicon, Temecula, California, USA, Cat. # MAB360), which was previously validated in fish (Forlano et al. 2001; Xing et al. 2017) and rabbit anti-rat TH antibody (1:500; Chemicon, Tamecula, California, USA, Cat. # AB152), which was previously validated in fish (Yamamoto et al. 2011; Xing et al. 2017) overnight at 4 °C. Sections were then washed for 2× 10 min in PBS and incubated with a donkey anti-rabbit Alexa fluor 488 (1:500; Invitrogen Molecular Probes, Invitrogen, Burlington, Ontario Canada) and goat anti-mouse Alexa fluor 596 (1:500; Invitrogen Molecular Probes, Invitrogen, Burlington, Ontario Canada) for 1 h at RT. The sections were then washed three times in 1× PBS with 0.2% Triton X-100 for 5 min and mounted with antifading medium Vectashield with DAPI (Vector Laboratories, Burlingame, California, USA) and kept in the dark until visualization of slides with a confocal microscope.

Quantification of BrdU and TH labelled cells

The BrdU- and TH-positive cells were visualized using a fluorescent microscope (Zeiss Axiophot Microscope, Carl Zeiss Canada Ltd., Toronto, Ontario, Canada) at 20× magnification with the aid of an Olympus DP70 (Olympus, Center Valley, Pennsylvania, USA) camera. Positive cells were counted in the 4 and 7 dpi goldfish brains to quantify cellular proliferation using ImageJ1.43I software (Rasband 1997–2014). The total number of TH- and BrdU-positive cells in goldfish brain sections were counted using unbiased, single-blind, counting of cells in comparable sections using the disector technique (Guillery 2002). For the TH and BrdU counting, five sections of the ventral

telencephalic area at 4 and 7 dpi were analyzed per fish ($n = 5$ per group) where cells were counted the entire length of the ventricular surface along the ventral-dorsal axis of the left brain hemisphere using a specific counting frame of a width of $\sim 680 \pm 80$ µm stretching laterally from the ventricular surface to the apical surface. Labelled cells were localized according to the goldfish brain atlas of Peter and Gill (1975) and Hornby et al. (1987). Cell counting was confined within neuroanatomical borders of the target area. The total number of positive cells between the treatment group and control group were first tested for normality and then statistically compared using a Student's t test (two-tailed) in SPSS Statistics (version 21). p-values ≤0.05 were considered statistically significant. All data are presented as mean + SEM.

Results

Effects of MPTP on female goldfish swimming behaviour

Female goldfish were treated with MPTP and their swimming movements were measured following MPTP injection. As shown in **Fig. 1**, control goldfish traveled an average of ~1250 cm/5 min and rested for ~14 s during the test period. However, there was an observable decrease in total distance traveled and an increase in resting time following the injection of MPTP. At 3 dpi of MPTP goldfish decreased distance traveled by ~85% ($p < 0.0001$) (**Fig. 1**). There was a significant increase in resting time ($p = 0.0011$) at 3 dpi (**Fig. 1b**). Thereafter, the total distance traveled by MPTP-treated goldfish started to recover towards control levels at 7 dpi but was still significantly different compared with control ($p = 0.0178$). Reversible changes were evident for the resting time, which approached control levels of 7 dpi ($p = 0.2981$).

### Effects of MPTP on TH and BrdU immunoreactivity in the female goldfish ventral telencephalon

Confocal imaging of control and MPTP-treated female goldfish brain sections at 4 and 7 dpi revealed TH- and BrdU-positive cell labelling in the Vd of the telencephalon. **Figure 2b** shows BrdU-positively labelled cells (red) located laterally and dorsally from the ventricular surface to the Vd area at 4 dpi of MPTP. Confocal imaging also revealed that BrdU-positive cells are colocalized with DAPI (blue nuclear stain) thereby indicating that BrdU was incorporated into newly generated cells in the female goldfish brain (**Fig. 3a**). Several double-labelled BrdU- and TH-positive cells were also found in the 4 dpi of MPTP fish group (**Figs. 3b** and **3c**).

The total number of TH-positive neurons in the ventral telencephalon, which includes the Vd, was counted. At 4 dpi, TH-positive cells significantly decreased by 27% compared with control fish ($p = 0.02$) (**Fig. 4a**). No significant differences in TH-immunolabelling were observed at 7 dpi ($p = 0.69$) compared with controls (**Fig. 4c**). At 4 dpi, BrdU-positive cells significantly increased ~1.9-fold compared with control fish ($p = 0.02$) (**Fig. 4b**). In contrast, no significant differences in BrdU immunolabelling were observed at 7 dpi ($p = 0.69$) (**Fig. 4d**).

TH and GFAP immunoreactivity in the female goldfish ventral telencephalon

Immunoreactivity of TH and GFP in control goldfish ventral telencephalon, a region negatively impacted by MPTP toxicity (Goping et al. 1995) and one of which contains large amounts of RGCs (Pellegrini et al. 2016), reveals a close anatomical relationship between catecholamine neurons (TH) and GFAP fibers (~5 µm) (**Fig. 5**). Dense GFAP immunoreactivity (red) was observed along the surface of the telencephalon ventricle with fibers extending ventrally towards the Vd where TH-immunoreactive catecolaminergic cells (green) are located.
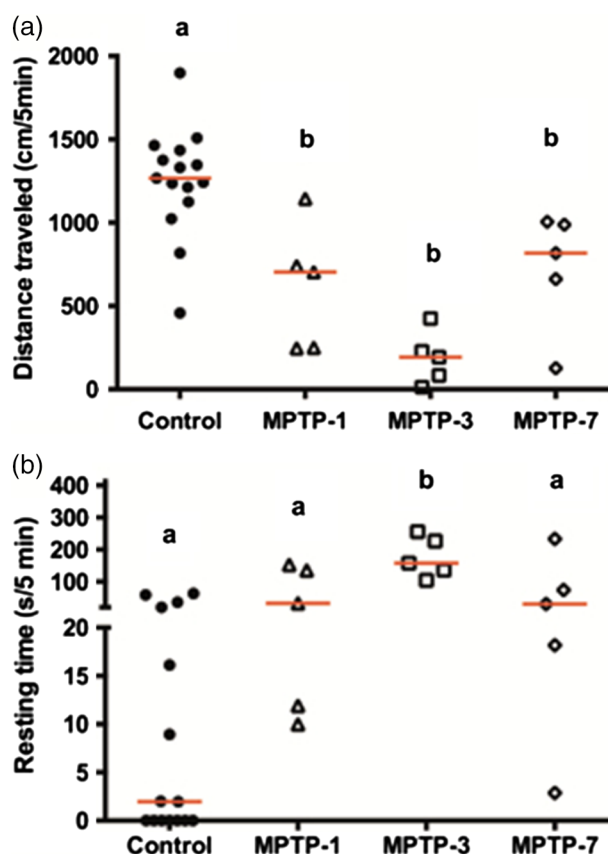
Fig. 1. Time course for onset and recovery of swimming activity of female goldfish from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity. Goldfish in groups of five were treated on day 0 with MPTP (50 μg/g body weight, intraperitoneal (i.p.)) or with 0.6% saline. (*a*) At 1, 3, and 7 d post-MPTP injection fish were tested for the distance travelled in a 5-min period according to Logger Pro 3 tracking software. Red bars represent median values ($n = 5$). One-way ANOVA followed by Tukey's test was performed to determine the effects of MPTP treatment. Treatment groups marked by different letters have significantly different total distance travelled ($p < 0.0001$). (*b*) Resting time during each 5-min period is presented as determined by the Logger Pro 3 tracking program. Data were not normally distributed so the distribution and median values (red bars) are presented. A Kruskal–Wallis test was performed followed by Dunn's multiple comparison test to determine the effects of MPTP treatment. Treatment groups marked by different letters have significantly different resting time compared with the control group ($n = 5$ per group; $p = 0.0011$).

Discussion

The behavioural analysis indicated that goldfish treated with MPTP exhibit PD motor deficits consistent with previous reports (Pollard et al. 1992; Adeyemo and Youdim 1993; Weinreb and Youdim 2007). A significant ~85% decrease in total distance traveled was observed in MPTP-treated goldfish 3 dpi, coincident with the maximal loss of DA in the forebrain, which has been reported previously (Poli et al. 1992; Pollard et al. 1992; Lu et al. 2014). Youdim et al. 1992 also reported profound bradykinesia in goldfish at 3 dpi of MPTP. We also observed a significant increase in total resting time in MPTP-injected goldfish at 3 dpi. Goldfish began significant recovery in total swimming distance and resting time by 7 dpi. A similar recovery of total distance traveled and resting time was reported at 7 and 5 dpi, respectively (Weinreb and Youdim 2007). The recovery of normal motricity suggests a
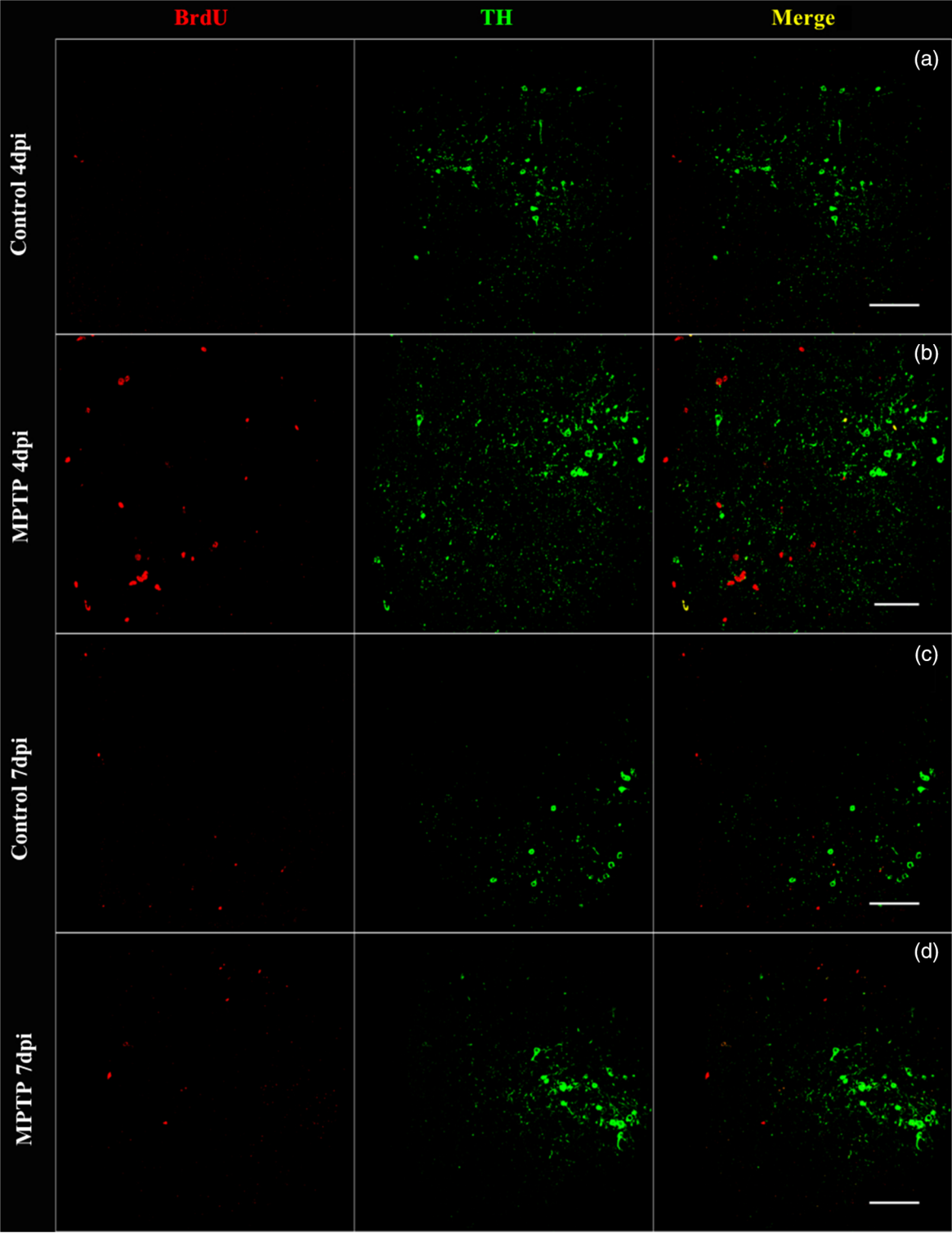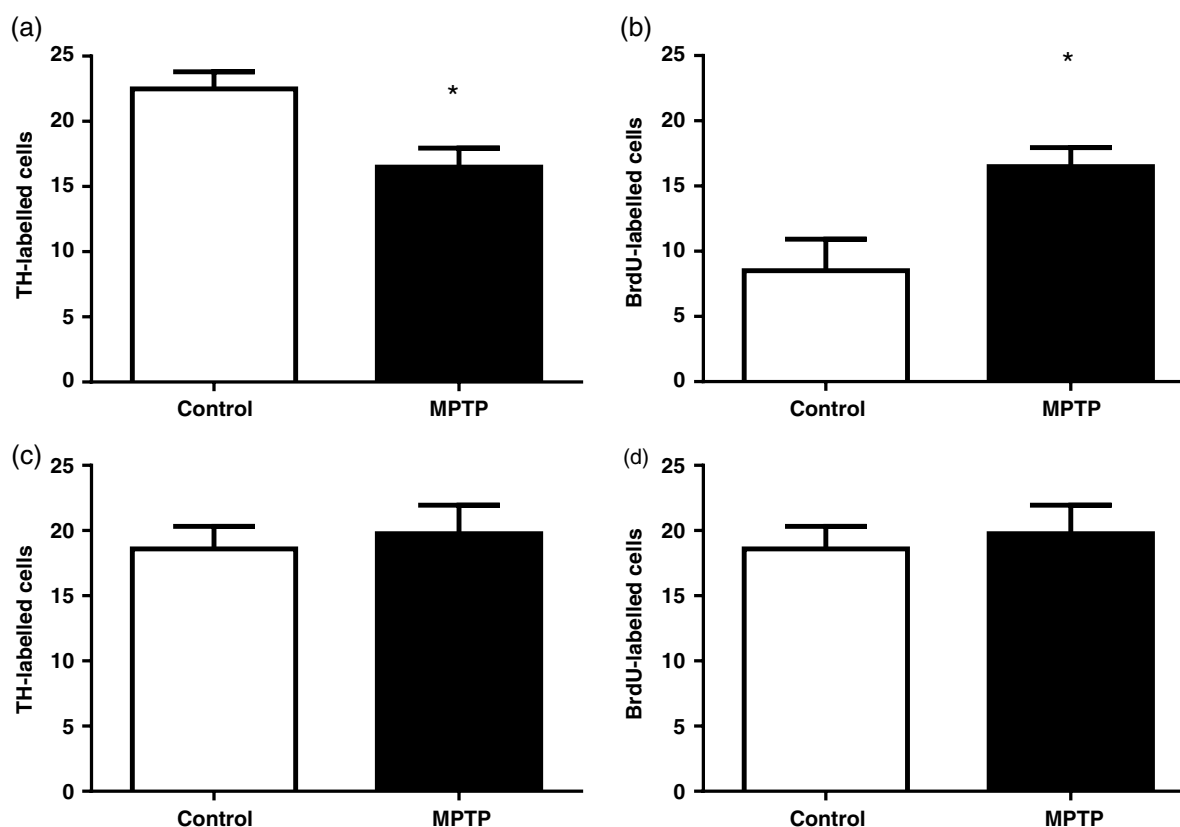
Fig. 2. Confocal imaging illustrating effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on bromodeoxyuridine (BrdU)-positive cells (red) and tyrosine hydroxylase (TH)-positive cells (green) in the area telencephali pars dorsalis (Vd) of female goldfish. The images show positively stained BrdU and TH cells in (*a*) control goldfish at 4 d post-injection (dpi); (*b*) MPTP-treated goldfish at 4 dpi; (*c*) control goldfish at 7 dpi; and (*d*) MPTP-treated goldfish at 7 dpi. Scale bars = 100 μm.
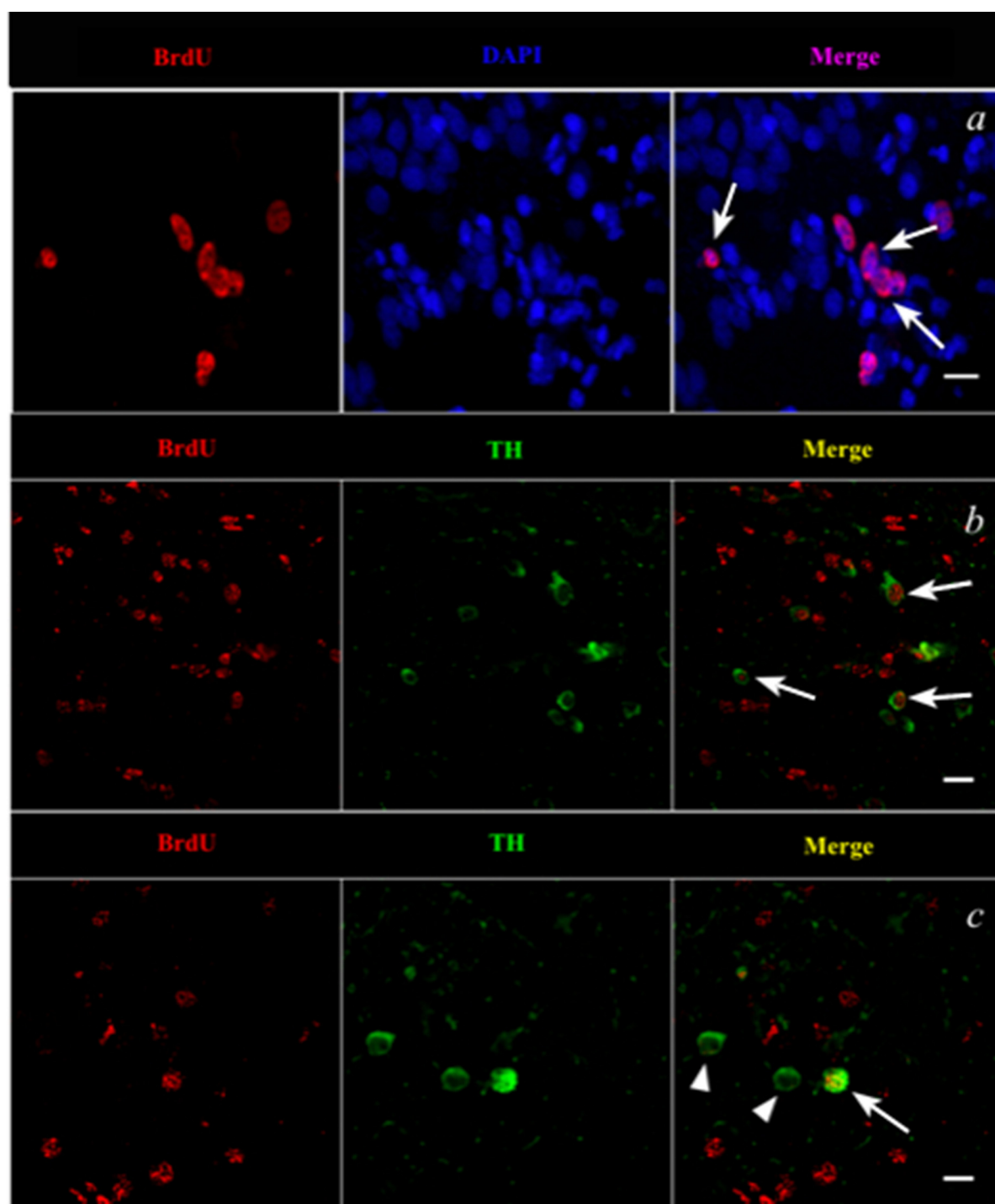
Fig. 3. Confocal imaging of double-labelling of bromodeoxyuridine (BrdU) and tyrosine hydroxylase (TH) in the female goldfish telencephalon. Confocal imaging of (*a*) double-labelled BrdU (red) and 4,6-diamino-2-phenylindole (DAPI, blue) nuclear staining; (*b*) and (*c*) double-labelled BrdU-positive cells (red) and TH-positive cells (green) in the area telencephali pars dorsalis (Vd) of female goldfish. Arrows show double-labelled cells and arrowheads show TH-positive and BrdU-negative cells. Scale bars = 10 μm.

Fig. 4. The effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on the number of tyrosine hydroxylase (TH)-positive cells and bromodeoxyuridine (BrdU)-positive cells in the female goldfish ventral telencephalon. Female goldfish exposed to MPTP after 4 d had a significant decrease of (*a*) TH-labelled cells and a significant increase of (*b*) BrdU-labelled cells compared with control fish. There were no significant changes observed in (*c*) TH-labelled cells or (*d*) BrdU-labelled cells in female goldfish exposed to MPTP after 7 d. A Student's *t* test was performed to determine the effects of MPTP on TH and BrdU cell numbers. Treatment groups marked by asterisks are significantly different compared with the control group ($n = 5$ per group; $p = 0.02$ (*a*) and $p = 0.02$ (*b*)).

recovery of neurotransmitter levels or neuronal function, i.e., DA, and thus the ability of goldfish to reestablish important biological behaviours following neurotoxic insult.

The TH-positive cells in the ventral telencephalon were depleted by MPTP at 4 dpi. The greatest decrease of forebrain DA content following MPTP injection is reached at 3–5 d (Pollard et al. 1992; Adeyemo et al. 1993; Sloley and McKenna 1993; Goping et al. 1995; Hibbert et al. 2004) coincident with TH depletion observed in the goldfish ventral telencephalon in this study. The TH immunoreactivity was quantified in the goldfish ventral telencephalon and was significantly decreased by 27% at 4 dpi of MPTP. Pollard et al. (1996) and Goping et al. (1995) also reported similar decreases in TH-immmunoreactivity at 4 d after MPTP treatment. Goldfish have also been reported to recover DA brain content to baseline levels at 7–8 dpi of MPTP (Pollard et al. 1992; Adeyemo et al. 1993; Popesku 2009). Remarkably, we observed a significant 1.9-fold increase in BrdU-labelled cells at 4 d following MPTP, but no significant differences were observed at 7 dpi in comparison with controls. This indicates that goldfish have a rapid recovery from MPTP toxicity starting at 4 dpi. Furthermore, BrdU-positive cell counts returned to control levels at 7 dpi, providing further evidence that neuroregeneration occurs rapidly after the neurotoxic insult in this species. Future studies are needed to determine if the rate of cellular proliferation returns to control levels following MPTP injection.
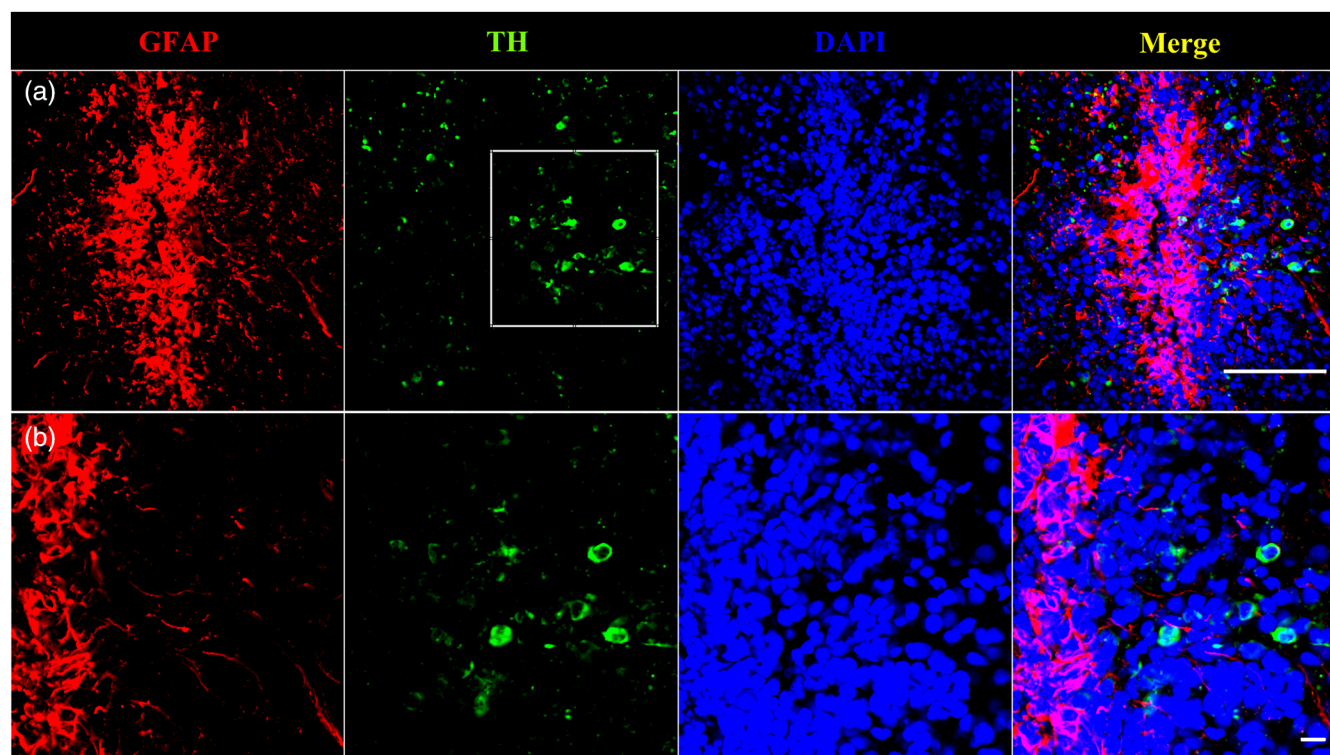
Fig. 5. Confocal imaging of immunoreactive glial fibrillary acidic protein (GFAP, red), tyrosine hydroxylase (TH, green) and 4,6-diamino-2-phenylindole (DAPI, blue) nuclear staining of the goldfish ventral telencephalon. Scale bars = (*a*) 20 μm and (*b*) 5 μm.

The short-term experiments conducted in this present study, where BrdU was injected 24 h prior to the 4 dpi of MPTP, allowed for sufficient time for BrdU to be available for incorporation into newly generated cells. Previous studies have shown that BrdU is readily available for uptake for ~4 h after intraperitoneal injection (Zupanc and Horschke 1995). Furthermore, the length of the cell cycle in the adult teleost *Astatotilapia burtoni* (Günther, 1894) was estimated to be ~25 h using proliferating cell nuclear antigen (PCNA) and BrdU labelling in the retina (Mack and Fernald 1997). Based on these studies, the positively labelled BrdU cells in the goldfish telencephalon likely label proliferative cells and their progeny if cellular division occurred. The longer-term experiment, where BrdU was available for a total of 4 d post-injection, proliferative cells that are actively dividing likely went through several cell cycles. Therefore the BrdU observed at this time point would likely label several cellular generations and also the original cell progeny. In this present study, we observed proliferation and lateral and dorsal migration of BrdU-positive cells towards the Vd area at 4 dpi. These newborn cells likely originated from the proliferative cellular pools along the ventricular surface where a large number of RGCs that can act as neural stem cells reside (Zupanc and Clint 2003; Mahler and Driever 2007; Pellegrini et al. 2007; März et al. 2010; Zupanc and Sîrbulescu 2011). At 7 dpi, the majority of proliferative cells remained near the ventricular area in the proliferative cell pools. A future experiment should be conducted to explore the possibility that BrdU remains in the goldfish brain for a longer period of time thus explaining why proliferative cells remained near the ventricle at 7 dpi.

Based on the observations of BrdU-positive cells in this study, we postulate that proliferative cells are completing or have completed their migration to the Vd following MPTP insult shortly after 4 dpi.

Previous studies have reported the occurrence of rapid migration of newborn cells following injury based on BrdU cellular labelling. For example, cellular proliferation in the zebrafish cerebellum following injury was observed where cells migrated to their final destination of the granular cell layer after 10 d (Zupanc et al. 2005). Ampatzis and Dermon (2007) also found BrdU-labelled cells in the migration state at 24 h post-injury in the zebrafish cerebellum. Another study of spinal cord injury in *A. leptorhynchus* reported a several fold increase in the rate of cell proliferation starting at 1 d post-injury and lasting several weeks (Zupanc and Ott 1999; Reimer et al. 2008; Sîrbulescu et al. 2009). It is important to note that seasonality and environmental conditions can influence the rate of cellular proliferation in the brain as observed in the electric fish *Brachyhypopomus gauderio* Giora and Malabarba, 2009 where breeding fish had 3–7 times more BrdU-positive cells in the brain compared with nonbreeding fish (Dunlap et al. 2011). Seasonality could also impact the rate of motor recovery observed in the behavioural experiment as fish with different reproductive stages were used.

Remarkably, we observed some colocalization of BrdU- and TH-positive cells in the Vd at 4 dpi. This provides direct evidence that TH-positive neurons are regenerating in the region exhibiting neuronal depletion following MPTP injections. There were also BrdU-positive cells that did not stain for TH. These cells could still be migrating to their final target site and did not yet express TH. It is also possible that other non-TH expressing neuronal cell types could be differentiating in the Vd following a neurotoxic insult. Future studies are needed to fully characterize these newborn cells in the telencephalon.

The close proximity (<5 μm) of RGC fibers and TH-positive cells suggests that the newly generated cells may be migrating along RGC fibers to their final destination. Xing et al. 2017 showed that the injection of MPTP in goldfish induces an upregulation of mRNAs for glial cell-derived neurotrophic factor and brain-derived neurotrophic factor as well as genes involved in DA neuron development
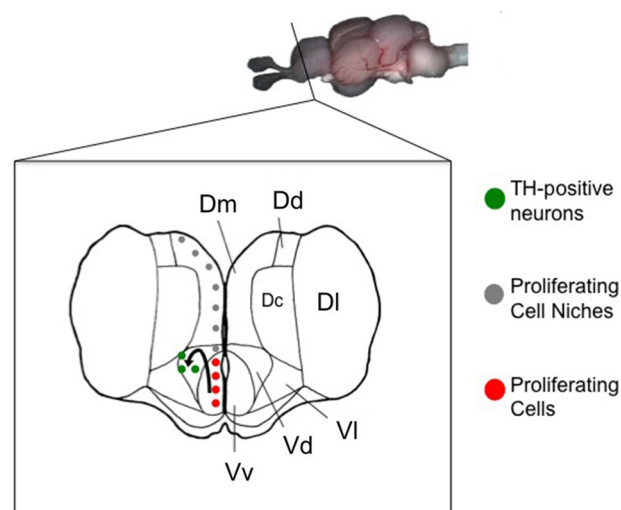
Fig. 6. Proposed tyrosine hydroxylase (TH) neuronal regeneration pathway following neurotoxic insult in the goldfish telencephalon. The area observed and targeted during the study was the area telencephali pars dorsalis (Vd). Progenitor cells (red dots) proliferate, migrate along radial glial cells (black arrow) and differentiate into TH-positive cells in the telencephalic Vd area. Grey dots represent various progenitor cell niches in the goldfish telencephalon. Vv, area ventralis telencephali pars ventralis; Vl, area ventralis telencephali pars lateralis; Dl, area dorsalis telencephali pars lateralis; Dd, area dorsalis telencephali pars dorsalis; Dm, area dorsalis telencephali pars medialis; Dc, area dorsalis telencephali pars centralis.

such as TH, dopamine transporter (slc6a3), and paired-like homeodomain transcription factor 3 (pitx3) in both the goldfish hypothalamus and telencephalon. These neurotrophic factors are produced by glial cells in teleosts and are probably involved in DA neuronal survival, decreasing DA neuronal apoptosis and promoting axonal growth and regeneration (Lin et al. 1993; Lykissas et al. 2007; Pellegrini et al. 2007; Yasuhara et al. 2007; Xing et al. 2017). This provides further evidence that the newly generated TH cells are most likely regenerating from a RGC pool along the ventricular surface of the telencephalon and migrating to damaged sites, e.g., the Vd, to restore catecholaminergic function (Fig. 6).

Acknowledgements

Author contributions

MJV and VLT conceived and designed the study. MJV, LX, and CCE performed the experiments/collected the data. MJV, LX, and CCE analyzed and interpreted the data. VLT contributed resources. MJV and VLT drafted or revised the manuscript.

Competing interests

VLT is currently serving as a Subject Editor for FACETS, but was not involved in review or editorial decisions regarding this manuscript.

Data accessibility statement

All relevant data are within the paper.

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