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Trimethyltin intoxication induces the migration of ventricular/subventricular zone cells to the injured murine hippocampus

Blair C. Weig[a], Jason R. Richardson[a,b], Herbert E. Lowndes[a], Kenneth R. Reuhl[a,*]

[a] Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy and Joint Program in Toxicology, Rutgers Biomedical Health Sciences, Piscataway, NJ, United States
[b] Department of Pharmaceutical Sciences, Northeast Ohio Medical University, Rootstown, OH, United States

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ABSTRACT

Following the postnatal decline of cell proliferation in the mammalian central nervous system, the adult brain retains progenitor cells with stem cell-like properties in the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampus. Brain injury can stimulate proliferation and redirect the migration pattern of SVZ precursor cells to the injury site. Sublethal exposure to the neurotoxicant trimethyltin (TMT) causes dose-dependent necrosis and apoptosis in the hippocampus dentate gyrus and increases SGZ stem cell proliferation to generate new granule cells. To determine whether SVZ cells also contribute to the repopulation of the TMT-damaged dentate gyrus, 6-8 week old male C3H mice were injected with the carbocyanine dye spDiI and bromodeoxyuridine (80 mg/kg; ip.) to label ventricular cells prior to TMT exposure. The presence of labeled cells in hippocampus was determined 7 and 28 days after TMT exposure. No significant change in the number of $BrdU^+$ and $spDiI^+$ cells was observed in the dentate gyrus 7 days after TMT treatment. However, 28 days after TMT treatment there was a 3–4 fold increase in the number of spDiI-labeled cells in the hippocampal hilus and dentate gyrus. Few $spDiI^+$ cells stained positive for the mature phenotypic markers NeuN or GFAP, suggesting they may represent undifferentiated cells. A small percentage of migrating cells were $BrdU^+/spDiI^+$, indicating some newly produced, SVZ- derived precursors migrated to the hippocampus. Taken together, these data suggest that TMT-induced injury of the hippocampus can stimulate the migration of ventricular zone-derived cells to injured dentate gyrus.

1. Introduction

Cell proliferation in the developing central nervous system of rodents declines rapidly during the perinatal period, with the final stages of hippocampal and cerebellar neurogenesis largely completed by postnatal day 21. However, some neurogenesis persists in the adult rodent brain, primarily through the activity of stem cells localized in the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the hippocampus (Gage, 2000; reviewed by Ming and Song, 2011). Slowly dividing radial glia-derived neural stem cells residing in the subventricular zone of the lateral ventricle give rise neural precursors that migrate to the olfactory bulbs and once there, disperse radially and differentiate into olfactory interneurons (Merckle et al., 2004). This long distance tangential migration, referred to as the rostral migratory stream, is facilitated by a network of astrocytic tube-like processes that create both an attachment scaffold and molecular guidance cues to migrating neuroblasts (Doetsch et al., 1997; Conover et al., 2000). The migration of SVZ precursors is confined to the rostral migratory stream under physiological conditions, however, injury or neurodegenerative processes can induce progenitor cell proliferation and migration of SVZ-derived neuroblasts toward affected non-olfactory brain regions (De Marchis et al., 2004; Nakatomi et al., 2002; Arvidsson et al., 2002; Zhang et al., 2004a; Yamashita et al., 2006; Goings et al., 2004).

A second neurogenic environment is located in the subgranular zone (SGZ) of the hippocampal dentate gyrus which generates new cells throughout life (Scharfman 2000). Within the SGZ are quiescent radial glia-like cells (Seri et al., 2001; Filippov et al., 2003), coined type-1 cells, that give rise to mitotically active type 2a multipotent stem cells. These cells produce lineage restricted

* Corresponding author at: Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy and Joint Program in Toxicology, Rutgers Biomedical Health Sciences, Piscataway, NJ, United States
E-mail address: Reuhl@eohsi.rutgers.edu (K.R. Reuhl).

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neural progenitors (type 2b cells) that generate neuroblasts (type 3 cells) destined to become new granule cell neurons. Unlike SVZ-derived neuroblasts, hippocampal neuroblasts only migrate short distances into the granule cell layer (GCL) of the dentate gyrus where they differentiate into granule cells. Progenitor proliferation rate and neurogenesis in the hippocampal SGZ are acutely responsive to injury, neurological and chemical factors including physical activity (van Praag et al., 1999), environmental enrichment (Nilsson et al., 1999), neurotransmitters (Cameron et al., 1995; Brezun and Daszuta 1999; Brazel et al., 2005), gonadal and adrenal hormones (Gould et al., 1992; Cameron and Gould, 1994; Cameron et al., 1998; Galea et al., 2013) and antidepressants (Malberg et al., 2000; Santarelli et al., 2003). The effect of injury on proliferation and neurogenesis in the hippocampus is dependent on the injury model and the extent of the injury. Further, if the injury involves alteration of the neurogenic environment, it can impart abnormal remodeling through ectopic localization of new cells or disproportionate gliogenesis at the expense of neurogenesis (Parent et al., 1998; Ferland et al., 2002; Scott et al., 2000; Monje et al., 2002; Madsen et al., 2003; Scharfman et al., 2000; Gray and Sundstrom, 1998; Rola et al., 2006; Wang et al., 2012).

In vitro studies of isolated hippocampal stem cells reveal multipotency upon exposure to appropriate factors (Palmer et al., 1997), but *in vivo* multipotency has not been clearly demonstrated. Numerous models have been proposed to describe the identity and mechanism of lineage fate progression for neural stem cells (reviewed by Ming and Song, 2011). Both asymmetric and symmetric cell division have been observed; however, it is clear that there is a loss of stem cell numbers with aging. It has been proposed that SGZ stem cells may irreversibly enter a quiescent state, undergo cell death, or leave the stem cell pool after a finite number of cell divisions and subsequently differentiate into a post-mitotic astrocyte (Encinas et al., 2011), resulting in a diminished hippocampal neuronal cell density in the aged rodent. Injury or other extraneous factors may radically accelerate turnover in the stem cell pool, potentially leading to a premature depletion of viable stem cells later in life if no mechanism exists to replenish this pool. Perhaps to conserve this finite stem cell capacity, hippocampal neurogenesis occurs at a much lower frequency in comparison to SVZ neurogenesis, which has been shown to undergo asymmetric division to maintain the stem cell pool (Morshead et al., 1998; Zhang et al., 2004b).

Trimethyltin (TMT) is a potent hippocampal neurotoxicant that preferentially damages regions of the limbic system in rodents (Chang et al., 1983). In adult mice, TMT induces extensive apoptosis and necrosis within the hippocampal dentate gyrus granule cell layer within 24–36 h after exposure (Chang et al., 1982; Fiedorowicz et al., 2001). Harry et al. (2004) first demonstrated that TMT-induced injury stimulates a strong neurogenic response by endogenous stem cells in the murine hippocampal subgranular zone. Further studies showed the utility of this injury model for studying neurogenesis and repair mechanisms in the hippocampus (Ogita et al., 2005, 2012; Corvino et al., 2005; McPherson et al., 2011; Yoneyama et al., 2014). The current study tests the hypothesis that TMT-induced injury of the mouse dentate gyrus triggers migration of cells from ventricular neurogenic regions to the lesion site as part of a potential repair response.

2. Materials and methods

2.1. Animals and TMT exposure

Male C3H mice aged 6–8 weeks (Taconic, NY) were housed in polycarbonate cages with wood chip bedding and maintained according to AALAC guidelines. Trimethyltin chloride (TMT, Sigma, St. Louis, MO) was dissolved in normal saline at a concentration of 2.5 mg/ml. A working solution was prepared in saline (0.25 mg/ml) and a single intraperitoneal injection was administered to mice at a dose (2.7 mg/kg) that would induce tremor activity within 24–36 h and recovery by 72–94 h post-dose. Groups of 5–8 mice were used for generating saline- and TMT-injected brain samples.

2.2. spDiI and BrdU labeling of ependymal/subventricular cells

The method of intraventricular injection of spDiIC18(3) ([1,1-dioctadecyl-6,6-di(4-sulfophenyl)-3,3,3,3-tetramethylindocarbocyanine], Life Technologies) was described previously (Johansson et al., 1999). Briefly, mice were sedated by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (12 mg/kg). An incision was made along the midline of the cranial surface and 3 ul spDiI (0.2% w/v in DMSO) was injected i.c.v. into one lateral ventricle 2 mm below the dura mater at 0.5 mm posterior and 0.7 mm lateral to bregma (Paxinos and Franklin, 2001). The incision was closed using Vetbond tissue adhesive (3 M Animal Care Products) and mice were permitted to recover from surgery for 48 h prior to TMT injection.

To track the migration of rapidly proliferating cells including mitotically active cells in the SVZ (i.e. type C cells; Doetsch et al., 1997), mice were dosed with bromodeoxyuridine (BrdU; 50 mg/kg; ip) every two hours for twelve hours. Twenty-four hours after the last BrdU dose, the mice were then injected with TMT (2.7 mg/kg; ip) and returned to normal housing. The mice were euthanized by CO_2 asphyxiation 7 days and 28 days later; the brain was removed and flash frozen at −70 °C for subsequent immunohistological analysis.

2.3. Tissue sectioning and immunohistochemistry

Twelve-micrometer cryostat sections were prepared from stereotaxically-defined regions of hippocampus (-1.46 to 2.40 mm relative to bregma), mounted on slides and post-fixed by immersion in 4% *para*-formaldehyde at room temperature for 15 min. Antigen retrieval was performed before mounting onto Shandon coverplates (Thermo Scientific, MA). Samples were incubated overnight at 4 °C with primary antibodies prepared in blocking buffer containing 3% donkey serum and 0.3% Tween-20. Primary antibodies used were sheep anti-BrdU (5ug/ml; Fitzgerald Industries, Inc.), anti-GFAP (1:100; Sigma) for astrocytes, anti-NeuN (1:100; Millipore) for neurons, anti-cd11b (1:200; BD Pharmingen) for microglia, and anti-collagen IV (1:100; Pierce) for staining blood vessels. For BrdU staining, slides were acid digested in 2.5 M HCl at 37 °C for 30 min. HCl-digested samples were then neutralized in 10 mM sodium borate solution (pH 8) for 5 min before incubation with sheep anti-BrdU primary antibody in blocking buffer. NeuN primary antibody staining was performed overnight at 4 °C after the antigen retrieval step used for BrdU staining. For GFAP and BrdU co-staining, slides were microwaved for 15 min in 10 mM sodium citrate (pH 5), cooled to room temperature, and then incubated with BrdU and GFAP primary antibody overnight at 4 °C. Slides were then incubated for 45 min at room temperature with Alexa Fluor-conjugated secondary antibodies (1:300; Invitrogen, CA) prepared in blocking buffer.

2.4. Imaging and cell quantification

Tissue sections were visualized by epifluorescence using a Zeiss Axiophot microscope equipped with a CoolSnap digital camera and Image-Pro Plus image analysis software (Advanced Imaging Concepts, Princeton, NJ). Coronal sections of matched anatomical levels along the rostro-caudal axis were used for analysis. Quantification was performed only in hemispheres contralateral to spDiI injection (icv) on 8–10 sections/animal with an