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DYNAMIC IMAGING REVEALS THAT BRAIN-DERIVED NEUROTROPHIC FACTOR CAN INDEPENDENTLY REGULATE MOTILITY AND DIRECTION OF NEUROBLASTS WITHIN THE ROSTRAL MIGRATORY STREAM

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Abstract—Neuronal precursors generated in the subventricular zone (SVZ) migrate through the rostral migratory stream (RMS) to the olfactory bulb (OB). Although, the mechanisms regulating this migration remain largely unknown. Studies have shown that molecular factors, such as brain-derived neurotrophic factor (BDNF) emanating from the OB, may function as chemoattractants drawing neuroblasts toward their target. To better understand the role of BDNF in RMS migration, we used an acute slice preparation from early postnatal mice to track the tangential migration of GAD65-GFP labeled RMS neuroblasts with confocal time-lapse imaging. By quantifying the cell dynamics using specific directional and motility criteria, our results showed that removal of the OB did not alter the overall directional trajectory of neuroblasts, but did reduce their motility. This suggested that additional guidance factors present locally within the RMS region also contribute to this migration. Here we report that BDNF and its high affinity receptor, tyrosine kinase receptor type 2 (TrkB), are indeed heterogeneously expressed within the RMS at postnatal day 7. By altering BDNF levels within the entire pathway, we showed that reduced BDNF signaling changes both neuroblast motility and direction, while increased BDNF levels changes only motility. Together these data reveal that during this early postnatal period BDNF plays a complex role in regulating both the motility and direction of RMS flow, and that BDNF comes from sources within the RMS itself, as well as from the olfactory bulb. Published by Elsevier Ltd on behalf of IBRO.

Key words: RMS, BDNF, SVZ, imaging, neuroblast, motility.

The migration of rostral migratory stream (RMS) neurons from the subventricular zone (SVZ) to the olfactory bulb (OB) continues throughout life (Altman, 1969; Luskin, 1993; Lois and Alvarez-Buylla, 1994; Wichterle et al., 2001), but the elements controlling this process are not fully understood. Studies have shown that the vascular network within the RMS may act as a source of molecular factors (Leventhal et al., 1999; Snapyan et al., 2009), and as a scaffold for migration (Whitman et al., 2009). In addition, many molecular signals influencing RMS migration have been identified, including slits (Wu et al., 1999, Nguyen-Ba-Charvet et al., 2004), semaphorins (Ito et al., 2008; Melendez-Herrera et al., 2008), netrins (Hamasaki et al., 2001; Murase and Horwitz, 2002), ephrins (Conover et al., 2000), and growth factors (Paratcha et al., 2006; Chiaramello et al., 2007; Garzotto et al., 2008). However, it is not clear where all these signals originate, how they relate to one another, or whether they distinctly modulate direction or motility. Recent studies demonstrated that molecules, such as the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Bolteus and Bordey, 2004), metalloproteases (Bovetti et al., 2007), and the adhesion molecule PSA-NCAM (Tomasiewicz et al., 1993; Chazal et al., 2000), can indeed alter neuroblast motility without affecting the direction of migration. These findings suggest that separate mechanisms may be used to control different aspects of the migration process.

One candidate molecule implicated in regulating migration through the RMS is brain-derived neurotrophic factor (BDNF), which is present in the OB from late embryonic periods through adulthood (Maisonpierre et al., 1990). BDNF is a pleiotropic signaling molecule involved in the survival, proliferation and differention of many neural cells types (Ernfors et al., 1994; Jones et al., 1994; Lindsay, 1996). Studies using explant culture assays have shown that BDNF acts as a chemoattractant for RMS neuroblasts derived from early postnatal tissue (Chiaramello et al., 2007). Together, these findings have suggested that BDNF secreted by the OB may provide a targeting signal for migrating neuroblasts. Interestingly, studies in adult mice have also shown that RMS neuroblasts can migrate in the absence of the OB (Kirschenbaum et al., 1999), and that BDNF is present within the RMS itself (Bath et al., 2008; Galvao et al., 2008; Snapyan et al., 2009). These findings raise questions about the role of the OB as a source of migration factors, the specific effect BDNF exerts on migrating cells, and the function of BDNF arising within the RMS.

Our study examines the role BDNF plays in regulating RMS neuroblast migration during the early postnatal period using time-lapse imaging to track and quantify distinct aspects of neuroblast motility. We show that removal of the OB does not alter the direction of migrating cells but does reduce neuroblast motility. By contrast, altering BDNF concentration or disrupting its signaling can affect both the

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Abbreviations: BDNF, brain derived neurotrophic factor; GAD65, glutamic acid decarboxylase 65kDa promoter; GFP, green fluorescent protein; OB, olfactory bulb; RMS, rostral migratory stream; SVZ, subventricular zone; TrkB, tyrosine kinase receptor type 2.

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direction and motility of migrating neuroblasts. Our data indicate that BDNF signaling likely occurs through the TrkB receptor, which we find is expressed within the RMS in a non-homogeneous pattern similar to BDNF. In addition, we show that reducing BDNF levels affects motility in a similar fashion to inhibition of TrkB activity.

EXPERIMENTAL PROCEDURES

Animals

Wildtype C57BL/6 mice were used for *in vivo* injections. Time-lapse imaging used GAD65-GFP transgenic mice which express green fluorescent protein (GFP) under the control of the glutamic acid decarboxylase 65kDa promoter (GAD65) as described previously (Lopez-Bendito et al., 2004). All procedures conformed to National Institute of Neurological Disorders and Stroke's Animal Care and Use Committee guidelines.

In vivo injections

Wildtype mice, 7–9 days old, were initially anesthetized with an i.p. injection of ketamine (Bioniche Teoranta, Galway, Ireland; 0.04 mg/kg)/xylazene (Ben Venue Labs., Bedford, OH, USA; 0.0008 mg/kg) mixture for craniotomy, and then maintained with isofluorane (Baxter, Deerfield, IL, USA; 1–2%) during injection. Mice were then recovered in a heated incubator for 4–5 h before being sacrificed and processed for histology. Dextran-tetramethylrhodamine (TMR) 3000 molecular weight (Invitrogen, CA, USA) was loaded into a quartz micropipette (10 μm tip diameter) and injected using iontophoresis (+10 μA, 200 ms duration, 0.4 Hz, 120 pulses) into the RMS at stereotaxic coordinates 0.8 mm lateral to midline, 1.5 mm anterior to bregma, and depth 2.5 mm.

Acute brain slice experiments

Transgenic GAD65-GFP mice, 6–8 days old, were decapitated, and their brains were dissected in ice cold artificial cerebral spinal fluid (ACSF) containing (in mM): NaCl 124, KCl 3, $MgSO_4$ 1.3, $CaCl_2$ 2, NaHCO3 26, NaH_2PO_4 1.25, dextrose 10, while oxygenated with 95% O2 5% CO_2. Tissue was then sectioned sagittally (300 μm thick) using a Vibratome (3000, Vibratome, MO, USA) in ice cold oxygenated ACSF. Fluorescent sections containing an intact SVZ–RMS–OB pathway were selected and maintained in oxygenated ACSF at RT for no longer than 30 min until imaged. Removal of the OB was performed on acute slice sections bathed in ice-cold, oxygenated ACSF, under a dissecting microscope (Leica, IL, USA) using a scalpel. Signaling pathways were perturbed using bath application of either anti-BDNF-IgG (10 μg/mL, Millipore, MA, USA), BDNF (100 ng/mL, R&D, MN, USA), or k-252a (2 μM, Tocris, MO, USA). In all experiments, the sections were incubated with these solutions for 1 h prior to image acquisition.

Immunohistochemistry

All animals were sacrificed with an overdose i.p. injection of ketamine (0.25 mg/kg), and perfused intracardially with cold 1× PBS followed by 4% paraformaldehyde (PFA) in PBS. Dissected brains were post-fixed in 4% PFA, followed by cryoprotection in 30% sucrose overnight at 4 °C. Tissue was sectioned on a freezing microtome (SM 2000R, Leica, IL, USA) at 40 μm. Antibody staining was carried out as described previously (Marks et al., 2006) using the following primary antibodies: rabbit anti-BDNF, 1:1000 (Novus, CO, USA); rabbit anti-TrkB, 1:1000 (Millipore, MA, USA); or rabbit anti-p75NTR, 1:1000 (Millipore, MA, USA). Secondary and tertiary antibodies included biotin conjugated donkey anti-rabbit, 1.75 mg/mL, and Cy3 conjugated streptavidin, 1.75 mg/mL (Jackson Immunoresearch, PA, USA) which were applied at RT for 2 h. Sections were then rinsed in PBS and mounted on Superfrost slides (VWR, PA, USA) using Vectashield-DAPI mounting medium (Vector Labs, CA, USA).

In situ hybridization

Procedure was carried out as described in (Schaeren-Wiemers and Gerfin-Moser, 1993). Briefly, brains from P7 mice were dissected and immediately frozen in O.C.T. Compound (Sakura, CA, USA). Fresh frozen sections (16 μm) were cut on a cryostat then fixed in 4% PFA for 20 min and probed for BDNF mRNA using a digoxigenin (Roche, IN, USA) labeled riboprobe that was generated from a portion of the mouse BDNF coding sequence (1–588 bp). Slides were hybridized at 65 °C; washed at 68 °C. Signal was detected following overnight incubation at 4 °C with anti-digoxygenin antibody (Roche, IN, USA) and NBT/BCIP (Promega, WI, USA) solutions at room temperature.

Imaging fixed sections

Slides containing sections either labeled with antibody or injected with rhodamine were imaged at low and high magnification. Low magnification fluorescent images were collected on an Axiovert 200 microscope with a halogen lamp (Carl Zeiss, NY, USA) using a 10× (NA=0.5) objective, Cy3 (570 nm) or FITC (520 nm) emission filters, and a Qcolor 5 camera (Olympus, PA, USA). High magnification images were collected with an LSM 510 Axioskop 2 confocal microscope (Carl Zeiss, NY, USA) using a 40× (NA=1.3) or 63× (NA=1.4) oil immersion lens, and either an argon (488 nm) laser to excite GFP or a HeNe (543 nm) laser to excite Cy3 or Rhodamine. Confocal z-stacks (1 μm z-step) were collected in the RMS, midway between the SVZ and the OB, and co-localization of GFP and immunofluorescence was confirmed in three dimensions (although presented here as representative single slices).

In-vivo time-lapse imaging

Acute brain slices were loaded into a heated perfusion chamber (RC22c, Warner, CT, USA), maintained at 32 °C and secured with a string weight, while being constantly perfused (approximately 0.5 mL/min) with oxygenated ACSF. After a 1 h pre-incubation period in the chamber, the central portion of the RMS midway between the SVZ and OB was imaged on a confocal microscope using a 488 nm Argon laser to excite GFP fluorescence, and a 20× water immersion lens (NA=0.5, Carl Zeiss, NY, USA). A 40 μm stack (six z-slices) was collected every minute for 3 h. Slices were also rotated 180° to control for direction of perfusion flow, which revealed no discernable difference.

Image processing cell tracking and analysis

Each image z-stack was flattened (Zeiss software) to create a tiff image sequence of 180 frames, which was transferred to MATLAB (Mathworks, MA, USA) and filtered using noisecomp (Kovesi, 2000). Single migrating cells were automatically tracked using ImarisTrack module (Bitplane, MN, USA). Non-migrating cells outside the RMS were identified by unchanging morphology and used as an internal reference point to correct for tissue motion artifacts ("Drift Correction" function). The soma of migrating neurons in the RMS, were tracked by assigning an x–y position at each time point based upon a unique track reference number. Tracks with a displacement greater than two standard deviations above the combined mean for all tracked cells in a given experiment were then selected for further analysis to reduce the number of non-migrating cells in our population. All tracks from four experiments within each experimental condition were then pooled and analyzed with custom MATLAB programs. For each track, displacement was determined by measuring the distance from the

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