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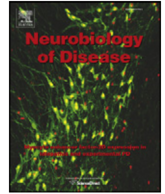

Neurogenesis upregulation on the healthy hemisphere after stroke enhances compensation for age-dependent decrease of basal neurogenesis

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ABSTRACT

Stroke is a leading cause of death and disability worldwide with no treatment for the chronic phase available. Interestingly, an endogenous repair program comprising inflammation and neurogenesis is known to modulate stroke outcome. Several studies have shown that neurogenesis decreases with age but the therapeutic importance of endogenous neurogenesis for recovery from cerebral diseases has been indicated as its ablation leads to stroke aggravation and worsened outcome. A detailed characterization of the neurogenic response after stroke related to ageing would help to develop novel and targeted therapies. In an innovative approach, we used the DCX-Luc mouse, a transgenic model expressing luciferase in doublecortin-positive neuroblasts, to monitor the neurogenic response following middle cerebral artery occlusion over three weeks in three age groups (2, 6, 12 months) by optical imaging while the stroke lesion was monitored by quantitative MRI. The individual longitudinal and noninvasive time profiles provided exclusive insight into age-dependent decrease in basal neurogenesis and neurogenic upregulation in response to stroke which are not accessible by conventional BrdU-based measures of cell proliferation. For cortico-striatal strokes the maximal upregulation occurred at 4 days post stroke followed by a continuous decrease to basal levels by three weeks post stroke. Older animals effectively compensated for reduced basal neurogenesis by an enhanced sensitivity to the cerebral lesion, resulting in upregulated neurogenesis levels approaching those measured in young mice. In middle aged and older mice, but not in the youngest ones, additional upregulation of neurogenesis was observed in the contralateral healthy hemisphere. This further substantiates the increased propensity of older brains to respond to lesion situation. Our results clearly support the therapeutic relevance of endogenous neurogenesis for stroke recovery and particularly in older brains.

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1. Introduction

Adult neurogenesis in the neurogenic niches, namely the hippocampal subgranular zone and the subventricular zone (SVZ) of the lateral ventricles, continuously produces new cells for neuronal replacement in the hippocampus and the olfactory bulb (Garcia-Verdugo et al., 1998). However, endogenous neurogenesis declines with age (Ahlenius et al., 2009; Brown et al., 2003; Couillard-Despres et al., 2009; Hamilton et al., 2013; Luo et al., 2006). Age-dependent changes are detectable on all levels of neurogenesis, i.e. reduced numbers of neural stem cells (NSCs) (Enwere et al., 2004; Luo et al., 2006; Shook et al.,

Abbreviations: ANOVA, Analysis of variance; AUC, area under curve; BLI, Bioluminescence imaging; DCX, doublecortin; MRI, magnetic resonance imaging; M, months; MCAO, middle cerebral artery occlusion; NPC, neuronal precursor cell; SVZ, subventricular zone.

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2012), decreased NSC proliferation, reduced neural progenitor cell and neuroblast proliferation (Tropepe et al., 1997) accompanied by structural changes of the SVZ architecture (Luo et al., 2006; Shook et al., 2012). The greatest reduction in cell genesis occurs between young adulthood and middle age with only modest additional decline during later senescence (reviewed in Hamilton et al., 2013).

Although adult neurogenesis by itself is not sufficient for complete recovery from brain injury such as stroke, its ablation aggravates infarct volume and worsens general stroke outcome (Butti et al., 2012; Sun et al., 2012; Wang et al., 2012). The endogenous NSCs represent an excellent therapeutic target for neurodegenerative diseases and provide the adult brain with a possibility of self-repair with low-level intervention (Lindvall and Kokaia, 2011). Pharmacological stimulation of endogenous neurogenesis with growth factors improved tissue replacement in rodent stroke models (Erlandsson et al., 2011; Kolb et al., 2007; Lai et al., 2008). It is therefore of high therapeutic relevance to better understand the mechanisms and dynamics of neurogenesis in response to brain injuries which widely lack effective treatments.

As neurodegenerative diseases or stroke occur predominantly in elderly people, the question arises whether endogenous neurogenesis of the aged brain is still beneficial despite its age-related decrease and if it can be employed for therapeutic interventions. Although the aged brain retains its capacity to respond with increased neurogenesis to brain injury (Darsalia et al., 2005; Jin et al., 2004), this response was reported in the rat to be altered in terms of intensity and timing (Jin et al., 2004; Sato et al., 2001). However, a clear description of the age-related changes in the neurogenic response to brain injury is not yet available, largely due to the situation that various studies investigated only discrete time points after stroke and assessed different phases of neurogenesis (Jin et al., 2004; Sato et al., 2001; Takasawa et al., 2002; Tang et al., 2014).

Therefore, we followed the spontaneous neurogenic response to stroke at the level of doublecortin (DCX) expressing neuroblasts in three different age groups spanning the time period with the steepest decline in adult basal neurogenesis: 2, 6 and 12 months. In a novel approach, bioluminescence imaging was used to characterize the neurogenic response temporally and quantitatively in vivo. The DCX-Luc transgenic mice with luciferase gene expression restricted to neural progenitor cells allowed for repetitive measurements of individual animals. We used quantitative magnetic resonance imaging (MRI) to assess the stroke lesion over the experimental time period of 3 weeks in order to group animals based on similar ischemic territory. Using this noninvasive approach, we report here new insight into discriminating between the age dependent decline of neurogenesis and the neurogenic response to stroke. Our investigation contributes to the elucidation of important factors for recovery processes such as time window of maximal neurogenic response and contribution of subventricular zones on both hemispheres.

2. Materials and methods

2.1. Animals

Homozygous male DCX-Luc mice (n = 72) with C57BL/6 albino background (B6(Cg)-Tyrc-2 J/J) were used for all experiments. These mice include the human DCX promoter which controls the wildtype firefly luciferase, and were characterized in detail earlier (Couillard-Despres et al., 2008). Mice were of three different age groups: 2 months ("2 M", n = 28), 6 months ("6 M", n = 29) and 12 months ("12 M", n = 27). All animal experiments comply with the ARRIVE guidelines (Kilkenny et al., 2010) and were conducted according to the national, German and Austrian, guidelines in accordance with the European Council Directive 2010/63/EU, and were approved by the local authorities (Landesamt für Natur, Umwelt und Verbraucherschutz North Rhine-Westphalia, reference number 84-02.04.2011.A123; Salzburger Landesregierung, Abteilung 9 für Gesundheit und Sport 20901-TVG/84). Animals were housed in individually ventilated cages under 12 h light/12 h darkness cycle with access to water and food ad libitum.

2.2. MCAO model

Focal cerebral ischemia was induced using the filament model as described by Bahmani et al. (Adamczak et al., 2014; Bahmani et al., 2011). Briefly, mice were anesthetized with 1–2% isoflurane in an O_2/N_2O (30:70%) gas mixture and received a subcutaneous (s.c.) injection of 4 mg/kg Carprofen (Rimadyl) for analgesia. Silicon rubber-coated filaments (Doccol Corporation, Sharon, MA USA) with tip diameters of 170 μm, 190 μm, and 200 μm were used for the 2 M, 6 M and 12 M group, respectively, to block the blood flow to the middle cerebral artery (MCA). Animals were allowed to recover during the 30 min occlusion in a temperature stable box (MediHeat, Peco Services Ltd., Brough, UK) and subsequently re-anesthetized to initiate reperfusion by filament removal. The common carotid artery (CCA) was permanently ligated. Sham animals received only a permanent ligation of the CCA. Following these surgical interventions, body weight was monitored daily for 1 week after stroke.

2.3. Experimental design and group allocation

The study consisted of two groups: the BLI group for the longitudinal neurogenesis time profile generation, and a separate histology group for the immunohistochemical analysis at the time point of maximal neurogenic response after stroke (determined in the first group to be at day 4 post stroke).

Animals in the BLI group received a pre-MCAO measurement for bioluminescence imaging (BLI) and MRI. This allows the evaluation of the pre-stroke distribution of DCX+ neuronal precursor cells (NPCs) via bioluminescence imaging (BLI) signal, as well as the detection of any abnormalities in brain anatomy via MRI. The number of DCX+ cells is a valid measure of neurogenesis as we reported previously (Couillard-Despres et al., 2005). On day 0, animals underwent surgery (MCAO n = 54, SHAM n = 18) as described above. Animals were measured by MRI for the evaluation of the stroke lesion at 2, 7, and 21 days after surgery. Bioluminescence imaging for NPC distribution was performed at 4, 7, 14, and 21 days after stroke. Animals were then sacrificed for immunohistological evaluation of the brain at day 21 after stroke.

Based on the MRI measurement at day 2 after surgery, animals were included (n = 33) or excluded (n = 39) from the study. For group homogeneity, only animals with cortico-striatal ischemic lesion were included in the study. Exclusion criteria were: i) ischemic lesion covering only striatum without cortical involvement, ii) stroke involving the hippocampus, iii) no successful stroke induction on T2-weighted MRI at 48 h, iv) death, v) lesion after sham surgery, or vi) anatomic abnormalities before stroke induction detected on T2-weighted MRI (Supplementary Fig. 1). Finally, group sizes were as follows: "2 M" MCAO n = 5, "2 M" SHAM n = 5, "6 M" MCAO n = 6, "6 M" SHAM n = 4, "12 M" MCAO n = 9, "12 M" SHAM n = 4 (Supplementary Fig. 1). It should be noted that death after MCAO or sham surgery was not affected by aging (death after stroke: 2 M – 1; 6 M – 3; 12 M – 1; death after sham surgery: 2 M – 0; 6 M – 1; 12 M – 1).

Animals in the histology group received MCAO, as described above, and were sacrificed for histological evaluation of the brains at day 4 after MCAO, the time point determined by the BLI group to be the time point of maximal neurogenesis. For this group, mice of 2 months (MCAO n = 3, SHAM n = 3) and 12 months (MCAO n = 3, SHAM n = 3) of mixed gender were used to cover the extreme ends of the neurogenesis age dependence.

2.4. Magnetic resonance imaging (MRI)

Experiments were performed on a horizontal 11.7 Tesla Bruker BioSpec 117/16USR system (Bruker Biospin, Ettlingen, Germany) with

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