Neuroscience Letters 548 (2013) 280–285

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

FGF2 and FGFR1 signaling regulate functional recovery following cuprizone demyelination☆

Amanda J. Mierzwa[a,b], Yong-Xing Zhou[a,1], Norah Hibbits[a,c], Adam C. Vana[a,2], Regina C. Armstrong[a,b,c,*]

[a] Department of Anatomy, Physiology and Genetics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, United States
[b] Center for Neuroscience and Regenerative Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, United States
[c] Graduate Program in Neuroscience, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, United States

HIGHLIGHTS

- Cuprizone demyelination reduces sensorimotor coordination and increases social interaction.
- Fgf2 null mice have improved running speed on complex wheels after acute or chronic demyelination.
- Fgfr1 knockdown in the oligodendrocyte lineage during chronic demyelination improves running.
- Fgf2 deletion and Fgfr1 knockdown do not alter social interaction.
- Fgf2 deletion and Fgfr1 knockdown promote remyelination and improve functional recovery.

ARTICLE INFO

Article history:
Received 22 March 2013
Received in revised form 16 April 2013
Accepted 3 May 2013

Keywords:
Remyelination
Corpus callosum
Wheel activity
Sociability
Growth factor
Multiple sclerosis

ABSTRACT

In demyelinating diseases, such as multiple sclerosis, remyelination offers the potential to recover function of viable denuded axons by restoring saltatory conduction and/or protecting from further damage. Mice with genetic reduction of fibroblast growth factor 2 (Fgf2) or Fgf receptor 1 (Fgfr1) exhibit dramatically improved remyelination following experimental demyelination with cuprizone. The current studies are the first to test neurobehavioral outcomes with these gene deletions that improved remyelination. The cuprizone protocols used did not produce overt abnormalities but did reduce bilateral sensorimotor coordination (complex wheel task) and increase sociability (two chamber apparatus with novel mouse). A significant effect of genotype was observed on the complex wheel task but not in the sociability apparatus. Specifically, complex wheel velocities for Fgf2 nulls improved significantly after removal of cuprizone from the diet. This improvement in Fgf2 null mice occurred following either acute (6 weeks) or chronic (12 weeks) demyelination. Plp/CreERT:Fgfr1$^{fl/fl}$ mice administered tamoxifen at 10 weeks of cuprizone treatment to induce Fgfr1 knockdown also showed improved recovery of running velocities on the complex wheels. Therefore, constitutive deletion of Fgf2 or Fgfr1 knockdown in oligodendrocyte lineage cells is sufficient to overcome impairment of sensorimotor coordination after cuprizone demyelination.

1. Introduction

Remyelination, which may restore nerve conduction and protect axons, is significantly greater in early stage multiple sclerosis (MS) lesions than in chronic disease [5,6]. Failed differentiation of oligodendrocyte lineage cells (OLCs) may contribute to poor remyelination in chronic MS lesions and prolonged neurological deficits [9]. Multiple molecular signaling pathways inhibit differentiation of oligodendrocyte progenitor (OP) cells and limit remyelination in experimental models [1]. Modifying inhibitory signals in lesion areas could potentially enhance functional recovery in MS patients by improving the remyelination capacity of immature OLCs that persist in MS lesions [4,9].

Our earlier studies showed increased expression of FGF2 and FGFR1 in demyelinated lesions [2,3,12,15]. OP differentiation is

Abbreviations: Cup, cuprizone; FGF2, fibroblast growth factor 2 ligand; Fgf2, gene encoding murine fibroblast growth factor 2; FGFR1, fibroblast growth factor receptor 1; Fgfr1, gene encoding murine fibroblast growth factor receptor 1; fl, floxed gene; MOG, myelin oligodendrocyte protein; MS, multiple sclerosis; OLCs, oligodendrocyte lineage cells; Plp/CreERT, transgene encoding the murine proteolipid protein promoter driving Cre recombinase fused to mutated estrogen receptor; Tam, tamoxifen.

* Corresponding author at: Department of Anatomy, Physiology and Genetics, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814, United States. Tel.: +1 301 295 3205.
E-mail address: regina.armstrong@usuhs.edu (R.C. Armstrong).
[1] Current address: Florida State University College of Medicine, 1300 Miccosukee Road, Tallahassee, FL 32308, United States.
[2] Current address: Department of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, United States.

0304-3940/$ – see front matter © 2013 The Authors. Published by Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.05.010

increased during remyelination in Fgf2 null mice [14]. FGF2 inhibits OP differentiation through activation of FGFR1 [20]. Genetic reduction of Fgf2 and Fgfr1signaling increased oligodendrogenesis and remyelination and also reduced axon damage in the corpus callosum after chronic cuprizone demyelination [2,18,21].

The current studies are the first to evaluate recovery of function in Fgf2 null mice as well as in mice with Fgfr1 knockdown in OLCs after cuprizone demyelination. A complex wheel task differentiates cuprizone treated mice due to impaired bilateral sensorimotor coordination that reflects corpus callosum dysfunction [7,16]. This complex wheel assessment detects deficits during acute and chronic cuprizone demyelination and with latent axon loss in the corpus callosum [7,10,11]. In addition, social interaction is evaluated as a distinct behavioral domain that is altered with cuprizone demyelination [7].

2. Materials and methods

2.1. Mice and cuprizone demyelination

All procedures were approved by the USUHS IACUC. Fgf2 and Plp/CreERT:Fgfr1$^{fl/fl}$ mice were bred, fed cuprizone and treated with tamoxifen as previously described [2,21].

2.2. Wheel running

Cohorts of up to 12 mice were tested on the wheels simultaneously as previously detailed [7].

2.3. Sociability

A sociability apparatus (Ugo Basile, Italy) along with AnyMaze software (Stoelting Co., Wood Dale, IL) was used to quantify sociability [13]. During a 15-min habituation period, mice explore between two chambers (top and bottom) – each has one side empty and the other contains a wire cage mouse carrier. An unfamiliar mouse is placed within the carrier in the top chamber during a subsequent 15-min social period.

2.4. Myelination status

Immunohistochemistry for myelin oligodendrocyte glycoprotein (MOG) was performed and quantified as described previously [2].

2.5. Data analysis

Prism (GraphPad Software, La Jolla, CA) was used to perform ANOVA followed by Tukey's multiple comparison test for statistical analyses. Cohorts were run simultaneously for gene deletion and control comparisons by investigators blinded to condition. Behavioral cohorts included 3–6 mice per condition.

3. Results

3.1. Complex wheel assessment of bilateral sensorimotor coordination in Fgf2 null mice

Cohorts of Fgf2 null and control mice were assessed on the complex wheel task (Figs. 1 and 2). Fgf2 wild type mice show reduced maximum velocities during acute cuprizone treatment that do not improve significantly after return to normal chow (Fig. 2A), consistent with our results in C57BL/6 mice [7]. In contrast, Fgf2 null mice improve significantly during the recovery period on normal chow (Fig. 2B). With chronic cuprizone demyelination, both Fgf2 wild type and null mice show impairment during acute and chronic cuprizone treatment periods (Fig. 2C and D). Notably, only Fgf2 null mice exhibit significant improvement following this chronic demyelination (Fig. 2D).

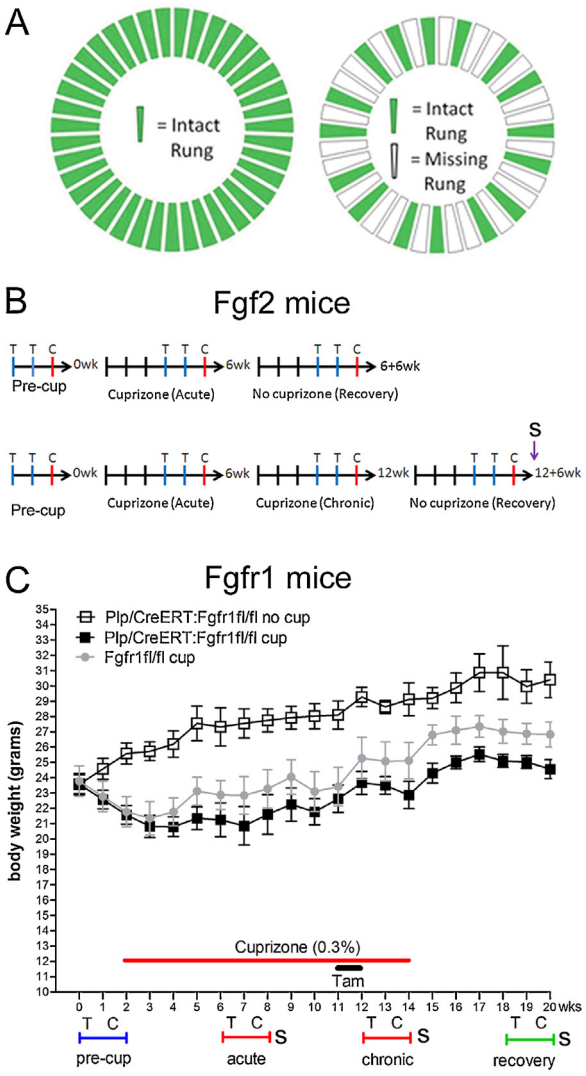

Fig. 1. Experimental design. (A) The running wheel was configured either as a "training wheel" with all rungs in place (left) or as a "complex" wheel with a non-uniform rung distribution (right) to increase the task difficulty. (B) Fgf2 mice behavioral assessment timelines showing weeks with training (T) or complex (C) wheels and sociability (S) testing. (C) Fgfr1 mice behavioral assessments shown weekly along body weight record. Cuprizone ingestion significantly reduced body weight in Plp/CreERT:Fgfr1$^{fl/fl}$ mice ($p < 0.001$ "cup" vs. "no cup") and Fgfr1$^{fl/fl}$ mice ($p < 0.001$ compared to Plp/CreERT:Fgfr1$^{fl/fl}$ "no cup"). Tamoxifen (tam) administration did not adversely affect body weight.

An additional cohort of Fgf2 null mice was fed 0.3% cuprizone attempting to further differentiate the demyelination deficits from the recovery phase (data not shown). As with 0.2% cuprizone, this 0.3% dose did not produce overt changes in behavior or appearance. Similar to the data presented for 0.2% cuprizone (Fig. 2), significant improvement was again observed with return to normal chow after chronic demyelination with 0.3% cuprizone in Fgf2 null mice ($p < 0.05$ for recovery vs. chronic). A similar extent of remyelination was also observed. After chronic demyelination and a 6-week recovery period on normal chow, 87.45% ± 1.18 of the area of the corpus callosum immunostained for MOG in Fgf2 null mice treated with 0.3% cuprizone as compared to 87.84% ± 4.72 with 0.2% cuprizone ($p = 0.9308$).