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Effects of cross-rearing with social peers on myelination in the medial prefrontal cortex of a mouse model with autism spectrum disorder

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Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impaired social interaction, poor communication skills, and repetitive/restrictive behaviors. Recent studies have indicated that early rehabilitative intervention can alleviate the symptoms of individuals with ASD. However, it remains unknown whether rehabilitative intervention can restore brain structures such as myelin, which generally shows abnormalities in individuals with ASD. Therefore, in the present study, we used a mouse model of ASD (BTBR mice) that demonstrated asocial behaviors and hypomyelination in the medial prefrontal cortex (mPFC) to investigate whether interaction with social peers (C57BL/6J mice) has an effect on myelination. We found that housing with C57BL/6J mice after weaning through adulthood increased the myelin thickness in mPFC, but not in the motor cortex, of BTBR mice. There was no effect of cross-rearing with C57BL/6J mice on axon

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diameter in mPFC of BTBR mice. This finding suggests that early rehabilitative intervention may alleviate myelin abnormalities in mPFC as well as clinical symptoms in individuals with ASD.

Keywords: Neuroscience, Psychology, Psychiatry

1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by two core symptoms, namely impaired social interaction and communication, and repetitive behaviors. Despite a number of studies conducted to elucidate the remedy, there are few established treatments for symptom improvement. However, recent studies have indicated the effectiveness of rehabilitative interventions to alleviate the social problems of individuals with ASD, and substantial efforts have been devoted to the development of such rehabilitative interventions. The most common rehabilitative intervention is applied behavioral analysis (ABA), which is an intense behavioral intervention that improves intellectual and educational functioning (Lovaas, 1987). Several brain imaging studies using functional magnetic resonance imaging (fMRI) revealed that rehabilitative interventions, including computer-based facial affect recognition training, pivotal response treatment, and reading intervention, can improve ASD symptoms by affecting brain functions and connectivity (Calderoni et al., 2016). However, to the best of our knowledge, no studies have confirmed the effects of rehabilitative interventions on brain structures such as myelin, which is reportedly altered in individuals with ASD (Zikopoulos and Barbas, 2010).

Numerous mouse models of ASD have been developed and investigated to extrapolate the pathobiology of ASD. Among these, the BTBR mouse is a widely used model of ASD with substantial face validity that demonstrates impaired social interaction, aberrant ultrasonic vocalization as communication problems, increased grooming as a repetitive behavior, and abnormalities of immune cells and oligodendrocyte lineage cells (Yang et al., 2007, 2011; Heo et al., 2011; Stephenson et al., 2011). In order to examine the effects of rehabilitative intervention, BTBR mice were cross-fostered and cross-reared with C57BL/6J mice, which have high sociability and low repetitive behaviors. Interestingly, cross-fostering with C57BL/6J mice from birth through weaning did not alter the behaviors of BTBR mice (Yang et al., 2007); however, cross-rearing with C57BL/6J for 20 or 40 days after weaning significantly resolved sociability deficits (Yang et al., 2011). These findings indicated that social intervention after weaning can change ASD-like behaviors and, most probably, other associated brain functions.

Myelin is a laminated membrane structure surrounding axons produced by oligodendrocytes, and it accelerates the axonal conduction velocity (McKenzie et al., 2014). Multiple studies have revealed that oligodendrocytes and myelin are

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necessary in order to acquire proper brain functions (McKenzie et al., 2014); therefore, aberrant myelin formation in early life may be associated with the development of ASD-like symptoms. Given that oligodendrocytes and myelination are apparently sensitive to external stimuli during the developing stage (Makinodan et al., 2012), early interventions before the completion of myelination are most likely to alleviate myelin impairment as opposed to interventions later in life. Interestingly, it has been shown that medial prefrontal cortex (mPFC) myelination plays a key role in social interaction in mice (Liu et al., 2012, 2016; Makinodan et al., 2012), and the extent of myelination in mPFC of BTBR mice could be related to the impairment of social interaction.

From the above perspectives, we measured myelin thickness in the mPFC of C57BL/6J mice and BTBR mice, and aimed to validate whether cross-rearing with social peers after weaning can change myelination in mPFC, considering that myelination is subject to social experience in mPFC (Makinodan et al., 2012; Liu et al., 2012).

2. Methods

2.1. Mice and housing conditions

C57BL/6J (C57) mice and BTBR $T^{+}tf$/J (BTBR) mice (Charles River Lab Japan, Inc., Yokohama, Japan) were housed in a temperature- and humidity-controlled animal facility under a reversed light–dark cycle (lights on from 8:00–20:00). Only male mice were used in this study because male and female mice differently respond to social experience (Pinna et al., 2004). All animals were provided with food and water ad libitum throughout the experiments. All experimental protocols were approved by the Animal Care Committee of Nara Medical University and were in accordance with the policies established in the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to utilize alternatives to in vivo techniques, if available. Male C57BL/6J and BTBR mice were randomly assigned to three different housing conditions at the time of weaning (postnatal day 21; P21): four C57BL/6J mice in a cage (B6-only), four BTBR mice in a cage (BTBR-only), and two C57BL/6J mice and two BTBR mice in a cage (B6-mixed and BTBR-mixed) until P65. Since a previous study showed that cross-rearing with C57BL/6J for 20 or 40 days after weaning significantly resolved sociability deficits of BTBR mice (Yang et al., 2011), we chose longer cross-rearing (44 days) to evaluate myelination in the mPFC of mice.

2.2. Electron microscopy

At P65, four mice per group were intracardially perfused with a fixative (2% paraformaldehyde, 2.5% glutaraldehyde, and 0.1% picric acid in 100 mM

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cacodylate buffer). The brains were removed from the skulls and fixed in the same solution overnight. Coronal sections (500-μm thick) were then obtained to isolate mPFC (prelimbic and infralimbic) and the motor cortex (between Bregma 1.70 mm and 1.98 mm) in epoxy resin, and the layer V of each brain region was examined under transmission electron microscopy in 50-nm sections. Using this approach, multiple, nonoverlapping regions of the brain from each mouse were imaged. A g-ratio was calculated as the axon perimeter/(axon + myelin sheath) perimeter using ImageJ (NIH). Axons with a circularity ($4 \times \pi \times$ area/perimeter^2) of $\ll 6.0$ were excluded from analysis, because the perimeter would not precisely reflect myelin thickness in cases where the axons were cut at a slant. The g-ratio and axon diameter of more than 200 myelinated axons from four mice were measured. The images were acquired and analyzed by a person who was blind to the sample information.

2.3. Statistics

Log-rank test was used for statistical analyses with GraphPad Prism 5 (GraphPad Software Inc, La Jolla, California, USA). A *P*-value of $\ll 0.05$ was considered statistically significant.

3. Results

3.1. Effects of cross-rearing with C57BL/6J mice on hypomyelination in mPFC of BTBR mice

The g-ratio of each axon in mPFC was measured to evaluate myelination (Fig. 1a). We found that the g-ratios of myelinated axons in mPFC were significantly different in the four groups; B6-only, BTBR-only, B6-mixed, and BTBR-mixed (log-rank test, $P \ll 0.0001$, N = 4) (Fig. 1b, c, d), followed by Tukey's test indicating that myelin was thinner in mPFC of BTBR mice than in mPFC of B6 mice ($P \ll 0.05$). After cross-rearing with C57BL/6J mice from P21 to P65, BTBR mice presented g-ratio scores comparable with those for the B6-only and B6-mixed groups ($P > 0.05$, $P > 0.05$ respectively). The axon diameters were not significantly different among the B6-only, BTBR-only, B6-mixed, and BTBR-mixed groups (log-rank test, $P = 0.4821$, N = 4) (Fig. 1e).

3.2. Effects of cross-rearing with C57BL/6J mice on myelination in the motor cortex of BTBR mice

A previous report showed that social experience does not change myelination in the motor cortex, as opposed to that in mPFC (Makinodan et al., 2012). Therefore, we measured the g-ratio in the motor cortex for each of the four groups and found that the g-ratio of axons in the motor cortex was comparable between the B6-only and BTBR-only groups. Furthermore, cross-rearing with C57BL/6J mice did not

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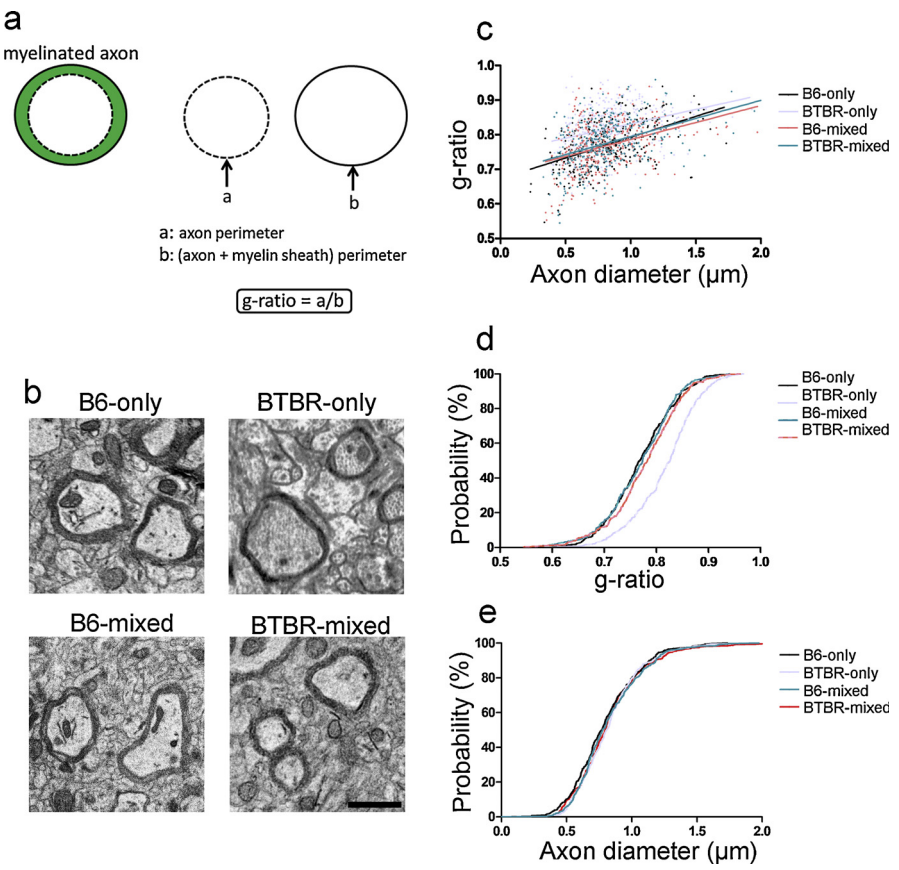

Fig. 1. An increase in the myelin thickness in the medial prefrontal cortex (mPFC) of BTBR mice after cross-rearing with C57BL/6J (B6) mice. (a) The image of g-ratio (b) The representative images of myelinated axons in the mPFC (c) The dot graph of g-ratio and axon diameter (d) The cumulative probability curve of g-ratio; At P65, the mPFC myelin is thinner in the BTBR-only group than in the B6-only group ($P \ll 0.05$). After housing with C57BL/6J mice from P21 through P65, the mPFC myelin thickness has increased in the BTBR-mixed group compared with that in the BTBR-only group ($P \ll 0.05$). After housing with BTBR mice from P21 through P65, there is no change in mPFC myelination in the B6-only group ($P > 0.05$). (f) The cumulative probability curve of g-ratio; There are no significant differences in the axon diameter among the B6-only, BTBR-only, B6-mixed, and BTBR-mixed groups ($P > 0.05$). More than 200 axons from four mice per group were analyzed. * $P \ll 0.05$. Scale bar: 1 μm. B6-only: C57BL/6J mice housed with B6 peers, BTBR-only: BTBR mice housed with BTBR peers, B6-mixed: C57BL/6J mice housed with BTBR mice, BTBR-mixed: BTBR mice housed with C57BL/6J mice.

change the myelin thickness in the motor cortex of BTBR mice. In other words, g-ratio scores were comparable among the four groups (Log-rank test, $P = 0.2719$, N = 4) (Fig. 2a, b, c). There were no significant differences in the axon diameter among the four groups (log-rank test, $P = 0.1179$, N = 4) (Fig. 2d).

4. Discussion

In the present study, we found that interaction with social peers, which has been shown to improve sociability (Yang et al., 2011), considerably influences

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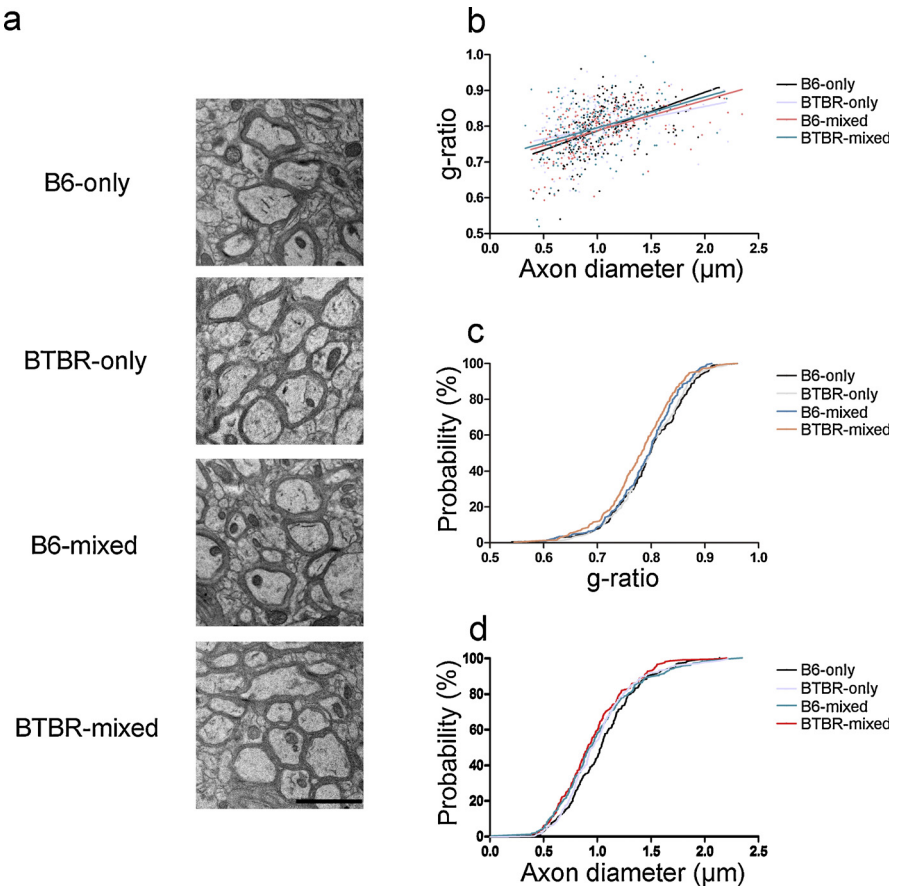

Fig. 2. No effects of cross-rearing with C57BL/6J (B6) mice on myelination in the motor cortex of BTBR mice. (a) The representative images of myelinated axons in the motor cortex (b) The dot graph of g-ratio and axon diameter (c) The probability curve of g-ratio; Myelin thickness is comparable among the B6-only, BTBR-only, B6-mixed, and BTBR-mixed groups ($P > 0.05$). (d) There are no differences in the axon diameter among the B6-only, BTBR-only, B6-mixed, and BTBR-mixed groups ($P > 0.05$). More than 200 axons from four mice per group were analyzed. Scale bar: 2 μm. B6-only: C57BL/6J mice housed with B6 peers, BTBR-only: BTBR mice housed with BTBR peers, B6-mixed: C57BL/6J mice housed with BTBR mice, BTBR-mixed: BTBR mice housed with C57BL/6J mice.

myelination in the BTBR mouse model of ASD. This finding supports the theory that rehabilitative interventions can affect brain structures as well as brain functions in individuals with ASD.

Individuals with ASD who have received intensive early intervention demonstrate improved long-term outcomes, and the effectiveness depends on the age at intervention. Earlier interventions such as ABA lead to more substantial resolution of ASD symptoms (Harris and Handleman, 2000). Although the biological mechanisms underlying the effects of early intervention remain unclear, recent findings propose that myelination in the relevant brain regions is a potential factor.

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First, we found that BTBR mice exhibit thinner myelin in mPFC, but not in the motor cortex, compared with C57BL/6J mice (Fig. 1a). This finding was similar to those in postmortem brain studies of individuals with ASD (Zikopoulos and Barbas, 2010). Because BTBR mice are generally considered genetic models of ASD (McFarlane et al., 2008), hypomyelination in mPFC could occur due to genetic mechanisms. The elevated expression of proinflammatory cytokines such as interleukin-6 in the brains of BTBR mice (Wei et al., 2016) may cause hypomyelination, as shown in our previous study (Makinodan et al., 2016). If so, myelin in the motor cortex of BTBR mice should also be thinner than that in the motor cortex of C57BL/6J mice. However, our study demonstrated comparable myelin thickness in the motor cortex between the two groups. Therefore, the extent of mPFC myelination may be dependent on social experience, considering that the sociability of BTBR mice is lower than that of C57BL/6J mice (Yang et al., 2011) and that mPFC is more sensitive to social experience than the motor cortex with regard to myelination and neuronal activities (Makinodan et al., 2012, 2017). Further studies are warranted to elucidate whether the myelinated axons are derived from projection neurons in the mPFC or afferents to mPFC. Since social isolation affects the activities of projection neurons in the mPFC (Yamamuro et al., 2017), the myelination of those axons is possibly susceptible to social experience.

Second, we found that social interaction with C57BL6/J mice increased the myelin thickness in mPFC of BTBR mice. Given an increase in the social interaction of BTBR mice after cross-rearing with C57BL6/J mice (Yang et al., 2011), the restoration of myelination could be attributed to mPFC neuronal activities in relation to social interaction (Yamamuro et al., 2017), on the basis of findings that myelination is axonal activity-dependent (Wake et al., 2011). On the other hand, the alteration of social experience in the present study did not change myelination in the motor cortex, indicating that myelination in the motor cortex is independent of social experience. Interestingly, cross-fostering of BTBR mice with C57BL/6J mice during the neonatal period produced no significant effects on ASD-like behaviors such as altered ultrasonic vocalization and repetitive behaviors (Yang et al., 2007), implying that social interaction with peers influences the symptoms of ASD more than the mother's care. These findings are consistent with the denial of the "refrigerator mother" theory and higher contribution of nonshared environmental factors compared with that of shared environmental factors with regard to the development of ASD (Sandin et al., 2014). Further studies should elucidate whether cross-fostering with C57BL/6J mice changes mPFC myelination in BTBR mice. It should be noted that after weaning, there is a limited time window for satisfactory effects of social peers on myelination in mPFC and ASD-like behaviors in BTBR mice.

In conclusion, the findings of the present animal study suggest that early rehabilitative interventions, particularly interaction with social peers, can modify

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the extent of myelination in mPFC although it remains unknown what changes myelination; the social behavior or context novelty, which could be related to alleviation of symptoms in individuals with ASD.

Declarations

Author contribution statement

Manabu Makinodan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Kazuki Okumura, Daisuke Ikawa,Yasunori Yamashita,Kazuhiko Yamamuro, Michihiro Toritsuka, Sohei Kimoto, Takahira Yamauchi, Takashi Komori, Yoshinori Kayashima, Hiroki Yoshino: Performed the experiments.

Akio Wanaka, Toshifumi Kishimoto: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Calderoni, S., Billeci, L., Narzisi, A., Brambilla, P., Retico, A., Muratori, F., 2016. Rehabilitative Interventions and Brain Plasticity in Autism Spectrum Disorders: Focus on MRI-Based Studies. Front. Neurosci. 10, 139.

Harris, S.L., Handleman, J.S., 2000. Age and IQ at intake as predictors of placement for young children with autism: a four- to six-year follow-up. J. Autism Dev. Disord. 30 (2), 137–142.

Heo, Y., Zhang, Y., Gao, D., Miller, V.M., Lawrence, D.A., 2011. Aberrant immune responses in a mouse with behavioral disorders. PloS One 6 (7) e20912.

http://dx.doi.org/10.1016/j.heliyon.2017.e00468

Liu, J., Dietz, K., DeLoyht, J.M., Pedre, X., Kelkar, D., Kaur, J., Vialou, V., Lobo, M.K., Dietz, D.M., Nestler, E.J., Dupree, J., Casaccia, P., 2012. Impaired adult myelination in the prefrontal cortex of socially isolated mice. Nat. Neurosci. 15 (12), 1621–1623.

Liu, J., Dupree, J.L., Gacias, M., Frawley, R., Sikder, T., Naik, P., Casaccia, P., 2016. Clemastine Enhances Myelination in the Prefrontal Cortex and Rescues Behavioral Changes in Socially Isolated Mice. J. Neurosci. 36 (3), 957–962.

Lovaas, O.I., 1987. Behavioral treatment and normal educational and intellectual functioning in young autistic children. J. Consult. Clin. Psychol. 55 (1), 3–9.

Makinodan, M., Ikawa, D., Miyamoto, Y., Yamauchi, J., Yamamuro, K., Yamashita, Y., Toritsuka, M., Kimoto, S., Okumura, K., Yamauchi, T., Fukami, S.I., Yoshino, H., Wanaka, A., Kishimoto, T., 2016. Social isolation impairs remyelination in mice through modulation of IL-6. FASEB J. 30 (12), 4267–4274.

Makinodan, M., Ikawa, D., Yamamuro, K., Yamashita, Y., Toritsuka, M., Kimoto, S., Yamauchi, T., Okumura, K., Komori, T., Fukami, S.I., Yoshino, H., Kanba, S., Wanaka, A., Kishimoto, T., 2017. Effects of the mode of re-socialization after juvenile social isolation on medial prefrontal cortex myelination and function. Sci. Rep. 7 (1), 5481.

Makinodan, M., Rosen, K.M., Ito, S., Corfas, G., 2012. A critical period for social experience-dependent oligodendrocyte maturation and myelination. Science 337 (6100), 1357–1360.

McFarlane, H.G., Kusek, G.K., Yang, M., Phoenix, J.L., Bolivar, V.J., Crawley, J. N., 2008. Autism-like behavioral phenotypes in BTBR T + tf/J mice. Genes Brain Behav. 7 (2), 152–163.

McKenzie, I.A., Ohayon, D., Li, H., de Faria, J.P., Emery, B., Tohyama, K., Richardson, W.D., 2014. Motor skill learning requires active central myelination. Science 346 (6207), 318–322.

Pinna, G., Agis-Balboa, R.C., Doueiri, M.S., Guidotti, A., Costa, E., 2004. Brain neurosteroids in gender-related aggression induced by social isolation. Crit. Rev. Neurobiol. 16 (1-2), 75–82.

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C.M., Reichenberg, A., 2014. The familial risk of autism. Jama 311 (17), 1770–1777.

Stephenson, D.T., O'Neill, S.M., Narayan, S., Tiwari, A., Arnold, E., Samaroo, H. D., Du, F., Ring, R.H., Campbell, B., Pletcher, M., Vaidya, V.A., Morton, D., 2011. Histopathologic characterization of the BTBR mouse model of autistic-like behavior reveals selective changes in neurodevelopmental proteins and adult hippocampal neurogenesis. Mol. Autism 2 (1), 7.

http://dx.doi.org/10.1016/j.heliyon.2017.e00468

Wake, H., Lee, P.R., Fields, R.D., 2011. Control of local protein synthesis and initial events in myelination by action potentials. Science 333 (6049), 1647–1651.

Wei, H., Ma, Y., Liu, J., Ding, C., Jin, G., Wang, Y., Hu, F., Yu, L., 2016. Inhibition of IL-6 trans-signaling in the brain increases sociability in the BTBR mouse model of autism. Biochim. Biophys. Acta 1862 (10), 1918–1925.

Yamamuro, K., Yoshino, H., Ogawa, Y., Makinodan, M., Toritsuka, M., Yamashita, M., Corfas, G., Kishimoto, T., 2017. Social Isolation During the Critical Period Reduces Synaptic and Intrinsic Excitability of a Subtype of Pyramidal Cell in Mouse Prefrontal Cortex. Cereb. Cortex, 1–13.

Yang, M., Perry, K., Weber, M.D., Katz, A.M., Crawley, J.N., 2011. Social peers rescue autism-relevant sociability deficits in adolescent mice. Autism Res. 4 (1), 17–27.

Yang, M., Zhodzishsky, V., Crawley, J.N., 2007. Social deficits in BTBR T + tf/J mice are unchanged by cross-fostering with C57BL/6J mothers. Int. J. Dev. Neurosci. 25 (8), 515–521.

Zikopoulos, B., Barbas, H., 2010. Changes in prefrontal axons may disrupt the network in autism. J. Neurosci. 30 (44), 14595–14609.

 http://dx.doi.org/10.1016/j.heliyon.2017.e00468