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Developmental expression profiles of axon guidance signaling and the immune system in the marmoset cortex: Potential molecular mechanisms of pruning of dendritic spines during primate synapse formation in late infancy and prepuberty (I)

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ABSTRACT

The synapse number and the related dendritic spine number in the cerebral cortex of primates shows a rapid increase after birth. Depending on the brain region and species, the number of synapses reaches a peak before adulthood, and pruning takes place after this peak (*overshoot-type* synaptic formation). Human mental disorders, such as autism and schizophrenia, are hypothesized to be a result of either too weak or excessive pruning after the peak is reached. Thus, it is important to study the molecular mechanisms underlying *overshoot-type* synaptic formation, particularly the pruning phase.

To examine the molecular mechanisms, we used common marmosets (*Callithrix jacchus*). Microarray analysis of the marmoset cortex was performed in the ventrolateral prefrontal, inferior temporal, and primary visual cortices, where changes in the number of dendritic spines have been observed. The spine number of all the brain regions above showed a peak at 3 months (3 M) after birth and gradually decreased (e.g., at 6 M and in adults). In this study, we focused on genes that showed differential expression between ages of 3 M and 6 M and on the differences whose fold change (FC) was greater than 1.2. The selected genes were subjected to canonical pathway analysis, and in this study, we describe axon guidance signaling, which had high plausibility. The results showed a large number of genes belonging to subsystems within the axon guidance signaling pathway, macrophages/immune system, glutamate system, and others. We divided the data and discussion of these results into 2 papers, and this is the first paper, which deals with the axon guidance signaling and macrophage/immune system. Other systems will be described in the next paper. Many components of subsystems within the axon guidance signaling underwent changes in gene expression from 3 M to 6 M so that the synapse/dendritic spine number would decrease at 6 M. Thus, axon guidance signaling probably contributes to the decrease in synapse/dendritic spine number at 6 M, the phenomenon that fits the *overshoot-type* synaptic formation in primates.

Microglial activity (evaluated by quantifying *AIF1* expression) and gene expression of molecules that modulate microglia, decreased at 6 M, just like the synapse/dendritic spine number. Thus, although microglial activity is believed to be related to phagocytosis of synapses/dendritic spines, microglial activity alone cannot explain how pruning was accelerated in the pruning phase. On the other hand, expression of molecules that tag synapses/dendritic spines as a target of phagocytosis by microglia (e.g., complement components) increased at 6 M, suggesting that these tagging proteins may be involved in the acceleration of pruning during the pruning phase.

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1. Introduction

Primates show a rapid increase in the synapse and/or dendritic spine number in infancy and early childhood, reaching a peak. After this peak is reached, a decrease is caused by pruning [1–5]. This primate type of synaptic change is called *overshoot-type* synaptic formation [5], which is not detectable in rodents. Recently, abnormalities in the generation and/or pruning of dendritic spines were implicated in several mental disorders including autism and schizophrenia. An autistic brain tends to show more synapse formation at the early age, with less pruning after the peak of the synaptic number is reached, whereas a schizophrenic brain tends to show more pruning on an average, after the peak of the synaptic number is reached [6]. To understand the mechanism underlying these mental disorders and to find possible therapeutic targets, it would be useful to identify molecular mechanisms underlying the *overshoot-type* synaptic formation, particularly of pruning after the peak is reached. Pruning does not exist in a pronounced form in rodents, which are the animal models most frequently used for studies of the molecular mechanisms underlying synaptogenesis [6].

Common marmosets become sexually mature earlier (1.5 years of age) than macaques (sexual maturation at approximately 4.5 years of age [8]). It is possible to make genetic modifications in the marmoset to create transgenic animals [9]; thus, marmosets appear to be a convenient model for examining molecular mechanisms of synapse formation in the primate brain.

In a previous study, we examined the postnatal development of basal dendritic trees and spines of layer III pyramidal cells in the primary visual sensory cortex (V1), visual association cortex (inferior temporal area, IT), and the vetrotalteral prefrontal cortex (PFC) in marmosets [7] and reported that all 3 areas show *overshoot-type* synaptic formation.

In this study, we examined gene expression of 3 cortical areas at 5 postnatal ages, using the same cortical areas and timing as described previously [7], on a custom-made gene chip with approximately 23,000 marmoset probes. We selected statistically significant differences in the expression of genes between 3 and 6 months (3 M and 6 M) of age, mostly in PFC, IT, and V1, for identifying the molecular mechanisms underlying the pruning phase. Here, we report and discuss the selected genes that participate in axon guidance signaling, which is the most plausible (lowest *p* value) canonical signaling in our analysis (Table 1). We also discuss genes of the microglia and immune systems, which are likely to be involved in synaptic pruning in the cerebral cortex and in plausible canonical signaling (Table 1).

Table 1
Canonical pathways most likely related to controlling dendritic spine number, based on differentially expressed genes between 3 and 6 months of age in the 3 brain regions.

Pathway	*p*	Gene name
Axonal guidance signaling	1.97E-06	*ADAM11, ADAM19, ADAMTS1, ADAMTS4, ARHGEF7, BMP3, BMP8A, COPS5, CXCR4, EPHA3, EPHA5, EPHA10, EPHB2, FZD1, FZD5, FZD8, GNA14, GNAZ, GNB4, GNG2, GNG4, GNG7, IGF1, KLC1, LIMK2, MKNK1, NFATC3, NRP2, PAK6, PIK3C2B, PIK3CG, PIK3R1, PLCB4, PLCD1, PLXND1, PPP3CA, PRKCG, RAC3, RASA1, RASSF5, ROBO2, ROCK2, SDC2, SEMA3B, SEMA3C, SEMA4D, SEMA4F, SEMA6A, SEMA6C, SEMA6D, SHANK2, SLIT1, TUBA1C, TUBB3, UNC5C, WNT16, WNT10A*
Hepatic fibrosis/hepatic stellate cell activation *(Macrophage system)	1.58E-03	*AGT, COL1A2, COL3A1, CYP2E1, EDNRB, FAS, FGFR1, FN1, IGF1, IGFBP3, IGFBP4, KDR, LAMA1, LEP, LEPR, PDGFRA, TGFBR2, TIMP2, TLR4, VCAM1*
Dendritic cell maturation *(Macrophage system)	9.32E-01	*COL1A2, COL3A1, FCGR1B, HLA-A, IRF8, LEP, LEPR, TLR4, TREM2*
†Microglia-specific genes		*AIF1, CX3CL1, CX3CR1, IL10RB, TGFBR2, TNFRSF19, NOS1AP*
Complement system	6.81E-03	*C1QB, C1QC, C5, CD46, CD55, CFB, MASP1*

Red letters indicate that expression at 3 months was stronger than at 6 months. Black text indicates the opposite.
* (Macrophage system): Genes not expressed in microglia were extracted from the original canonical pathway list.
† Microglia-specific genes were expressed in microglia but not in other macrophages.

2. Materials and methods

2.1. Animals and tissue preparation

We used common marmosets (*Callithrix jacchus*): 4 animals on postnatal Day 0 (0 D) and the remaining animals, each age group consisting of 3 animals [2 M, 3 M, 6 M, and >1.5 years old (adult); Table 2]. All experimental and animal care procedures were performed in accordance with the NIH guidelines [11] and the Guide for Care and Use of Laboratory Primates published by the National Institute of Neuroscience, National Center of Neurology and Psychiatry (2005, 2008).

2.2. Tissue processing

The animals were sedated with ketamine hydrochloride (Ketalar, 25 mg/kg i.m.; Daiichi Sankyo, Tokyo, Japan) and euthanized via overdose with sodium pentobarbital (Somnopentyl, 100 mg/kg i.p.; BCM International, Hillsborough, NJ). The animals were perfused intracardially with 0.1 M potassium phosphate-buffered saline (pH 7.2). Blocks of tissue from V1, IT, and PFC were excised as described previously [7], and immersed in RNAlater (Life Technologies Japan Ltd., Tokyo, Japan). Total RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. RNA concentrations in the samples were measured using the Amersham–Pharmacia spectrophotometer (Ultraspec 3100pro, Amersham Pharmacia Biotech, Uppsala, Sweden) with quantification and assessment of rRNA integrity using a 2100 Bioanalyzer (Agilent, Palo Alto, CA, USA).

2.3. Generation of common marmoset cDNA sequences and construction of the microarray

The DNA sequences for probes used in Marmo2 were constructed as follows. The human RefSeq sequences were BLAST searched against the marmoset 6× whole genome shotgun preliminary assembly sequence (Ver. 2.0.2) obtained from the public domain at the Genome Center of Washington University School of Medicine, and the human sequences were replaced with the marmoset ones by means of the BLAST output. After excluding repeated and multiple sequences, 19,117 human orthologous marmoset sequences were obtained. Those sequences were combined with cDNA sequences used for the design of the Marmo1 microarray [12] and newly cloned cDNAs from marmoset ES cells to make a total of 22,971 marmoset sequences, and were used for designing Affymetrix GeneChip probe sets. The microarray was constructed according to the GeneChip® CustomExpress® Array Program (Affymetrix, Santa Clara, CA, USA).

2.4. High-density oligonucleotide microarray analysis

Total RNA samples were prepared using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions, and concentrations were measured using the Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). rRNA integrity was assessed using a 2100 Bioanalyzer (Agilent). For microarray probing, reverse transcription, second-strand synthesis, and probe generation were all accomplished with the 3′IVT Express Kit (Affymetrix) following the manufacturer's protocol. In brief, from 100 ng of total RNA, the double-strand cDNA was synthesized. From the double-strand cDNA, biotin-labeled cRNA was prepared and 15 μg of labeled cRNA was fragmented. The Marmo2a520631F

Table 2
Characteristics of the animals used in the present study.

Age	Animal ID	Postnatal day	Sex	Body weight (g)
0 D	MA17	0	Male	28.9
	MA18	1	Female	32.0
	MA21	0	Female	24.0
	MA22	4	Male	24.5
2 M	MA14	61	Female	108.8
	MA15	61	Male	120.7
	MA07	68	Female	96.2
3 M	MA08	91	Male	103.0
	MA10	95	Female	142.7
	MA11	95	Female	155.7
6 M	MA09	184	Female	173.3
	MA12	195	Male	204.5
	MA13	208	Male	188.3
Adult	MA03	1032	Male	298.5
	MA01	1746	Male	332.6
	MA16	3906	Male	370.2

Note: 0 D, 0 days of age; 2 M, 2 months of age; 3 M, 3 months of age; 6 M, 6 months of age.

arrays were hybridized as described in the Gene Chip Expression Analysis Technical Manual (Affymetrix). The arrays were stained and washed using the GeneChip Hybridization, Wash, and Stain Kit (Affymetrix), while fluorescence was intensified using the antibody amplification method following the manufacturer's protocol. The arrays were scanned with a GeneChip Scanner 3000 (Affymetrix) and the obtained images were analyzed using Affymetrix microarray suite version 5 software (MAS 5). We then obtained tab-delimited files containing data on the relative levels of expression of transcripts (Signal) and the reliability of detection (Detection Call). The derived signal values were globally normalized and the control for all probes was set to 100.

2.5. Microarray data analysis

To examine genes specifically expressed in different cortical regions, average signal values of three replicates for each region were calculated. The gene expression data were analyzed using GeneSpring GX 11 software (Agilent). The signal intensity data were preprocessed using the RMA algorithm [13] and normalized using Affymetrix microarray suite version 5 software (MAS5). To improve the false discovery rate when gene probe sets were turned on or off, we restricted probe sets ($\geqslant$50% present), as described by McClintick and Edenberg [14]. Differentially expressed genes among the 3 areas and 5 time points were analyzed using 2-way analysis of variance (2-way ANOVA; p value computation: asymptotic; multiple testing correction: Benjamini and Hochberg false discovery rate; cutoff p value: 1%). In 2-way ANOVA, a main effect for gene expression of each gene was either only age or only brain region. The data showing a main effect of only age were analyzed using 1-way ANOVA with a *post hoc* multiple comparison test (Tukey's HSD method) to find genes differentially expressed between 3 M and 6 M in all brain regions examined. To avoid false positive results if gene distribution was potentially asymmetrical, we used the following approach. If differentially expressed genes were further filtered so that the absolute value of FC of gene expression between 3 M and 6 M is greater than 1.2, this method efficiently damps true gene-regulatory events in order to minimize their misleading effect on the normalization process. FC was calculated as follows: when gene expression at 3 M was greater than that at 6 M, the former was divided by the latter. When gene expression at 6 M was greater than that at 3 M, FC was calculated by dividing the former by the latter and was expressed as a negative value (−). Thus, negative FC values indicate that gene expression increased from 3 M to 6 M. Information on the selected genes is presented in Supplemental Table 1. To understand the biological implications of certain expression patterns, these data were analyzed using software IPA (Qiagen), which uses curated information gathered from scientific literature. We further confirmed the results of IPA by verifying original research articles.

3. Results and discussion

Based on the above criteria, 571 genes were expressed more strongly at 3 M than at 6 M and 1006 genes were expressed less strongly at 3 M than at 6 M (Supplemental Table 1) in all 3 brain regions examined.

3.1. Axon guidance signaling

In the canonical pathway analysis of IPA using the list above, axonal guidance signaling (Supplemental Table 2, Table 1) showed the lowest p value. Thus, axonal guidance signaling was the most plausible pathway based on genes differentially expressed between 3 M and 6 M. Although the list of canonical pathways (Supplemental Table 2) according to our data included axon guidance-related signaling (Eph, CXCR4, IGF-1, and Wnt/β-catenin signaling pathways), these pathways are components of the same system of axon guidance signaling. Thus, in this study, the genes that we discuss also include these subsystems as participants in axon guidance signaling. Table 3 shows that expression of genes belonging to axon guidance signaling changes from 3 M to 6 M so that synapse/dendritic spine number would decrease at 6 M (Table 3, [15–28]), although FC is, on an average, rather small. These results are suggestive of some role of axon guidance signaling in pruning approximately at 6 M.

3.2. Immune system

Microphages (part of microglia in the brain) and proteins that tag substrates in synapses to be eliminated by the immune system (e.g., complement components) play an important role in synaptic pruning [10]. Our canonical pathway analysis, as mentioned above, showed that the macrophage system and target-tagging components of the immune system were the pathways that were most likely to undergo changes between 3 M and 6 M (macrophage system, hepatic fibrosis/hepatic stellate cell activation as the 3rd most plausible pathway; dendritic cell maturation pathway as the 84th plausible pathway; target-tagging components of the immune system/complement system as the 8th most plausible pathway). In

Table 3
How different synapse number-related genes belonging to axon guidance signaling were expressed between 3 and 6 months, and expected effects of different expression of these genes on the number of synapses/dendritic spines.

Pathway within axon guidance signaling	Possible synapse number-related genes in a subpathway	Fold change (6 M/3 M)	Expected effect of gene expression change on synapse/dendritic spine number at 6 M compared to 3 M	Related references
Ephrin signaling	ROCK2	1.27	Decrease	[15]
	RAC3	1.21	Decrease	[16]
	EPHB2	1.47	Decrease	[17]
	LIMK2	1.20	Decrease	[18]
	RASA1	−1.24	Decrease	[19]
SHANK2 signaling	RAPGEF7	1.21	Decrease	[20]
IGF-1 signaling	CDC42	1.34	Decrease	[21]
Tubulin–KLC–CRMP2 signaling	TUBA1C	1.21	Decrease	[22]
	TUBB3	1.21	Decrease	[22]
	TUBB2A/TUBB2B	1.43	Decrease	[22]
	KLC1	1.23	Decrease	[22]
CXCR4 signaling	GNAZ	1.20	Decrease	[23]
Integrin signaling	RASSF5	−1.32	Decrease	[24]
Netrin/DCC signaling	MKNK1	1.22	Decrease	[25]
Netrin/UNC/DCC signaling	UNC5C	−1.25	Decrease	[26]
NGF receptor signaling	PRKCG	1.30	Decrease	[27]
Wnt5a/FZD signaling	PRKCG	1.30	Decrease	[27]
	NFATC3	1.23	Decrease	[28]

this section, we discuss the macrophage system and the target-tagging components of the immune system based on results of the canonical pathway analysis, which were confirmed in the brain only via the synthesis of the data published in scientific literature.

3.2.1. Microglia (macrophage) system

3.2.1.1. AIF1. Expression of *AIF1* [29], which is a microglia-specific gene that reflects the activity of this type of cells [30], decreased at 6 M (Table 1).

3.2.1.2. Microglia-modulating genes. Activity, motility, and release of cytokines by microglia are controlled by many molecules [10]. Within the list of the genes differentially expressed between 3 M and 6 M (Table 1), *CX3CR1*, *CX3CR1*, *TNFRSF19*, *PDGFRA*, *LEP*, *LEPR*, *IGFBP3*, *IGFBP4 TLR4*, *EDNRB*, *TIMP2*, *COL1A2*, *FN1*, *IRF8*, *PDGFRA*, and *TREM2* were downregulated at 6 M (FC: 1.22, 1.33, 1.26, 1.31, 1.32, 1.36, 2.00, 1.34, 1.26, 1.37, 1.49, 1.92, 1.32 1.37, 1.31, and 1.24, respectively). *TGRBR2*, which is an inhibitor of microglial activity, was upregulated (FC: −1.23) at 6 M. Downregulation or upregulation of the above genes is known to decrease activity, motility, and the release of cytokines by microglia [31–43], probably what happened at 6 M.

3.2.2. Target-tagging components of the immune system

3.2.2.1. Complement system. A complement protein, C3b participates in opsonization (enhances phagocytosis of antigens by microglia in the brain) [44]. C3-convertase promotes cleavage of C3 into C3a and C3b. *C1QB*, *C1QC*, and *CFB*, which are involved in the synthesis of C3 convertase [44], were upregulated relatively strongly (FC: −1.52, −2.24, and −2.05 respectively) at 6 M.

3.2.2.2. Other target-tagging components of the immune system. Both *HLA-A and FCGR1B*, signals for the elimination of synapses by microglia [45–47], were upregulated (FC: −1.28 and −1.57, respectively) at 6 M.

There appeared to be 2 tendencies of gene expression related to the immune system between 3 M and 6 M. (1) In general, microglial activity decreased at 6 M. This result suggests that although microglial activity is thought to be related to phagocytosis of synapses and dendritic spines, microglial activity alone could not explain how pruning was accelerated in the late pruning phase. (2) Genes (e.g., complement components) that are related to tagging of targets of microglial synapse pruning tended to be upregulated relatively strongly at 6 M. These data suggest that those genes performed important functions in implementation of the pruning phase in marmosets.

4. Conclusions

In summary, the axon guidance signaling pathway and immune system (a combination of microglial and complement components) probably contributes to the decrease in the number of synapses/spines by 6 M and may explain the *overshoot-type* synaptic formation in primates. Further experiments are required to elucidate how much these genes and subsystems contribute to the decrease in the spine number between postnatal 3 M and 6 M.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.01.024.

References

[1] G.N. Elston, T. Oga, I. Fujita, Spinogenesis and pruning scales across functional hierarchies, J. Neurosci. 29 (2009) 3271–3275.
[2] G.N. Elston, T. Oga, T. Okamoto, I. Fujita, Spinogenesis and pruning from early visual onset to adulthood: an intracellular injection study of layer III pyramidal cells in the ventral visual cortical pathway of the macaque monkey, Cereb. Cortex 20 (2010) 1398–1408.
[3] P.R. Huttenlocher, Synaptic density in human frontal cortex-developmental changes and effects of aging, Brain Res. 163 (1979) 195–205.
[4] M. Missler, A. Wolff, H.J. Merker, J.R. Wolff, Pre- and postnatal development of the primary visual cortex of the common marmoset. II. Formation, remodeling, and elimination of synapses as overlapping processes, J. Comp. Neurol. 333 (1993) 53–67.

[5] Z. Petanjek, M. Judaš, G. Šimic, et al., Extraordinary neoteny of synaptic spines in the human prefrontal cortex, Proc. Natl. Acad. Sci. USA 108 (2011) 13281–13286.
[6] P. Penzes, M.E. Cahill, K.A. Jones, et al., Dendritic spine pathology in neuropsychiatric disorders, Nat. Neurosci. 14 (2011) 285–293.
[7] T. Oga, H. Aoi, T. Sasaki, I. Fujita, N. Ichinohe, Postnatal development of layer III pyramidal cells in the primary visual, inferior temporal, and prefrontal cortices of the marmoset, Front. Neural Circuits 7 (2013) 31.
[8] G.J. Chellman, J.L. Bussiere, N. Makori, et al., Developmental and reproductive toxicology studies in nonhuman primates, Birth Defects Res. B 86 (2009) 446–462.
[9] E. Sasaki, H. Suemizu, A. Shimada, et al., Generation of transgenic non-human primates with germline transmission, Nature 459 (2009) 523–527.
[10] A. Miyamoto, H. Wake, A.J. Moorhouse, J. Nabekura, Microglia and synapse interactions: fine tuning neural circuits and candidate molecules, Front. Cell. Neurosci. 7 (2013) 70.
[11] Committee for the Update of the Guide for the Care and Use of Laboratory Animals, Guide for the Care and Use of Laboratory Animals, Eighth ed., Washington: *National Academic Press* 2011.
[12] T. Fukuoka, K. Sumida, T. Yamada, et al., Gene expression profiles in the common marmoset brain determined using a newly developed common marmoset-specific DNA microarray, Neurosci. Res. 66 (2010) 62–85.
[13] R.A. Irizarry, B. Hobbs, F. Collin, et al., Exploration, normalization, and summaries of high density oligonucleotide array probe level data, Biostatistics 4 (2003) 249–264.
[14] J.N. McClintick, H.J. Edenberg, Effects of filtering by present call on analysis of microarray experiments, BMC Bioinformatics 7 (2006) 49.
[15] Z. Zhou, Y. Meng, S. Asrar, et al., A critical role of Rho-kinase ROCK2 in the regulation of spine and synaptic function, Neuropharmacology 56 (2009) 81–89.
[16] S. Corbetta, S. Gualdoni, G. Ciceri, et al., Essential role of Rac1 and Rac3 GTPases in neuronal development, FASEB J. 23 (2009) 1347–1357.
[17] M. Henkemeyer, O.S. Itkis, M. Ngo, et al., Multiple EphB receptor tyrosine kinases shape dendritic spines in the hippocampus, J. Cell Biol. 163 (2003) 1313–1326.
[18] Y. Meng, Y. Zhang, V. Tregoubov, et al., Regulation of spine morphology and synaptic function by LIMK and the actin cytoskeleton, Rev. Neurosci. 14 (2003) 233–240.
[19] M. Soltau, K. Berhörster, S. Kindler, et al., Insulin receptor substrate of 53 kDa links postsynaptic shank to PSD-95, J. Neurochem. 90 (2004) 659–665.
[20] E. Park, M. Na, J. Choi, et al., The Shank family of postsynaptic density proteins interacts with and promotes synaptic accumulation of the beta PIX guanine nucleotide exchange factor for Rac1 and Cdc42, J. Biol. Chem. 278 (2003) 19220–19229.
[21] C.M. Cheng, R.F. Mervis, S.L. Niu, et al., Insulin-like growth factor 1 is essential for normal dendritic growth, J. Neurosci. Res. 73 (2003) 1–9.
[22] T. Kimura, H. Watanabe, A. Iwamatsu, K. Kaibuchi, Tubulin and CRMP-2 complex is transported via Kinesin-1, J. Neurochem. 93 (2005) 1371–1382.
[23] P. Sánchez-Blázquez, M. Rodríguez-Muñoz, E. de la Torre-Madrid, J. Garzón, Brain-specific Galphaz interacts with Src tyrosine kinase to regulate Mu-opioid receptor-NMDAR signaling pathway, Cell. Signal. 21 (2009) 1444–1454.
[24] J.M. Cantor, M.H. Ginsberg, D.M. Rose, Integrin-associated proteins as potential therapeutic targets, Immunol. Rev. 223 (2008) 236–251.
[25] B. Nicke, J. Bastien, S.J. Khanna, et al., Involvement of MINK, a Ste20 family kinase, in Ras oncogene-induced growth arrest in human ovarian surface epithelial cells, Mol. Cell 20 (2005) 673–685.
[26] E.D. Leonardo, L. Hinck, M. Masu, et al., Vertebrate homologues of *C. elegans* UNC-5 are candidate netrin receptors, Nature 386 (1997) 833–838.
[27] M. Moriya, S. Tanaka, Prominent expression of protein kinase C (gamma) mRNA in the dendrite-rich neuropil of mice cerebellum at the critical period for synaptogenesis, NeuroReport 5 (1994) 929–932.
[28] A. Freeman, A. Franciscovich, M. Bowers, et al., NFAT regulates pre-synaptic development and activity-dependent plasticity in *Drosophila*, Mol. Cell. Neurosci. 46 (2011) 535–547.
[29] M.V. Autieri, CDNA cloning of human allograft inflammatory factor-1: tissue distribution, cytokine induction, and mRNA expression in injured rat carotid arteries, Biochem. Biophys. Res. Commun. 228 (1996) 29–37.
[30] G. Liu, H. Ma, L. Jiang, Y. Zhao, Allograft inflammatory factor-1 and its immune regulation, Autoimmunity 40 (2007) 95–102.
[31] H. Wake, A.J. Moorhouse, A. Miyamoto, J. Nabekura, Microglia: actively surveying and shaping neuronal circuit structure and function, Trends Neurosci. 36 (2013) 209–217.
[32] J.T. Rogers, J.M. Morganti, A.D. Bachstetter, C.E. Hudson, M.M. Peters, B.A. Grimmig, E.J. Weeber, P.C. Bickford, C. Gemma, CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity, J. Neurosci. 31 (2011) 16241–16250.
[33] V.L. Jacobs, Y. Liu, J.A. De Leo, Propentofylline targets TROY, a novel microglial signaling pathway, PLoS One 7 (2012) e37955.
[34] J. Masuda, M. Tsuda, H. Tozaki-Saitoh, K. Inoue, Intrathecal delivery of PDGF produces tactile allodynia through its receptors in spinal microglia, Mol. Pain 5 (2009) 23.
[35] C.M. Fernández-Martos, P. González, F.J. Rodriguez, Acute leptin treatment enhances functional recovery after spinal cord injury, PLoS One 7 (4) (2012) e35594.
[36] D. Chesik, K. Glazenburg, N. Wilczak, F. Geeraedts, J. De Keyser, Insulin-like growth factor binding protein-1-6 expression in activated microglia, NeuroReport 15 (2004) 1033–1037.
[37] J. Zhang, N. Niu, M. Wang, M.A. McNutt, et al., Neuron-derived IgG protects dopaminergic neurons from insult by 6-OHDA and activates microglia through the FcγR I and TLR4 pathways, Int. J. Biochem. Cell Biol. 45 (2013) 1911–1920.
[38] M. Tonari, T. Kurimoto, T. Horie, T. Sugiyama, T. Ikeda, H. Oku, Blocking endothelin-B receptors rescues retinal ganglion cells from optic nerve injury through suppression of neuroinflammation, Invest. Ophthalmol. Vis. Sci. 53 (2012) 3490–3500.
[39] S. Soleman, M. Filippov, A. Dityatev, J. Fawcett, Targeting the neural extracellular matrix in neurological disorders, Neuroscience 253 (2013) 194–213.
[40] M.C. Seo, S. Kim, S.H. Kim, et al., Discoidin domain receptor 1 mediates collagen-induced inflammatory activation of microglia in culture, J. Neurosci. Res. 86 (2008) 1087–1095.
[41] M. Tsuda, T. Masuda, H. Tozaki-Saitoh, K. Inoue, P2X4 receptors and neuropathic pain, Front. Cell. Neurosci. 7 (2013) 191.
[42] M. Chertoff, K. Shrivastava, B. Gonzalez, L. Acarin, L. Giménez-Llort, Differential modulation of TREM2 protein during postnatal brain development in mice, PLoS One 8 (2013) e72083.
[43] M. Böttner, K. Krieglstein, K. Unsicker, The transforming growth factor-betas: structure, signaling, and roles in nervous system development and functions, J. Neurochem. 75 (2000) 2227–2240.
[44] C.A. Janeway Jr, P. Travers, M. Walport, et al. (Eds.), The Complement System and Innate Immunity, In Immunobiology: The Immune System in Health and Disease, fifth ed., Garland Science, New York, 2001.
[45] G.S. Huh, L.M. Boulanger, H. Du, P.A. Riquelme, T.M. Brotz, C.J. Shatz, Functional requirement for class1 MHC in CNS development and plasticity, Science 290 (2000) 2155–2159.
[46] M.D. Hulett, P.M. Hogarth, The second and third extracellular domains of FcgammaRI (CD64) confer the unique high affinity binding of IgG2a, Mol. Immunol. 35 (14–15) (1998) 989–996.
[47] Y. Quan, T. Möller, J.R. Weinstein, Regulation of Fcgamma receptors and immunoglobulin G-mediated phagocytosis in mouse microglia, Neurosci. Lett. 464 (1) (2009) 29–33.