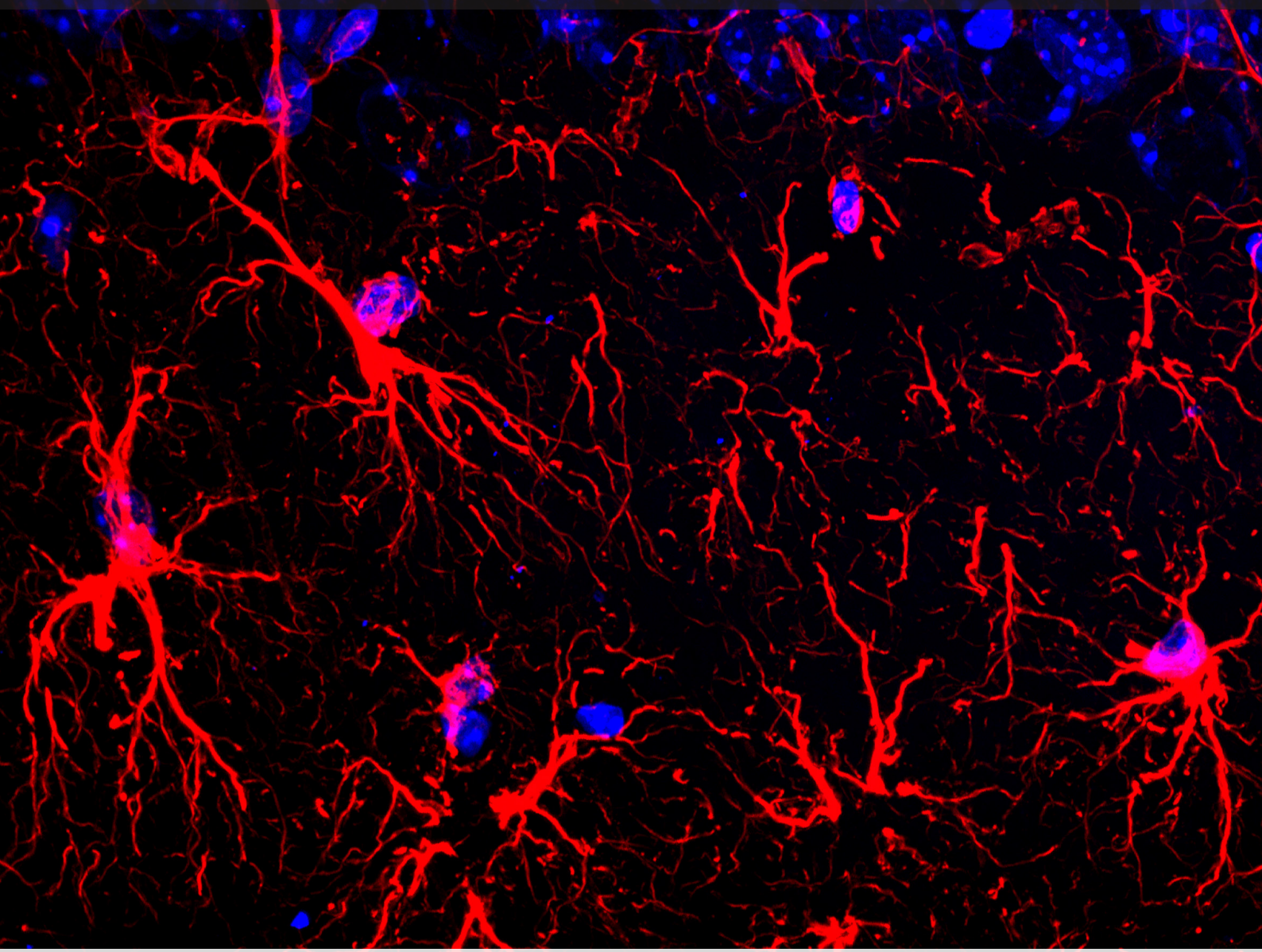
GLIAL PLASTICITY IN DEPRESSION

EDITED BY: João F. Oliveira, Catarina A. Gomes, Sandra H. Vaz, Nuno Sousa and Luisa Pinto

PUBLISHED IN: Frontiers in Cellular Neuroscience



frontiers Research Topics

frontiers

Frontiers Copyright Statement

© Copyright 2007-2016 Frontiers Media SA. All rights reserved.

All content included on this site, such as text, graphics, logos, button icons, images, video/audio clips, downloads, data compilations and software, is the property of or is licensed to Frontiers Media SA ("Frontiers") or its licensees and/or subcontractors. The copyright in the text of individual articles is the property of their respective authors, subject to a license granted to Frontiers.

The compilation of articles constituting this e-book, wherever published, as well as the compilation of all other content on this site, is the exclusive property of Frontiers. For the conditions for downloading and copying of e-books from Frontiers' website, please see the Terms for Website Use. If purchasing Frontiers e-books from other websites or sources, the conditions of the website concerned apply.

Images and graphics not forming part of user-contributed materials may not be downloaded or copied without permission.

Individual articles may be downloaded and reproduced in accordance with the principles of the CC-BY licence subject to any copyright or other notices. They may not be re-sold as an e-book.

As author or other contributor you grant a CC-BY licence to others to reproduce your articles, including any graphics and third-party materials supplied by you, in accordance with the Conditions for Website Use and subject to any copyright notices which you include in connection with your articles and materials.

All copyright, and all rights therein, are protected by national and international copyright laws.

The above represents a summary only. For the full conditions see the Conditions for Authors and the Conditions for Website Use.

ISSN 1664-8714
ISBN 978-2-88919-999-0
DOI 10.3389/978-2-88919-999-0

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.

Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.

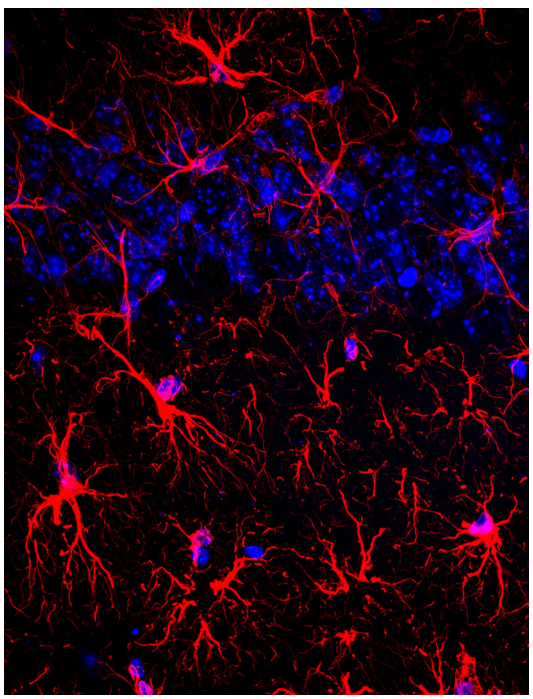
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: **researchtopics@frontiersin.org**

GLIAL PLASTICITY IN DEPRESSION

Topic Editors:
João F. Oliveira, University of Minho & Life and Health Sciences Research Institute/3B's—PT Government Associate Laboratory & Polytechnic Institute of Cávado and Ave, Portugal
Catarina A. Gomes, University of Coimbra, Portugal
Sandra H. Vaz, Universidade de Lisboa, Portugal
Nuno Sousa, University of Minho & Life and Health Sciences Research Institute/3B's—PT Government Associate Laboratory, Portugal
Luisa Pinto, University of Minho & Life and Health Sciences Research Institute/3B's—PT Government Associate Laboratory, Portugal



Astrocytes in the CA1 sub-region of the dorsal hippocampus of C57Bl6 mice. The astrocytic marker glial fibrillary acidic protein (GFAP) is stained in red and the nuclei are stained in blue (DAPI). Image by Oliveira, João Filipe et al.

Major depression is a highly prevalent disorder that poses a significant social burden in society nowadays. The pathophysiology of this disease is still poorly understood but growing evidence suggests that impaired neuron and glial plasticity may be a key underlying mechanism for the precipitation of the disorder.

One of the most surprising findings in this field was the involvement of glial cells in the pathophysiology of major depression and in the action of antidepressants, namely in mechanisms related with adult neurogenesis imbalances or dendritic arborization impairments. In particular, several works refer to alterations in the morphology and numbers of astrocytes, microglia and oligodendrocytes in the context of depression in human patients or animal models of depression. These observations were linked to functional evidences and suggested to underlie the pathophysiology of depression. Among others, these include impairments in the cross-talk between glia and neurons, changes in the level of neurotransmitter or immunoactive substances, myelination status, synapse formation, maintenance, or elimination.

In addition to the implication of glia in the pathophysiology of depression, a number of studies is ascribing glia pathways to classically accepted antidepressant mechanisms. Therefore, it is noteworthy to elucidate the role of glia in the effect provided by antidepressant treatment in order to better understand secondary effects and elucidate alternative targets for treatment.

Citation: Oliveira, J. F., Gomes, C. A., Vaz, S. H., Sousa, N., Pinto, L., eds. (2016). Glial Plasticity in Depression. Lausanne: Frontiers Media. doi: 10.3389/978-2-88919-999-0

Table of Contents

frontiers in Cellular Neuroscience

EDITORIAL
published: 17 June 2016
doi: 10.3389/fncel.2016.00163

Editorial: Glial Plasticity in Depression

João F. Oliveira[1,2,3], Catarina A. Gomes[4,5], Sandra H. Vaz[6,7], Nuno Sousa[1,2] and Luisa Pinto[1,2]*

[1] Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga, Portugal, [2] Life and Health Sciences Research Institute/3B's—PT Government Associate Laboratory, Braga/Guimarães, Portugal, [3] DIGARC, Polytechnic Institute of Cávado and Ave, Barcelos, Portugal, [4] Faculty of Medicine, Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, [5] Institute for Biomedical Imaging and Life Sciences and Faculty of Medicine, University of Coimbra, Coimbra, Portugal, [6] Faculdade de Medicina, Instituto de Medicina Molecular, Universidade de Lisboa, Lisboa, Portugal, [7] Faculdade de Medicina, Instituto de Farmacologia e Neurociências, Universidade de Lisboa, Lisboa, Portugal

Keywords: glia, depression, astrocyte, microglia, oligodendrocites

The Editorial on the Research Topic

Glial Plasticity in Depression

Depression is a highly prevalent disorder that poses a significant social burden to society. Despite continued advances toward the understanding of the pathophysiology of this disease, its molecular/cellular underpinnings remain elusive, which may be at the basis of the lack of effective treatment strategies. Among the different lines of research, recent literature suggests that impaired neuron and glial plasticity may be a key underlying mechanism in the precipitation of the disorder. Surprisingly, glial cells appear to be involved both in the pathophysiology of major depression and in the action of antidepressants. In particular, several works refer to alterations in the morphology and numbers of astrocytes, microglia, and oligodendrocytes in the context of depression, in human patients, and animal models of depression. These observations are linked to functional evidences, such as impairments in the cross-talk between glia and neurons, changes in the level of neurotransmitter or immunoactive substances, myelination status, and synapse formation, maintenance or elimination.

This Research Topic highlights the roles played by neurons, astrocytes, and microglia in depressive disorder(s). Polyakova et al. begin by suggesting the astrocytic S100B as a novel marker of minor depression, specifically in males, which could help to understand its pathophysiology. The study point outs the possible relevance of glial cells in the modulation of brain neuron-glia networks at least in some types of depression. Rial et al. explore the multiple interactions between glial and neuronal cells at the synaptic level, which may be impaired in depressive-like conditions. The authors address how purines may be used to restore synaptic efficacy by modulating glia-neuron bidirectional communication, possibly reverting depressive-like behaviors. Jo et al. discuss the importance of the glial-mediated immune modulation through cytokine signaling that may trigger depressive episodes, and Branchi et al. highlight that by participating actively in the modulation of the extracellular environment microglia are an integral part of brain plasticity and, therefore, appear to account directly for the precipitation of the depressive disorder. Brites and Fernandes point out that the disruption of secreted extracellular vesicles, an alternative form of neuro-glial signaling, may also underlie depressive behavior. Collectively the research indicates that these novel forms of neuro-glia communication may represent a novel approach for modulation of the brain networks and, thus, its manipulation may allow the development of autologous therapies for depression.

OPEN ACCESS

Edited and reviewed by:
Christian Hansel,
University of Chicago, USA

****Correspondence:***
João F. Oliveira
joaooliveira@ecsaude.uminho.pt

Received: *19 May 2016*
Accepted: *02 June 2016*
Published: *17 June 2016*

Citation:
Oliveira JF, Gomes CA, Vaz SH, Sousa N and Pinto L (2016) Editorial: Glial Plasticity in Depression. Front. Cell. Neurosci. 10:163. doi: 10.3389/fncel.2016.00163

In addition to the implication of glia in the pathophysiology (and treatment) of depression, a number of studies associates glia-related pathways to classically accepted antidepressant mechanisms. Specifically, in this Research Topic, Di Benedetto et al. suggest a link between fluoxetine modulation of aquaporin four levels in astrocytes with consequences for astrocyte morphology and re-establishment of a functional glia-vasculature interface, which may underlie its antidepressant effect. Interestingly, the decreased levels of astrocyte-specific connexin 43 (Cx43) appear to be related with antidepressant and anti-anxiolytic phenotypes as suggested by Quesseveur et al. Moreover, the authors suggest that the inactivation of Cx43 might induce beneficial effects through an attenuation of the stress response, "avoiding" depressive symptoms. In accordance, Jeanson et al. explored the effect of antidepressants on the functional status of astrocytic connexins, showing a complex pattern of responses that links astrocytes to the mode of action of these drugs. Exploring the use of deep brain stimulation (DBS) as an alternative for treatment-resistant depression patients, Etiévant et al. discuss the beneficial role of astrocytes in the process based on the absence of a DBS-effect after pharmacological lesion of astrocytes.

Overall, the excellent reflections and novel data sets that make up this Research Topic provide evidence for a role of glial cells, namely microglia and astrocytes, in the mechanisms underlying depression and the effect of antidepressants. Collectively, the data indicates that despite the rapid advancement of the field there is still a long way to go. The study of glial cells will continue to reveal novel and more effective therapeutic mechanisms for depression and its symptomatology.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

JO and LP received fellowships from the Foundation for Science and Technology (FCT) and their work is funded by FCT (SFRH/BD/101298/2014 to JO and IF/01079/2014 to LP) and Bial Foundation (207/14 for JO and 427/14 for LP) projects. SV is supported by FCT (SFRH/BPD/81627/2011). CG is supported by FCT (SFRH/BPD/63013/2009). This work was co-funded by the Life and Health Sciences Research Institute (ICVS), and Northern Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER) (projects NORTE-01-0145-FEDER-000013 and NORTE-01-0145-FEDER-000023). This work has been also funded by FEDER funds, through the Competitiveness Factors Operational Programme (COMPETE), and by National funds, through the (FCT), under the scope of the project POCI-01-0145-FEDER-007038.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Oliveira, Gomes, Vaz, Sousa and Pinto. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Cellular Neuroscience

ORIGINAL RESEARCH
published: 09 October 2015
doi: 10.3389/fncel.2015.00406

First evidence for glial pathology in late life minor depression: S100B is increased in males with minor depression

Maryna Polyakova[1,2,3], Christian Sander[2,3], Katrin Arelin[1,3], Leonie Lampe[1,3], Tobias Luck[3,4], Melanie Luppa[3,4], Jürgen Kratzsch[3,5], Karl-Titus Hoffmann[6], Steffi Riedel-Heller[3,4], Arno Villringer[1,3,7], Peter Schoenknecht[2,3] and Matthias L. Schroeter[1,3,7]*

[1] Department of Neurology, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany, [2] University Clinic for Psychiatry and Psychotherapy, Leipzig University, Leipzig, Germany, [3] LIFE—Leipzig Rsearch Center for Civilization Diseases, Leipzig University, Leipzig, Germany, [4] Institute of Social Medicine, Occupational Health and Public Health (ISAP), Leipzig University, Leipzig, Germany, [5] Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, Leipzig University, Leipzig, Germany, [6] Department of Neuroradiology, Leipzig University, Leipzig, Germany, [7] Clinic for Cognitive Neurology, University of Leipzig, Leipzig, Germany

Minor depression is diagnosed when a patient suffers from 2 to 4 depressive symptoms for at least 2 weeks. Though minor depression is a widespread phenomenon, its pathophysiology has hardly been studied. To get a first insight into the pathophysiological mechanisms underlying this disorder we assessed serum levels of biomarkers for plasticity, glial and neuronal function: brain-derived neurotrophic factor (BDNF), S100B and neuron specific enolase (NSE). 27 subjects with minor depressive episode and 82 healthy subjects over 60 years of age were selected from the database of the Leipzig population-based study of civilization diseases (LIFE). Serum levels of BDNF, S100B and NSE were compared between groups, and correlated with age, body-mass index (BMI), and degree of white matter hyperintensities (score on Fazekas scale). S100B was significantly increased in males with minor depression in comparison to healthy males, whereas other biomarkers did not differ between groups ($p = 0.10$–0.66). NSE correlated with Fazekas score in patients with minor depression ($r_S = 0.436$, $p = 0.048$) and in the whole sample ($r_S = 0.252$, $p = 0.019$). S100B correlated with BMI ($r_S = 0.246$, $p = 0.031$) and with age in healthy subjects ($r_S = 0.345$, $p = 0.002$). Increased S100B in males with minor depression, without alterations in BDNF and NSE, supports the glial hypothesis of depression. Correlation between white matter hyperintensities and NSE underscores the vascular hypothesis of late life depression.

Keywords: minor depression, late life depression, S100B, BDNF, NSE, glia, white matter hyperintensities, biomarker

OPEN ACCESS

Edited by:
Luisa Pinto,
University of Minho, Portugal

Reviewed by:
Hermona Soreq,
The Hebrew University of Jerusalem, Israel
Eva Benito,
Deutsches Zentrum Für Neurodegenerative Erkrankungen, Germany

****Correspondence:***
Maryna Polyakova
polyakova@cbs.mpg.de

Received: *31 July 2015*
Accepted: *25 September 2015*
Published: *09 October 2015*

Citation:
Polyakova M, Sander C, Arelin K, Lampe L, Luck T, Luppa M, Kratzsch J, Hoffmann K-T, Riedel-Heller S, Villringer A, Schoenknecht P and Schroeter ML (2015) First evidence for glial pathology in late life minor depression: S100B is increased in males with minor depression. Front. Cell. Neurosci. 9:406. doi: 10.3389/fncel.2015.00406

INTRODUCTION

Minor depression is a widespread phenomenon in late life (Hegerl and Schoenknecht, 2009; Polyakova et al., 2015). According to the fourth edition of the diagnostic statistical manual of mental disorders (DSM-IV) a person suffering from two to four depressive symptoms for at least 2 weeks has a minor depressive episode. For diagnosis of minor depressive disorder one additionally has

to exclude a history of major depression (American Psychiatric Association, 2000). In clinical practice patients with minor depressive symptoms may represent an independent minor depressive episode or a subsyndromal stage of major depression (Park et al., 2010). Every fourth patient with minor depression develops major depression within 2 years after diagnosis (Lyness et al., 1999) and 13% of subjects with minor depression have attempted suicide at least once (Eaton et al., 1995). With regard to these data proper diagnosis and management of minor depression might become an approach to prevent a more severe depressive disorder.

Although plenty of studies have been conducted to elucidate the etiology of major depression, the pathophysiology of minor depression is still unknown. Possible research directions include the glial, neurotrophic and vascular hypotheses of depression. Alterations of peripheral biomarkers of brain structure and function might shed light on the pathological changes in central mechanisms. Brain derived neurotrophic factor (BDNF), S100B and neuron specific enolase (NSE) are among the most studied biomarkers in affective disorders, in particular major depressive disorder (Schroeter et al., 2002; Hetzel et al., 2005; Andreazza et al., 2007; Schroeter and Steiner, 2009; Kalia and Silva, 2015).

BDNF, associated with plasticity in the central and peripheral nervous system, is decreased in serum in acute major depressive episodes and restored in remission (Molendijk et al., 2014). The glial marker protein S100B is elevated during major depressive episodes and decreased following successful treatment (Schroeter et al., 2013). Thus, fluctuations in serum levels of BDNF and S100B seem to be state markers for major depression. This is supported by powerful meta-analyses including a very high number of subjects (Schroeter et al., 2008; Polyakova et al., 2015). NSE is a marker for neuronal injury. In contrast to BDNF and S100B, serum NSE levels seem to be stable in depression suggesting mainly glial dysfunction (Schroeter et al., 2013). However, a recent publication reported increased NSE levels in cerebrospinal fluid (Schmidt et al., 2015), leaving more space for discussion.

Due to clinical similarities with major depression, we expect similar biomarker changes in minor depression. Since BDNF levels do not correlate with depression severity (Molendijk et al., 2011), decreased serum BDNF might also be observed in minor depression. In this disorder it might reflect impaired constitutive or activity-dependent BDNF expression, resulting in impaired brain plasticity. Increased S100B in minor depression may indicate early glial pathology that precedes specific neuronal changes such as in major depression (Rajkowska, 2000). Unaltered (comparing to healthy controls) NSE should confirm that there is no neuronal damage in minor depression.

To further explore the etiology of minor depression we analyzed serum levels of BDNF, S100B and NSE in subjects with minor depression and healthy control subjects from the Leipzig population-based study of civilization diseases (LIFE). Serum levels of BDNF, S100B and NSE were considered as primary outcomes. In analogy to major depression we hypothesized a decrease of BDNF and an increase of S100B, without changes of NSE in minor depression.

An association between late life minor depression and the vascular hypothesis of depression (Taylor et al., 2013) was investigated in explorative analyses by correlating white matter hyperintensities to serum markers. In order to control for confounding variables we correlated age and body mass index (BMI) with serum markers. Correlation of serum markers with clinical and imaging parameters, such as age, BMI and extent of white matter hyperintensities were considered as secondary outcomes.

MATERIALS AND METHODS

Participants

Twenty seven subjects 60 years and older satisfying the DSM-IV criteria for minor depressive episode and eighty two healthy control subjects were selected from the LIFE study. LIFE study includes a representative sample from the Leipzig population (Loeffler et al., 2015). All of the participants provided their written informed consent in accordance with the Declaration of Helsinki before participation in the study. The study was approved by the ethics board of the Medical Faculty of the University of Leipzig. At the moment of subjects' selection the LIFE study database included 1617 subjects over 60 years of age. Every subject underwent structured psychiatric interview, neuropsychological testing, clinical examination, blood sampling and scanning with multimodal magnetic resonance imaging (MRI).

Diagnostic Criteria and Laboratory Procedures

Minor depressive episode according to DSM-IV criteria was diagnosed based on the structured clinical interview for DSM-IV axis I disorders (SCID). White matter hyperintensities were rated by experienced neuroradiologists using the Fazekas scale (Fazekas et al., 1987) in fast fluid-attenuated inversion recovery (FLAIR) images.

Blood samples were collected from the subject's cubital vein at the first day of the study. The mean time between blood sampling and psychiatric interview was 10.0 (4.3) days for subjects with minor depression and 13.0 (9.0) for healthy subjects. All samples were collected uniformly in the morning, following overnight fasting. Serum was prepared using the standard operating procedures. In brief, samples were left for 45 min for clotting, followed by a centrifugation step (10 min, 2750 g, 15°C). Samples were then filled in straws (CryoBioSytems IMV, France) by an automatic aliquoting system (DIVA, CryoBioSytems IMV, France). After that serum samples were stored at −80°C. To minimize freeze-thaw cycles, samples were sorted in a cryogenic work bench (temperatures below −100°C) and automatically stored in tanks with a coolable top frame in the gas phase of liquid nitrogen (Askion, Germany; Loeffler et al., 2015).

S100B and NSE were measured with monoclonal 2-site immunoluminometric assays performed on the fully mechanized system LIAISON (Diasorin, Dietzenbach, Germany). The detection limit for the assays was 0.02 μg/l and 0.04 μg/l (described in detail elsewhere (Streitbuerger et al., 2012). BDNF

was measured in serum with an ELISA manufactured by R&D systems (Wiesbaden, Germany). The sensitivity of the assay was 20 ng/L leading to a measuring range of 62.5 until 4000 ng/L. Interassay coefficients of variation were between 9.4 and 11.1% for mean BDNF concentrations between 362 and 2079 ng/L. Note that serum samples were diluted 1:20 before measuring them with the assay.

Statistical Analyses

Statistical analyses were performed using SPSS version 22 (IBM, Chicago, IL, USA). Complex assessment of the data distributions were performed including visual assessment of the histograms, skewness and kurtosis of the data, as well as by Kolmogorov–Smirnoff and Shapiro-Wilk test. Since the protein levels were non-normally distributed and different numbers of subjects were included in patients' and controls' groups we applied non-parametric Mann-Whitney U test for evaluation of group differences. The differences in demographic factors were assessed by independent *t*-test or by chi-square test. The impact of sex differences and a history of depression were assessed by subgroup analyses. The correlation analyses between serum markers, clinical/imaging and demographic data were performed by calculating Spearmen correlation coefficients. We expected directed changes for BDNF and S100B in minor depression in comparison with control subjects, therefore one-tailed α-level for statistical significance was set at 0.05 for these biomarkers. For NSE and the correlation analyses two-tailed α-level at 0.05 was chosen. The statistical power was calculated using G-power 3.1.9.2. (Faul et al., 2009). Generally, data are presented as means and standard deviations (SD). Dot plots represent the distributions of the protein levels and their medians.

RESULTS

The demographics, clinical and imaging data, and serum marker levels of the patients and healthy control subjects are presented in **Table 1**. Both cohorts were matched for age, education, BMI and the extent of white matter hyperintensities as measured with the Fazekas scale.

BDNF, S100B and NSE did not differ between patients in minor depressive episode and healthy control subjects (**Table 1**). Since the two groups differed with regard to sex, we conducted additionally sex-specific analyses. **Figure 1** illustrates results with dot plots for the three serum marker proteins across the whole groups, and specifically for each sex. When the analysis was stratified by sex we observed significantly increased S100B ($p = 0.034$) in males with minor depressive episode (0.092 μg/l [0.012]) in comparison with healthy male controls (0.067 μg/l [0.004]).

As depicted in the **Figure 2**, serum S100B levels were significantly lower in healthy males (0.067 μg/l [0.004]) in comparison with healthy females (0.115 μg/l [0.029]; $p = 0.01$), whereas there was no sex difference in the minor depression group (male: 0.091 μg/l [0.12]; female: 0.088 μg/l [0.011]; $p = 0.53$). Removal the abovementioned female outlier did not affect the differences between healthy males and females for S100B. BDNF and NSE did not differ neither between the groups stratified by sex (males $p = 0.13$–0.95; females $p = 0.40$–0.42), nor when male and female subjects were compared within the minor depression subgroup ($p = 0.10$–0.98).

Similarly, presence of the history of major depression did not affect the levels of BDNF, S100B or NSE in the minor depression group ($p = 0.10$–0.50); neither in comparison with healthy controls ($p = 0.13$–0.38; **Figure 3**).

As presented on **Figure 1** one female control subject presented with extremely high S100B value, above three SD of the group. To control for the impact of this subject on the analysis of S100B we performed an additional analysis of S100B excluding this subject's data. In this analysis we observed a trend, $p = 0.078$, for increased S100B in the whole minor depression group (0.088 μg/l [0.043]) in comparison with healthy controls (0.074 μg/l [0.032]).

We observed a positive correlation between S100B and BMI in the whole sample ($r_s = 0.204$, $p = 0.04$), and in healthy subjects ($r_s = 0.246$, $p = 0.03$), and a positive correlation between S100B

TABLE 1 | Demographical, clinical/imaging data and serum markers in patients and healthy control subjects.

	Whole group		Males		Females	
	MinD	HC	MinD	HC	MinD	HC
Number (with a history of depression)	27 (14)	82	7 (3)	58	21 (11)	24
Age	71.2 (4.5)	70.0 (4.1)	71.4 (4.8)	70.3 (4.1)	71.1 (4.5)	69.6 (4.3)
Sex (male/female)	7/21***	51/31***				
Education (<12years/>12 years)	24/4	58/24	5/2	37/15	19/2	22/9
Fazekas score (0/1/2/3)	6/16/5/0	25/45/12/0	2/4/0/0	17/27/7/0*	4/12/5/0	8/18/5/0
BMI (kg/m^2)	27.1 (4.9)	28.1 (4.6)	26.7 (2.1)	27.8 (3.7)	27.2 (5.7)	28.7 (5.9)
BDNF (μg/L)	25.8 (5.4)	25.2 (5.9)	29.6 (14.2)	24.7 (4.3)	26.1 (4.9)	26.1 (7.8)
NSE (μg/L)	11.8 (2.6)	11.9 (2.1)	11.9 (2.9)	11.7 (2.3)	11.7 (2.6)	12.2 (1.7)
S100B (μg/L)	0.088 (0.043)	0.086 (0.11)	0.088 (0.03)*	0.067 (0.03)*†	0.088 (0.05)	0.12 (0.16)†

*MinD, minor depression; HC, healthy controls; BMI, body-mass index; BDNF, brain derived neurotrophic factor; NSE, neuron specific enolase; †Significant difference between males and females at p < 0.05; *Significant difference between minor depression and healthy controls group at p < 0.05; ***Significant difference between minor depression and healthy controls group at p < 0.001; Student's t-test for age and body mass index, chi-square test for categorical data, Mann-Whitney U test for BDNF, NSE, S100B.*

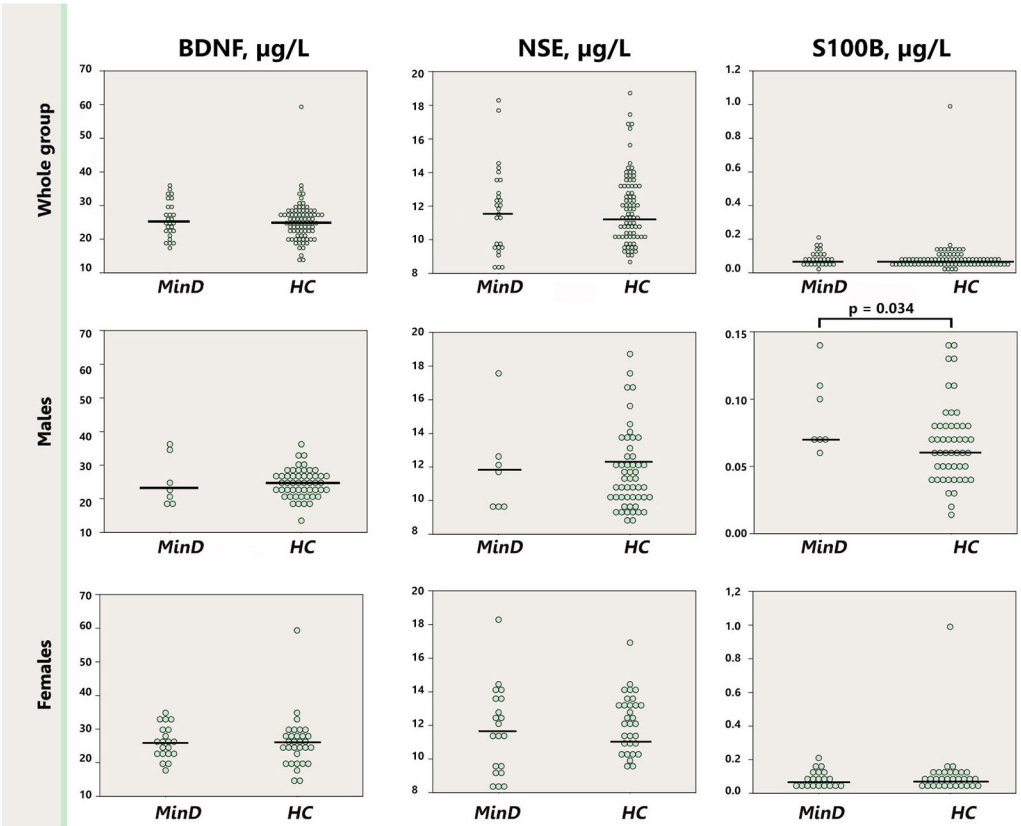


FIGURE 1 | Dot plot for the distribution of serum markers' levels in subjects and healthy controls (first row), separately for males (second row), and females (third row). Median levels of the serum markers are depicted with black horizontal lines. Note that the distribution of S100B in males is depicted on a zoomed scale. MinD, minor depression; HC, healthy controls; BDNF, brain derived neurotrophic factor; NSE, neuron specific enolase.

and age in the whole sample ($r_s = 0.229$, $p = 0.02$) and in healthy control subjects ($r_s = 0.345$, $p = 0.002$).

A significant positive correlation was found between age and the degree of white matter hyperintensities as measured with the Fazekas score both, in the whole sample ($r_s = 0.425$, $p < 0.001$), and in subgroups (minor depression: $r_s = 0.462$, $p = 0.04$; healthy controls: $r_s = 0.421$, $p < 0.001$). With regard to serum markers, Fazekas score correlated positively with NSE in the whole sample ($r_s = 0.252$, $p = 0.02$) and in patients with minor depression ($r_s = 0.436$, $p = 0.048$). In the healthy control sample the Fazekas score correlated positively only with S100B ($r_s = 0.261$, $p = 0.04$).

Finally, we examined whether our groups were large enough to detect the predicted impact of minor depression on serum BDNF and S100B. The statistical power calculations using G-Power for Mann-Whitney tests were based on the previous meta-analyses of BDNF and S100B alterations in major depression (Schroeter et al., 2013; Polyakova et al., 2015). These calculations lead to required sample sizes of $n = 36$ per group for BDNF and $n = 5$ per group for S100B.

DISCUSSION

In this study, for the first time to our knowledge, we evaluated serum levels of BDNF, S100B and NSE in subjects with minor depressive episode. We found evidence for increased S100B in males with minor depression without any alterations of NSE, which was in agreement with our hypotheses. BDNF was unchanged, although we expected a decrease in analogy to major depression. In assessment of the secondary outcomes we observed a positive correlation between NSE and Fazekas score in minor depression and in the whole sample. S100B correlated positively with age and BMI in the whole sample and in healthy controls.

Our hypotheses were initially built on data derived from major depression studies. In minor depression we didn't detect the hypothesized difference for BDNF. One explanation of such a negative finding might be that neurotrophic functions are not impaired at the subthreshold level of depression. Then the substantial differences in the pathophysiology of these disorders arise. Nevertheless, one might also argue that the sample size was too low. The calculation of the required sample size using G-Power for BDNF indeed showed that our minor depressive group might have been underpowered (27 subjects instead of the 36 required). In this study we reached only 75% of statistical power. To solve the power issue future studies should involve larger sample size.

For S100B the sample size was obviously large enough to detect the expected group effects (27 subjects instead of five

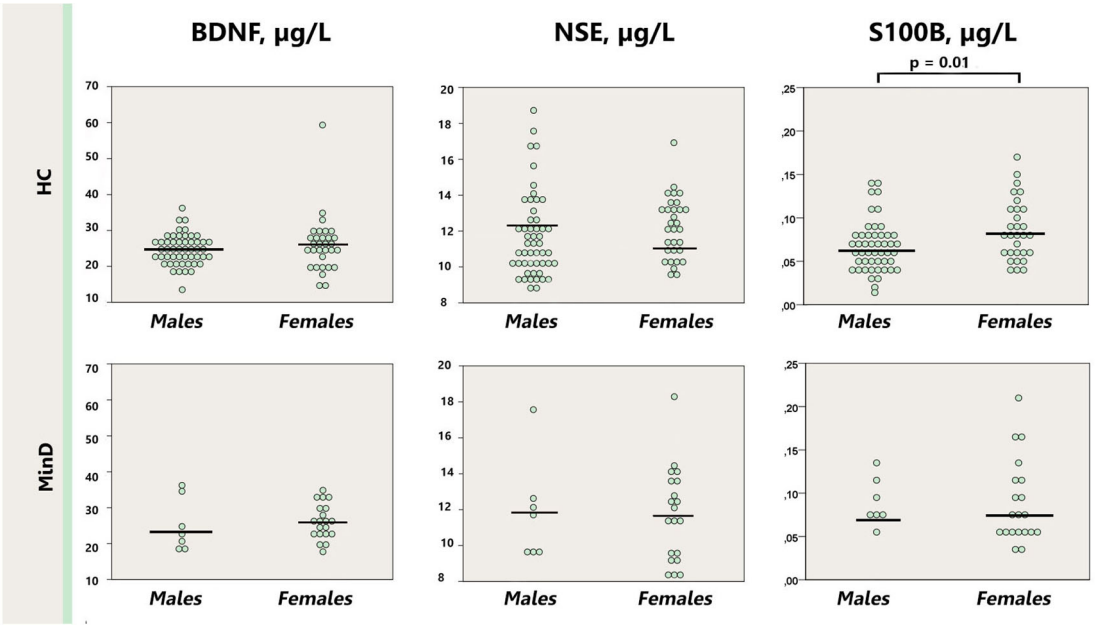


FIGURE 2 | Dot plot for the distribution of serum markers' levels in healthy males and females (first row), and males and females with minor depression (third row). Median levels of the serum markers are depicted with black horizontal lines. Note that the outlier from the healthy females group is not depicted on the S100B plot. MinD, minor depression; HC, healthy controls; BDNF, brain derived neurotrophic factor; NSE, neuron specific enolase.

required). Indeed, we observed a trend for increased S100B in the whole minor depression group and significantly increased S100B in males with minor depression in comparison with control subjects. Though we did not rule out potential non-brain sources of S100B in our study, this finding points to the similarities between major and minor depression. Moreover, the fact that differences in S100B are less prominent than in major depression suggests that clinical presentation mirrors to some extend molecular changes.

The findings we describe are based on the concept that serum S100B changes are related to alterations in the brain. However, S100B, as well as BDNF, and NSE might originate from non-brain sources. For instance, various subtypes of leukocytes can secrete S100B (Miki et al., 2013; Fujiya et al., 2014; Moutsatsou et al., 2014). Thrombocytes are the largest source for serum BDNF (Fujimura et al., 2002), adipocytes produce both S100B and BDNF (Fujiya et al., 2014; Huang et al., 2014), finally NSE may originate from damaged peripheral nerves (Li et al., 2013). Because we did not assume relevant biases related to these potentially confounding sources in minor depression, we did not control for potential non-brain sources of the serum markers in our study. Note that we compared subjects with minor depression to matched healthy control subjects and considered, therefore, differences and not absolute values of

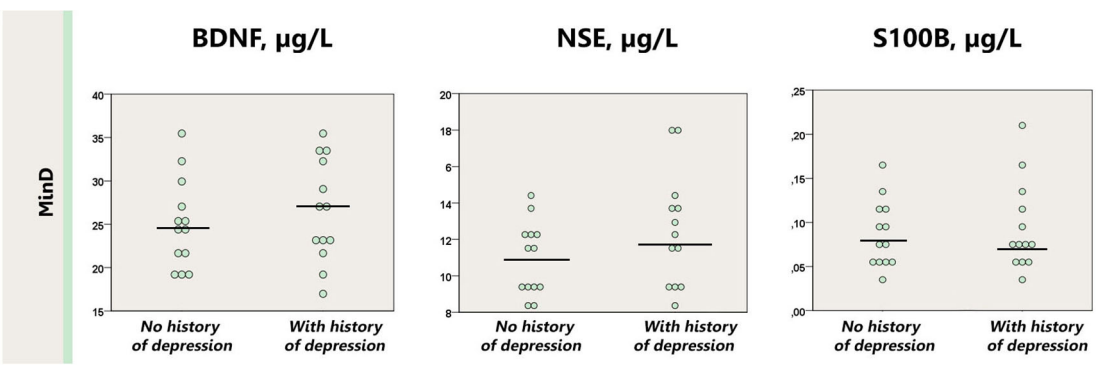


FIGURE 3 | Dot plot for the distribution of serum markers' levels in subjects with minor depression with or without a history of depression. Median levels of the serum markers are depicted with black horizontal lines. MinD, minor depression, HC, healthy controls; BDNF, brain derived neurotrophic factor; NSE, neuron specific enolase.

S100B. Moreover, changes of S100B in leukocytes have been shown only in bipolar disorder (Moutsatsou et al., 2014), whereas for unipolar depression, which is more relevant for our data, no studies are available in the literature so far. Future studies might transcend these limitations by including larger and more strictly selected cohorts and controlling for non-brain sources of these markers.

The concept of leakage from the brain obviously has its limitations. S100B, as well as BDNF, and NSE might originate from non-brain sources. Various subtypes of leucocytes can secrete S100B (Miki et al., 2013; Fujiya et al., 2014; Moutsatsou et al., 2014), thrombocytes are the largest source for serum BDNF (Fujimura et al., 2002), adipocytes produce both S100B and BDNF (Fujiya et al., 2014; Huang et al., 2014), finally NSE originate from damaged peripheral nerves (Li et al., 2013).

Interestingly, S100B was not different between males and females with minor depression, rather it differed between healthy males and females. This finding is in line with previous studies showing no sex differences in major depression (Arolt et al., 2002; Hetzel et al., 2005), but contradicts another one showing increased S100B in females with major depression (Yang et al., 2008). The differences between our and the former study might be attributed to the differences in the studied samples with regard to disease severity and age. Further studies are in agreement with our finding for healthy subjects by showing higher S100B in healthy female than male adults (Streitbuerger et al., 2012) and children (Gazzolo et al., 2003). Overall, the literature on effects of gender on S100B did not reach consensus so far. Whether gender differences in S100B reflect the differences in susceptibility to disease and whether S100B is a gender-specific marker of minor depression needs more systematic assessment.

S100B, as an index for glial alterations, is modified by age in major depression (Schroeter et al., 2011). In minor depression we did not find a correlation between S100B and age. Instead, S100B correlated positively with age in healthy controls. This finding is in line with cerebrospinal fluid studies (van Engelen et al., 1992; Nygaard et al., 1997), but contradicts later serum studies (Wiesmann et al., 1998; Portela et al., 2002). One potential reason for these differences is again the different disease severity. According to Rajkowska's observations development of depressive disorder starts with glial alterations and progresses with age (Rajkowska, 2000). If late life minor depression is a subtle manifestation of major disorder, absence of correlation between S100B and age in minor depression might add to Raikowska's hypothesis.

A weak positive correlation between S100B and BMI was not surprising. S100B is secreted by adipocytes and is involved in the pathogenesis of obesity as shown *in vitro* (Fujiya et al., 2014) and *in vivo* (Buckman et al., 2014). Positive correlation of serum S100B with BMI was previously reported in a combined sample of cognitively intact lean and obese subjects (Steiner et al., 2010a) and in subjects with schizophrenia (Steiner et al., 2010b). In our study the positive correlation in the whole sample was likely driven by the healthy subgroup, with no such association in minor depressive episode. As correlations between S100B and age/BMI were detected only in healthy subjects but not in the minor depression group, and both cohorts were matched regarding mean age and BMI, we assume that age and BMI did not drive the S100B effects in minor depression.

The finding of positive correlation between S100B and white matter hyperintensities in healthy subjects is in agreement with a study by Streitbuerger et al. (2012). These authors reported an association between serum S100B and the diffusion tensor imaging parameters fractional anisotropy and radial diffusivity in white matter tracts in healthy females. From the biological point of view increased secretion of S100B might reflect neuroinflammation that accompanies neuronal damage (Kabadi et al., 2015).

We detected also a positive correlation between serum NSE and Fazekas score in the whole sample and in the minor depression subgroup. NSE, a peripheral marker of neuronal damage, might be either of central (Cheng et al., 2014) or peripheral origin (Li et al., 2013). In major depression a central origin is suggested by the correlation with white matter hyperintensities. Finally, the extent of white matter hyperintensities correlated with age in both cohorts, healthy and minor depressive subjects, which is in line with the literature (Nyquist et al., 2015). In combination with the correlation between white matter hyperintensities and the neuronal injury marker NSE in minor depression, our data might support the vascular hypothesis of late life depression (Taylor et al., 2013; Taylor, 2014).

Limitations

As already discussed our study was limited by a relatively small sample size, which might have hampered, in particular, the detection of BDNF effects. Another limitation might be the inclusion of patients having a history of depression. Thus, not all patients could be qualified as having minor depressive disorder. We addressed this issue in the subgroup analysis and found that inclusion of the subjects with a history of depression did not affect our results. In fact, such a sample mirrors a real life situation when psychiatrists need to make a clinical judgement and select a treatment strategy. Note, moreover, that our subjects were chosen from a representative population study.

The findings we describe are based on the concept that serum S100B changes are related to alterations in the brain. However, S100B, as well as BDNF, and NSE might originate from non-brain sources. For instance, various subtypes of leukocytes can secrete S100B (Miki et al., 2013; Fujiya et al., 2014; Moutsatsou et al., 2014). Thrombocytes are the largest source for serum BDNF (Fujimura et al., 2002), adipocytes produce both S100B and BDNF (Fujiya et al., 2014; Huang et al., 2014), finally NSE may originate from damaged peripheral nerves (Li et al., 2013). Because we did not assume relevant biases related to these potentially confounding sources in minor depression, we did not control for potential non-brain sources of the serum markers in our study. Note that we compared subjects with minor depression to matched healthy control subjects and considered, therefore, differences and not absolute values of S100B. Moreover, changes of S100B in leukocytes have been shown only in bipolar disorder (Moutsatsou et al., 2014), whereas for unipolar

depression, which is more relevant for our data, no studies are available in the literature so far. Future studies might transcend these limitations by including larger and more strictly selected cohorts and controlling for non-brain sources of these markers.

CONCLUSION

In this study we made a first attempt to assess serum levels of BDNF, S100B, and NSE in minor depression. We found evidence for increased glial marker S100B in males with minor depression and a similar trend in the whole minor depressive group, but no significant evidence of BDNF and NSE alterations. The positive correlation of NSE with Fazekas score as a measure for white matter hyperintensities in minor depression supports the vascular hypothesis of late life depression.

AUTHOR CONTRIBUTIONS

MP, PS, MLS have designed the study, analyzed and interpreted the data, drafted and revised the manuscript content; MP and CS selected the subjects from LIFE study database, JK was responsible for the laboratory detection of the serum markers; LL and KTH were responsible for ratings of white matter hyperintensities, CS, KA, ML, TL, SRH, AV contributed to data acquisition. All authors have critically reviewed the manuscript and approved its final version. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This study has been supported by the International Max Planck Research School on Neuroscience of Communication (IMPRS NeuroCom; MP), by LIFE—Leipzig Research Center for Civilization Diseases at the University of Leipzig—funded by the European Union, European Regional Development Fund and by the Free State of Saxony within the framework of the excellence initiative (CZ, KA, TL, SRH, AV, PS and MLS), by the German Consortium for Frontotemporal Lobar Degeneration, funded by the German Federal Ministry of Education and Research (MLS), and by the Parkinson's Disease Foundation (MLS; Grant No. PDF-IRG-1307).

REFERENCES

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edn, *Text Revision*. Washington, DC.

Andreazza, A. C., Cassini, C., Rosa, A. R., Leite, M. C., de Almeida, L. M., Nardin, P., et al. (2007). Serum S100B and antioxidant enzymes in bipolar patients. *J. Psychiatr. Res.* 41, 523–529. doi: 10.1016/j.jpsychires.2006.07.013

Arolt, V., Peters, M., and Rothermundt, M. (2002). Neuroplasticity in major depression may be indicated by S100B. *Eur. Psychiatry* 17, 159S–159S. doi: 10.1016/s0924-9338(02)80691-5

Buckman, L. B., Anderson-Baucum, E. K., Hasty, A. H., and Ellacott, K. L. (2014). Regulation of S100B in white adipose tissue by obesity in mice. *Adipocyte* 3, 215–220. doi: 10.4161/adip.28730

Cheng, F., Yuan, Q., Yang, J., Wang, W., and Liu, H. (2014). The prognostic value of serum neuron-specific enolase in traumatic brain injury: systematic review and meta-analysis. *PLoS One* 9:e106680. doi: 10.1371/journal.pone.0106680

Eaton, W. W., Badawi, M., and Melton, B. (1995). Prodromes and precursors–epidemiologic data for primary prevention of disorders with slow onset. *Am. J. Psychiatry* 152, 967–972. doi: 10.1176/ajp.152.7.967

Faul, F., Erdfelder, E., Buchner, A., and Lang, A.-G. (2009). Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behav. Res. Methods* 41, 1149–1160. doi: 10.3758/brm.41.4.1149

Fazekas, F., Chawluk, J. B., Alavi, A., Hurtig, H. I., and Zimmerman, R. A. (1987). MR signal abnormalities at 1.5-T in Alzheimer dementia and normal aging. *AJR Am. J. Roentgenol.* 149, 351–356. doi: 10.2214/ajr.149.2.351

Fujimura, H., Altar, C. A., Chen, R., Nakamura, T., Nakahashi, T., Kambayashi, J., et al. (2002). Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thromb. Haemost.* 87, 728–734.

Fujiya, A., Nagasaki, H., Seino, Y., Okawa, T., Kato, J., Fukami, A., et al. (2014). The Role of S100B in the interaction between adipocytes and macrophages. *Obesity (Silver Spring)* 22, 371–379. doi: 10.1002/oby.20532

Gazzolo, D., Michetti, F., Bruschettini, M., Marchese, N., Lituania, M., Mangraviti, S., et al. (2003). Pediatric concentrations of S100B protein in blood: age- and sex-related changes. *Clin. Chem.* 49, 967–970. doi: 10.1373/49.6.967

Hegerl, U., and Schoenknecht, P. (2009). Subdiagnostic depression. Are there treatments with clinically relevant effects? *Nervenarzt* 80, 532–539. doi: 10.1007/s00115-008-2622-z

Hetzel, G., Moeller, O., Evers, S., Erfurth, A., Ponath, G., Arolt, V., et al. (2005). The astroglial protein S100B and visually evoked event-related potentials before and after antidepressant treatment. *Psychopharmacology (Berl)* 178, 161–166. doi: 10.1007/s00213-004-1999-z

Huang, T., Larsen, K. T., Ried-Larsen, M., Moller, N. C., and Andersen, L. B. (2014). The effects of physical activity and exercise on brain-derived neurotrophic factor in healthy humans: a review. *Scand. J. Med. Sci. Sports* 24, 1–10. doi: 10.1111/sms.12069

Kabadi, S. V., Stoica, B. A., Zimmer, D. B., Afanador, L., Duffy, K. B., Loane, D. J., et al. (2015). S100B inhibition reduces behavioral and pathologic changes in experimental traumatic brain injury. *J. Cereb. Blood Flow Metab.* doi: 10.1038/jcbfm.2015.165 [Epub ahead of print].

Kalia, M., and Silva, J. C. E. (2015). Biomarkers of psychiatric diseases: current status and future prospects. *Metabolism* 64, S11–S15. doi: 10.1016/j.metabol.2014.10.026

Li, J., Zhang, H., Xie, M., Yan, L., Chen, J., and Wang, H. (2013). NSE, a potential biomarker, is closely connected to diabetic peripheral neuropathy. *Diabetes Care* 36, 3405–3410. doi: 10.2337/dc13-0590

Loeffler, M., Engel, C., Ahnert, P., Alfermann, D., Arelin, K., Baber, R., et al. (2015). The LIFE-Adult-Study: objectives and design of a population-based cohort study with 10,000 deeply phenotyped adults in Germany. *BMC Public Health* 15: 691. doi: 10.1186/s12889-015-1983-z

Lyness, J. M., King, D. A., Cox, C., Yoediono, Z., and Caine, E. D. (1999). The importance of subsyndromal depression in older primary care patients: prevalence and associated functional disability. *J. Am. Geriatr. Soc.* 47, 647–652. doi: 10.1111/j.1532-5415.1999.tb01584.x

Miki, Y., Gion, Y., Mukae, Y., Hayashi, A., Sato, H., Yoshino, T., et al. (2013). Morphologic, flow cytometric, functional and molecular analyses of S100B positive lymphocytes, unique cytotoxic lymphocytes containing S100B protein. *Eur. J. Haematol.* 90, 99–110. doi: 10.1111/ejh.12036

Molendijk, M. L., Bus, B. A., Spinhoven, P., Penninx, B. W., Kenis, G., Prickaerts, J., et al. (2011). Serum levels of brain-derived neurotrophic factor in major depressive disorder: state-trait issues, clinical features and pharmacological treatment. *Mol. Psychiatry* 16, 1088–1095. doi: 10.1038/mp.2010.98

Molendijk, M. L., Spinhoven, P., Polak, M., Bus, B. A., Penninx, B. W., and Elzinga, B. M. (2014). Serum BDNF concentrations as peripheral manifestations of depression: evidence from a systematic review and meta-analyses on 179 associations (N=9484). *Mol. Psychiatry* 19, 791–800. doi: 10.1038/mp.2013.105

Moutsatsou, P., Tsoporis, J. N., Salpeas, V., Bei, E., Alevizos, B., Anagnostara, C., et al. (2014). Peripheral blood lymphocytes from patients with bipolar disorder demonstrate apoptosis and differential regulation of advanced glycation end products and S100B. *Clin. Chem. Lab. Med.* 52, 999–1007. doi: 10.1515/cclm-2013-0978

Nygaard, O., Langbakk, B., and Romner, B. (1997). Age- and sex-related changes of S-100 protein concentrations in cerebrospinal fluid and serum in patients with no previous history of neurological disorder. *Clin. Chem.* 43, 541–543. doi: 10.1016/s0303-8467(97)81581-8

Nyquist, P. A., Bilgel, M., Gottesman, R., Yanek, L. R., Moy, T. F., Becker, L. C., et al. (2015). Age differences in periventricular and deep white matter lesions. *Neurobiol. Aging* 36, 1653–1658. doi: 10.1016/j.neurobiolaging.2015.01.005

Park, J. H., Lee, J. J., Lee, S. B., Huh, Y., Choi, E. A., Youn, J. C., et al. (2010). Prevalence of major depressive disorder and minor depressive disorder in an elderly Korean population: Results from the Korean Longitudinal Study on Health and Aging (KLoSHA). *J. Affect. Disord.* 125, 234–240. doi: 10.1016/j.jad.2010.02.109

Polyakova, M., Stuke, K., Schuemberg, K., Mueller, K., Schoenknecht, P., and Schroeter, M. L. (2015). BDNF as a biomarker for successful treatment of mood disorders: a systematic & quantitative meta-analysis. *J. Affect. Disord.* 174, 432–440. doi: 10.1016/j.jad.2014.11.044

Portela, L. V., Tort, A. B., Schaf, D. V., Ribeiro, L., Nora, D. B., Walz, R., et al. (2002). The serum S100B concentration is age dependent. *Clin. Chem.* 48, 950–952.

Rajkowska, G. (2000). Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. *Biol. Psychiatry* 48, 766–777. doi: 10.1016/s0006-3223(00)00950-1

Schmidt, F. M., Mergl, R., Stach, B., Jahn, I., and Schoenknecht, P. (2015). Elevated levels of cerebrospinal fluid neuron-specific enolase (NSE), but not S100B in major depressive disorder. *World J. Biol. Psychiatry* 16, 106–113. doi: 10.3109/15622975.2014.952776

Schroeter, M. L., Abdul-Khaliq, H., Diefenbacher, A., and Blasig, I. E. (2002). S100B is increased in mood disorders and may be reduced by antidepressive treatment. *Neuroreport* 13, 1675–1678. doi: 10.1097/00001756-200209160-00021

Schroeter, M. L., Abdul-Khaliq, H., Krebs, M., Diefenbacher, A., and Blasig, I. E. (2008). Serum markers support disease-specific glial pathology in major depression. *J. Affect. Disord.* 111, 271–280. doi: 10.1016/j.jad.2008.03.005

Schroeter, M. L., Sacher, J., Steiner, J., Schoenknecht, P., and Mueller, K. (2013). Serum S100B represents a new biomarker for mood disorders. *Curr. Drug Targets* 14, 1237–1248. doi: 10.2174/13894501113149990014

Schroeter, M. L., and Steiner, J. (2009). Elevated serum levels of the glial marker protein S100B are not specific for schizophrenia or mood disorders. *Mol. Psychiatry* 14, 235–237. doi: 10.1038/mp.2008.85

Schroeter, M. L., Steiner, J., and Mueller, K. (2011). Glial pathology is modified by age in mood disorders - a systematic meta-analysis of serum S100B *in vivo* studies. *J. Affect. Disord.* 134, 32–38. doi: 10.1016/j.jad.2010.11.008

Steiner, J., Schiltz, K., Walter, M., Wunderlich, M. T., Keilhoff, G., Brisch, R., et al. (2010a). S100B serum levels are closely correlated with body mass index: an important caveat in neuropsychiatric research. *Psychoneuroendocrinology* 35, 321–324. doi: 10.1016/j.psyneuen.2009.07.012

Steiner, J., Walter, M., Guest, P., Myint, A. M., Schiltz, K., Panteli, B., et al. (2010b). Elevated S100B levels in schizophrenia are associated with insulin resistance. *Mol. Psychiatry* 15, 3–4. doi: 10.1038/mp.2009.87

Streitbuerger, D.-P., Arelin, K., Kratzsch, J., Thiery, J., Steiner, J., Villringer, A., et al. (2012). Validating serum S100B and neuron-specific enolase as biomarkers for the human brain - a combined serum, gene expression and MRI study. *PLoS One* 7:e43284. doi: 10.1371/journal.pone.0043284

Taylor, W. D. (2014). Clinical practice. Depression in the elderly. *N. Engl. J. Med.* 371, 1228–1236. doi: 10.1056/NEJMcp1402180

Taylor, W. D., Aizenstein, H. J., and Alexopoulos, G. S. (2013). The vascular depression hypothesis: mechanisms linking vascular disease with depression. *Mol. Psychiatry* 18, 963–974. doi: 10.1038/mp.2013.20

van Engelen, B. G., Lamers, K. J., Gabreels, F. J., Wevers, R. A., Van Geel, W. J., and Borm, G. F. (1992). Age-related changes of neuron-specific enolase, S-100 protein and myelin basic protein concentrations in cerebrospinal fluid. *Clin. Chem.* 38, 813–816.

Wiesmann, M., Missler, U., Gottmann, D., and Gehring, S. (1998). Plasma S-100b protein concentration in healthy adults is age- and sex-independent. *Clin. Chem.* 44, 1056–1058.

Yang, K., Xie, G.-R., Hu, Y.-Q., Mao, F.-Q., and Su, L.-Y. (2008). The effects of gender and numbers of depressive episodes on serum S100B levels in patients with major depression. *J. Neural Transm.* 115, 1687–1694. doi: 10.1007/s00702-008-0130-8

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Polyakova, Sander, Arelin, Lampe, Luck, Luppa, Kratzsch, Hoffmann, Riedel-Heller, Villringer, Schoenknecht and Schroeter. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution and reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Cellular Neuroscience

MINI REVIEW
published: 22 January 2016
doi: 10.3389/fncel.2015.00521

Depression as a Glial-Based Synaptic Dysfunction

*Daniel Rial[1,2], Cristina Lemos[1], Helena Pinheiro[1], Joana M. Duarte[1], Francisco Q. Gonçalves[1], Joana I. Real[1], Rui D. Prediger[2], Nélio Gonçalves[1], Catarina A. Gomes[1,3], Paula M. Canas[1], Paula Agostinho[1,3] and Rodrigo A. Cunha[1,3]**

[1] CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal, [2] Departamento de Farmacologia, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil, [3] Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Recent studies combining pharmacological, behavioral, electrophysiological and molecular approaches indicate that depression results from maladaptive neuroplastic processes occurring in defined frontolimbic circuits responsible for emotional processing such as the prefrontal cortex, hippocampus, amygdala and ventral striatum. However, the exact mechanisms controlling synaptic plasticity that are disrupted to trigger depressive conditions have not been elucidated. Since glial cells (astrocytes and microglia) tightly and dynamically interact with synapses, engaging a bi-directional communication critical for the processing of synaptic information, we now revisit the role of glial cells in the etiology of depression focusing on a dysfunction of the "quad-partite" synapse. This interest is supported by the observations that depressive-like conditions are associated with a decreased density and hypofunction of astrocytes and with an increased microglia "activation" in frontolimbic regions, which is expected to contribute for the synaptic dysfunction present in depression. Furthermore, the traditional culprits of depression (glucocorticoids, biogenic amines, brain-derived neurotrophic factor, BDNF) affect glia functioning, whereas antidepressant treatments (serotonin-selective reuptake inhibitors, SSRIs, electroshocks, deep brain stimulation) recover glia functioning. In this context of a quad-partite synapse, systems modulating glia-synapse bidirectional communication—such as the purinergic neuromodulation system operated by adenosine 5′-triphosphate (ATP) and adenosine—emerge as promising candidates to "re-normalize" synaptic function by combining direct synaptic effects with an ability to also control astrocyte and microglia function. This proposed triple action of purines to control aberrant synaptic function illustrates the rationale to consider the interference with glia dysfunction as a mechanism of action driving the design of future pharmacological tools to manage depression.

Keywords: depression, synapse, astrocytes, microglia, purines

OPEN ACCESS

Edited by:
Tycho M. Hoogland, Erasmus MC, Netherlands

Reviewed by:
Lisa Mapelli, University of Pavia, Italy
Anna Maria Pugliese, University of Florence, Italy

****Correspondence:***
Rodrigo A. Cunha
cunharod@gmail.com

Received: *11 November 2015*
Accepted: *27 December 2015*
Published: *22 January 2016*

Citation:
Rial D, Lemos C, Pinheiro H, Duarte JM, Gonçalves FQ, Real JI, Prediger RD, Gonçalves N, Gomes CA, Canas PM, Agostinho P and Cunha RA (2016) Depression as a Glial-Based Synaptic Dysfunction. Front. Cell. Neurosci. 9:521. doi: 10.3389/fncel.2015.00521

INTRODUCTION

Depression is the neuropsychiatric disorder with higher incidence worldwide, representing a major socio-economical burden (Kessler et al., 2003). Depressive conditions display heterogeneous presentations and are defined clinically based on different affective symptoms (sadness, desperation, apathy, anhedonia, sensation of discomfort) that decrease interest in daily

activities (Ferrari et al., 2013). Understanding the causes and neurobiological basis of depression remains a challenge, probably due to the lack of faithful animal models (Berton et al., 2012). The relation between cumulative stress and the incidence of depression (de Kloet et al., 2005) and the prevalence of depression in suicide completers (Coryell and Young, 2005), provide two windows of opportunities to indirectly study the neurobiological basis of depression using chronically stressed animals and brain samples from suicide completers.

PURPORTED BIOLOGICAL BASIS OF DEPRESSION

Depressive conditions have traditionally been considered to involve a deregulated hypothalamic-pituitary-adrenal (HPA) axis, leading to an aberrant impact of sustained increased levels of glucocorticoids (Sousa et al., 2008). However, recent studies in adrenalectomized animals (thus blunting the main source of glucocorticoids) revealed an impact of ghrelin rather than glucocorticoids in formatting the emotional disturbances associated with repeated stress (Meyer et al., 2014).

Another lead for the neurobiology of depression is based on the clinical use of serotonin-selective reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs) to manage depression, which hints at the dysfunction of the neuromodulation by serotonin and other biogenic amines in depression (Krishnan and Nestler, 2008). However, these drugs have a slow onset of action (over 2 weeks) and are effective in only *circa* 50% of patients, leaving open the possibility that they may influence different targets apart normalizing the levels of biogenic amines.

Neurotrophins and in particular brain-derived neurotrophic factor (BDNF) have also been linked to depression, based on the ability of BDNF to reactivate neuronal plasticity and on the association between serum BDNF levels and depression (Castrén, 2014). This should be cautiously evaluated since the plasma levels of substances might not reflect their brain levels and there is no clear association between depression and different polymorphisms of the *bdnf* gene (Gyekis et al., 2013). Also, excessive BDNF-induced plasticity can actually trigger an aberrant hyper-plasticity, as heralded by the key pathogenic role of BDNF in neuropathic pain (Trang et al., 2012).

SYNAPTIC DYSFUNCTION IN DEPRESSION

A major breakthrough in understanding depression was the observation that sub-anesthetic doses of ketamine, an NMDA receptor antagonist, can revert rapidly (within 90 min) symptoms of depression, with a long-lasting antidepressant effect (2 weeks; Berman et al., 2000; Zarate et al., 2006). Since NMDA receptors are the main switches to trigger synaptic plasticity (both long-term potentiation, LTP, and depression, LTD), this indicates that an abnormal plasticity at glutamatergic synapses underlies the expression of depressive symptoms (Duman and Aghajanian, 2012); indeed, chronically-stressed rodents display abnormal patterns of synaptic plasticity (both LTP and/or LTD) in brain areas involved in emotional processing, namely amygdala, ventral striatum, hippocampus and prefrontal cortex (Krishnan and Nestler, 2008). Animal studies also allowed identifying the molecular mechanisms of the antidepressant effect of ketamine, which involves the antagonism of NMDA (2B) receptors and the preservation of dendritic morphology and AMPA receptor trafficking through an mTOR pathway in the prefrontal cortex (Li et al., 2010). This joins other observations showing that riluzole and antagonists of types 2/3 or 5 metabotropic glutamate receptors, which control glutamatergic transmission, also display robust antidepressant effects (Machado-Vieira et al., 2009; Pilc et al., 2013). Altogether these observations support the hypothesis that depression results from the disruption of mechanisms controlling synaptic plasticity in afflicted regions (Duman and Aghajanian, 2012).

This de-regulation seems to translate into a destabilization and loss of synaptic connections. Indeed, repeated stress triggers a reduction of dendritic complexity in prefrontocortical and hippocampal neurons (Magariños et al., 1997; Sousa et al., 2000; Radley et al., 2006) and a selective loss of markers of excitatory synapses (Gilabert-Juan et al., 2012; Tzanoulinou et al., 2014; Kaster et al., 2015), which recover upon alleviation of "depressive"-like symptoms using SSRIs, exercise or enriched environment (Li et al., 2010, 2011; McEwen et al., 2012). Post-mortem brain samples of depressed patients also revealed a reduction in the size rather than number of prefrontocortical and hippocampal pyramidal neurons (Rajkowska et al., 1999; Stockmeier et al., 2004) accompanied by a decreased number of synaptic contacts (Kang et al., 2012). As occurs in stressed rodents, synaptic markers in frontolimbic area are also altered in patients with major depressive disorder (Feyissa et al., 2009; Zhao et al., 2012; Duric et al., 2013).

This pivotal role of the disruption of mechanisms controlling synaptic plasticity for the expression of depressive symptoms also has the attractive feature to allow bridging the different traditional explanations for the emergence of depression. In fact, glucocorticoids are well established to affect synaptic plasticity and to contribute for synaptic atrophy in several brain regions (Sousa et al., 2008). Likewise, BDNF is well recognized as a bolster of synaptic plasticity (Gray et al., 2013) and different biogenic amines, such as serotonin (Lesch and Waider, 2012), noradrenaline (Marzo et al., 2009) and dopamine (Tritsch and Sabatini, 2012) impact on synaptic plasticity in cortical regions.

However, although the pivotal role of aberrant synaptic plasticity successfully integrates different findings derived from patients and animal models of depression, it still fails to provide an explanation for the etiology of depression. This review proposes to focus on the emerging concept of the quad-partite synapse (Schafer et al., 2013), integrating both astrocytes and microglia as critical pillars of synaptic plasticity, to address the possible relevance of a mis-communication between glia and synapses, as a possible basis of depression.

THE "QUAD-PARTITE" SYNAPSE

Astrocytes were long considered as morphological and metabolic support cells, as testified by their importance in the synthesis

(Rose et al., 2013) and re-uptake of glutamate (Asztely et al., 1997; Arnth-Jensen et al., 2002), in buffering extracellular K^+ to control neuronal excitability (Wallraff et al., 2006), in neurovascular coupling (Viswanathan and Freeman, 2007; Petzold et al., 2008; Figley and Stroman, 2011) and transport (Rouach et al., 2008) and delivery of nutrients to active synapses (Magistretti et al., 1999; Pellerin et al., 2007). This concept has actually evolved to recognize astrocytes as dynamic players engaged in a bi-directional communication with synapses and able to actually format synaptic function with impact on the expression of behavior (Achour and Pascual, 2010; Allen, 2014; Oliveira et al., 2015). This interplay between synapses and astrocytes is so tight that it is difficult to disentangle if a synaptic dysfunction results from intrinsic modifications of neurons or from astrocytic modifications (Agostinho et al., 2010; Sanacora and Banasr, 2013; Crunelli et al., 2015; Verkhratsky et al., 2015).

Probably the first experimental support of an ability of astrocytes to respond to synaptic activity was provided by observations that astrocytes respond to glutamate by triggering a directional long-distance response, typified by a wave of variation of intracellular calcium (Cornell-Bell et al., 1990). The inter-astrocytic transport of calcium waves is possible thanks to the organized formation of a syncytium through different connexins pores, that form an alphabet still to be fully deciphered to understand this long-range directional communication of information through astrocytes (Wallraff et al., 2004; Theis and Giaume, 2012; Decrock et al., 2015). Astrocytes respond not only to glutamate, but to most neurotransmitters and neuromodulators, such as GABA, noradrenaline, acetylcholine or adenosine 5′-triphosphate (ATP; Volterra and Meldolesi, 2005; Haydon and Carmignoto, 2006). Astrocytic calcium waves can feedback to influence neuronal responses (Nedergaard, 1994; Parpura et al., 1994) and control synaptic strength (Jourdain et al., 2007; Perea and Araque, 2007) through the release of different mediators such as glutamate, ATP, D-serine, NO, neurotrophins, prostaglandins or cytokines to name a few (Volterra and Meldolesi, 2005; Haydon and Carmignoto, 2006). Several of these mediators are released from astrocytes in a vesicular manner, with SNARE complexes similar, but with some differences to neurons (e.g., synaptobrevin 2), able to sustain a quantal release (Bezzi et al., 2004; Pangrsic et al., 2007). The relevance of this astrocytic vesicular apparatus to control synaptic function is re-enforced by the observation that astrocytic processes enwrap synapses in a spatially organized manner, with a single astrocyte wrapping from 300 (in rodents) up to 90,000 (in humans) synapses (Bushong et al., 2002; Ogata and Kosaka, 2002; Oberheim et al., 2006) and this association of astrocytes with synapses was found to be an experience-dependent dynamic process (Genoud et al., 2006; Haber et al., 2006). This prompted the concept of the tri-partite synapse to recognize the importance of the astrocytic network as a new level of integration of information in neuronal networks (Araque et al., 1999, 2014; Halassa et al., 2007). Accordingly, synaptic plasticity processes are controlled by different gliotransmitters such as D-serine (Yang et al., 2003; Panatier et al., 2006; Henneberger et al., 2010), glutamate (Fellin et al., 2004), ATP (Koizumi et al., 2003; Zhang et al., 2003; Pankratov and Lalo, 2015) or adenosine (Newman, 2003; Pascual et al., 2005; Serrano et al., 2006) or by controlling glutamate clearance (Diamond, 2001; Tsvetkov et al., 2004; Omrani et al., 2009; Murphy-Royal et al., 2015). Accordingly, astrocytic function critically affects integrated brain responses such as sleep, mood or memory (Banasr and Duman, 2008; Halassa et al., 2009; Suzuki et al., 2011; Lima et al., 2014; Perea et al., 2014; Matos et al., 2015).

The addition of microglia to the number of synaptic players is more recent. Microglia coordinate brain innate immunity, displaying features characteristic of immune cells able to rapidly expand their population, to chemotaxically migrate to sites of injury and to trigger and sustain inflammatory responses through their chemokine and cytokine repertoire (Lynch, 2009; Kettenmann et al., 2011). Traditionally, microglia were considered to be "resting", becoming "activated" upon allostatic changes to coordinate immune-like responses (Perry and Gordon, 1988). Several studies revealed that the purported "resting"-state of microglia actually corresponds to an active surveying state, where microglia phylopodia dynamically interact with neurons and astrocytes with a regulatory and supportive role critical for brain homeostasis (Raivich, 2005; Hanisch and Kettenmann, 2007; Wake et al., 2013; Cherry et al., 2014). In particular, microglia dynamically interact with synapses in an activity-dependent manner (Biber et al., 2007; Kettenmann et al., 2013; Wake et al., 2013), to such as extent that the concept of a quad-partite synapse has been forwarded (Schafer et al., 2013). In fact, microglia are equipped with receptors for neurotransmitters (Pocock and Kettenmann, 2007), and excitatory transmission increases whereas inhibitory transmission decreases microglial processes dynamic (Fontainhas et al., 2011; Wong et al., 2011). Conversely, microglia can affect both excitatory and inhibitory transmission (Tsuda et al., 2003; Pascual et al., 2012) through the release of a variety of signals ranging from chemokines (Schafer et al., 2012), cytokines (Rebola et al., 2011), purines (Pascual et al., 2012; George et al., 2015), glutamate and D-serine (Scianni et al., 2013), NO (Zhan et al., 2014) or BDNF (Gomes et al., 2013; Parkhurst et al., 2013). The importance of this bi-directional communication between synapses and microglia is best heralded by the synaptic dysfunction observed upon genetic manipulation of microglia function (Roumier et al., 2004; Costello et al., 2011; Hoshiko et al., 2012), which can be direct or involve astrocytes (Pascual et al., 2012). Thus, microglia are critical for the dynamic synaptic carving that is essential to entrain adaptive brain function (Paolicelli et al., 2011; Ji et al., 2013; Cristovão et al., 2014; Zhan et al., 2014).

DYSFUNCTION OF GLIAL CELLS AND DEPRESSION

Numerous lines of evidence support the contention that a modification of astrocytes in frontolimbic regions is associated with depression (Altshuler et al., 2010; Rajkowska and Stockmeier, 2013; Peng et al., 2015). Most studies analyzing post-mortem brain samples from adult individuals with major

depressive disorder or suicide completers concur to conclude that there is a decreased number of astrocytic-like elements in frontolimbic structures (Ongür et al., 1998; Rajkowska et al., 1999; Cotter et al., 2002; Medina et al., 2015; Nagy et al., 2015; Torres-Platas et al., 2015). This is paralleled by an alteration of astrocytic morphology, typified by hypertrophic cell bodies (Rajkowska et al., 1999; Cotter et al., 2002; Torres-Platas et al., 2011, 2015), and a modification in frontolimbic regions of the density of different astrocytic markers, such as GFAP (Miguel-Hidalgo et al., 2000; Si et al., 2004; Schlicht et al., 2007; Gittins and Harrison, 2011), connexins (Ernst et al., 2011; Sun et al., 2012; Miguel-Hidalgo et al., 2014), aquaporin-4 (Rajkowska et al., 2013), GLT-1 and glutamine synthase (Choudary et al., 2005; Sequeira et al., 2009; Miguel-Hidalgo et al., 2010) and an increased release of S100β (e.g., Grabe et al., 2001; Schroeter et al., 2008). A causal relation between astrocytic dysfunction and depression is provided by animal studies showing that the selective destruction of frontocortical astrocytes with the gliotoxin L-α-aminoadipic acid is sufficient to trigger a depressive-like phenotype (Banasr and Duman, 2008); likewise, a depressive-like phenotype also emerges upon functional inhibition of astrocytes, such as upon downregulating synaptobrevin-2 thus blunting astrocytic vesicular release (Cao et al., 2013), upon altering connexin-mediated gap-junctions (Sun et al., 2012), upon knocking out IP3-receptor type-2 (Cao et al., 2013) or aquaporin-4 (Kong et al., 2014) or inhibiting astrocytic glutamate transporters (Bechtholt-Gompf et al., 2010; John et al., 2012). Notably, astrocytic function is affected by the signaling systems assumed as traditional culprits of depression, namely glucocorticoids (Yin et al., 2013), BDNF (Ye et al., 2011; Liu et al., 2015), serotonin (Hertz et al., 2015), noradrenaline (Madrigal et al., 2009; Pankratov and Lalo, 2015) or dopamine (Shao et al., 2013). Furthermore, treatments alleviating depressive symptoms can recover astrocytic function, such as SSRIs (Czéh et al., 2006; Schipke et al., 2011) or electroconvulsive shock (Iwata et al., 2011) and some are even critically dependent on astrocytic function, such as fluoxetine (Kong et al., 2009) or deep brain stimulation (Etiévant et al., 2015). Altogether, these observations indicate that a decreased astrocytic function in frontolimbic regions is necessary and sufficient for the emergence of depressive symptoms (**Figure 1**). This suggests a scenario where a defective astrocyte function

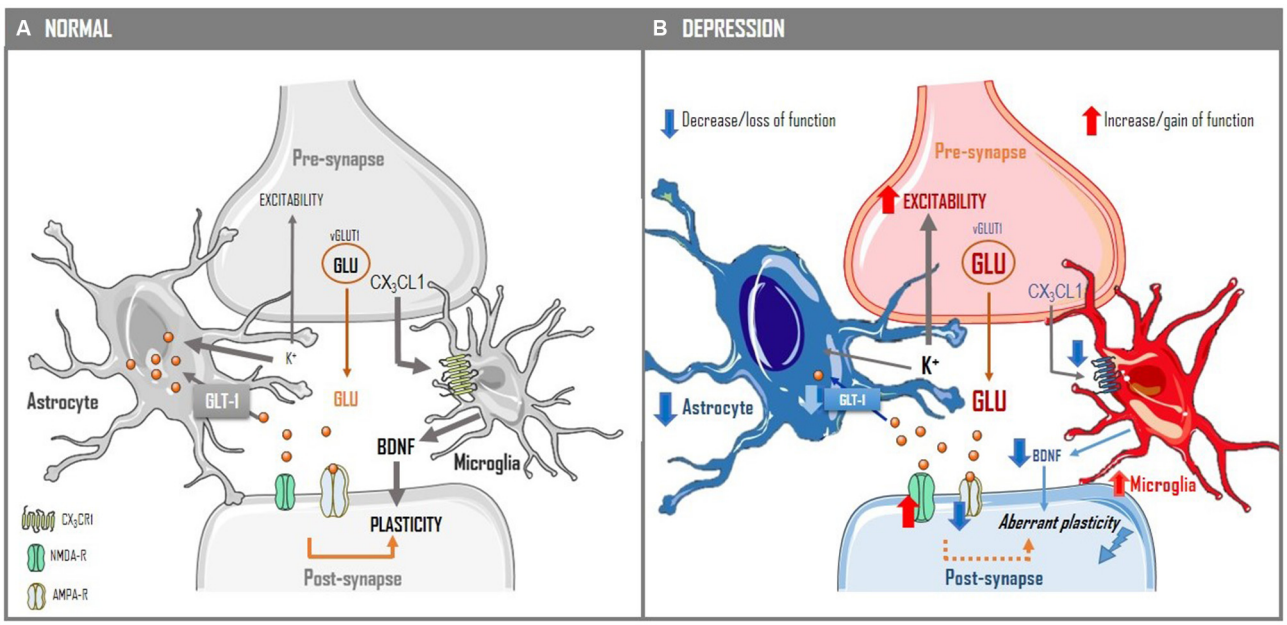


FIGURE 1 | Modifications of the quad-partite synapse in depression. The processing of information in synapses is not only defined by neurons, but also by glia cells, namely by astrocytes, which enwrap synapses, and microglia, which dynamically interact with synapses in an activity-dependent manner **(A)**. Thus, amongst other roles, astrocytes regulate both K^+ and the basal levels of glutamate, defining the basal excitability of neurons; also the extracellular levels of glutamate contribute to define the density of NMDA and AMPA receptors in the plasma membrane of the post-synaptic compartment, which trigger and sustain alterations of synaptic plasticity, respectively. The excitability of neurons, their set-up of plasma membrane glutamate receptors, namely of NMDA receptors, and the neurotrophins support provided by glial cells are critical to allow the implementation of adequate synaptic plasticity traits, i.e., an appropriate encoding of both long-term potentiation (LTP) and depression (LTD) according to specific patterns of incoming information. In depression **(B)** there is a modification of information processing in excitatory synapses in frontolimbic area: there is modification of astrocytes, typified by hypertrophic cell bodies and decreased complexity and activity of phylopodia, resulting in an hypofunction of astrocytes and a decreased ability to buffer K^+ and to clear extracellular glutamate; this leads to increased excitability, corresponding to greater noise in the circuit, and to an imbalance of NMDA and AMPA receptors, with an increased availability of NMDA receptors in dendrites. Additionally, there is a decreased signaling through the master chemokine CX3CL1 in microglia, which is associated (through mechanisms still undefined) to a shift of microglia towards a more pro-inflammatory profile associated with decreased microglia-derived BDNF release. These alterations of the quad-partite synapse, typified by the altered inter-relations between glial cells and neurons, are associated with an aberrant plasticity, typified by a shift in the stimuli triggering LTD and LTP, which translates into inadequate encoding of information in frontocortical circuits.

initially hampers synaptic plasticity, which then evolves into neuronal loss at advanced phases of depressive disorders.

Depression is also tightly associated with alterations of microglia and inflammation (Yirmiya et al., 2015; **Figure 1**). Thus, depressive patients exhibit increased levels of peripheral inflammatory markers (Raison et al., 2006; Howren et al., 2009); conversely, bolstering inflammation triggers a sickness behavior reminiscent of depression (Dantzer et al., 2008) and patients with inflammatory and autoimmune diseases often experience depression (Kiecolt-Glaser et al., 2015). Indeed, most studies converge to propose that microglia are morphologically altered in frontolimbic regions of depressed patients or suicide completers (Steiner et al., 2008; Schnieder et al., 2014; Torres-Platas et al., 2014; Setiawan et al., 2015). Likewise, repeated stress in rodents also triggers microglia dystrophy (Kreisel et al., 2014; Milior et al., 2015; Ślusarczyk et al., 2015) and the manipulation of microglia function, altering its dynamic (Kreisel et al., 2014) or hampering microglia-neuron communication via the CX3CR1-fractalkine pathway (Corona et al., 2010; Milior et al., 2015), alters stress responsiveness and depressive-like behavior. Furthermore, microglia function is affected by all the signaling systems traditionally associated with depression, namely glucocorticoids (Ros-Bernal et al., 2011), BDNF (Gomes et al., 2013), serotonin (Müller and Schwarz, 2007; Krabbe et al., 2012), noradrenaline or dopamine (Färber et al., 2005). Further highlighting the role of microglia in depression are the observations that antidepressants like ketamine (Walker et al., 2013), fluoxetine (Chung et al., 2011) or citalopram (Su et al., 2015) regulate microglia function and minocycline, an inhibitor of microglia, simultaneously recovers microglia function and emotional impairments (Hinwood et al., 2013). Thus, the available evidence indicates that microglia dysfunction is a core event in depression (**Figure 1**), affecting synaptic plasticity either directly (Paolicelli et al., 2011; Zhan et al., 2014) or indirectly through its ability to control astrocytic function (Pascual et al., 2012).

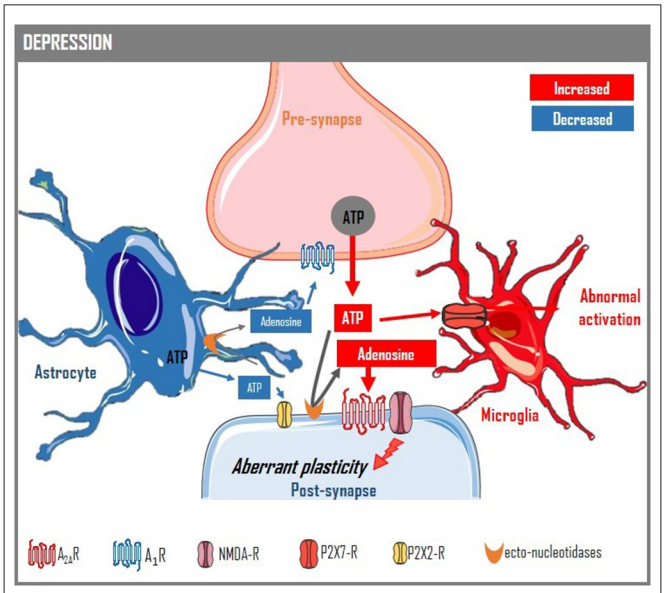


FIGURE 2 | Purine-based control of glia-neuron bidirectional communication in depression. Associated with astrocytic hypofunction in depression, there is a lower release of ATP and adenosine from astrocytes (Cao et al., 2013; Hines et al., 2013). This causes a deficient astrocyte-to-neuron activation of P2X2 (ATP) receptors (Cao et al., 2013) and a deficient activation of inhibitory adenosine A_1 receptors in neurons (Serchov et al., 2015), which density decreases upon chronic stress (Cunha et al., 2006; Kaster et al., 2015). In parallel, there is an increased excitability (increased noise), which bolsters the synaptic release of ATP (Cunha et al., 1996), an up-regulation of synaptic adenosine A_{2A} receptors (Cunha et al., 2006; Batalha et al., 2013; Kaster et al., 2015), which is associated with aberrant plasticity (Li et al., 2015), and an up-regulation of microglia P2X7 receptors, which contributes to microglia hyper-responsiveness upon depression (Stokes et al., 2015). This illustrates the role of the purinergic system in the control of the homeostasis of the quad-partite synapse and shows that maladaptive changes in the purine neuromodulation system occur upon depression that can be exploited therapeutically, such as increasing the release of ATP and adenosine from astrocytes, decreasing the neuronal activation of A_{2A} receptor or bolstering the neuronal activation of A_1 receptors or inhibiting microglia P2X7 receptors.

INTEGRATIVE ROLE OF PURINES IN THE QUAD-PARTITE SYNAPSE TO CONTROL DEPRESSION

In view of the key role of aberrant synaptic plasticity involving neuronal, astrocytic and microglia dysfunction (**Figure 1**), therapeutic strategies to manage depression should ideally target systems dedicated to the control of neuron-glia bidirectional communication. Purines operate one such system through the action of ATP and adenosine (**Figure 2**). ATP is released in a controlled manner from synaptic terminals, astrocytes and microglia and it is a documented signal to control astrogliosis, microglia dynamics and reactivity and synaptic transmission through ionotropic P2X1–7 and metabotropic P2Y1–13 receptors (reviewed in Rodrigues et al., 2015). Adenosine can be formed from the catabolism of extracellular ATP (Augusto et al., 2013) by ectonucleotidases located in synapses (Cunha, 2001) or released as such through bidirectional nucleoside transporters in synapses (Pinto-Duarte et al., 2005). Adenosine mainly activates inhibitory A_1 and facilitatory A_{2A} receptors (Fredholm et al., 2005) that act neuronally to control synaptic transmission and plasticity (Cunha, 2008) and also control astrocytic (van Calker and Biber, 2005; Matos et al., 2013, 2015) and microglia function (Rebola et al., 2011; Luongo et al., 2014; George et al., 2015).

Notably, both ATP and adenosine signaling have been implicated in the control of depressive conditions (**Figure 2**). Thus, a deficient astrocyte-derived ATP release providing an insufficient P2X2 receptor-mediated neuronal tonus was identified in chronically stressed mice (Cao et al., 2013); also, the prevention of excessive P2X7 receptor activation in glial cells ameliorates depressive-like conditions (Stokes et al., 2015) and P2X7 receptor polymorphisms lead to vulnerability to mood disorders (Bennett, 2007). The case for an involvement of adenosine is more robust. Thus, epidemiological studies show an inverse relation between the intake of moderate amounts of caffeine (an adenosine receptor antagonist) and the incidence of depression (Lucas et al., 2011) and suicide

(Lucas et al., 2014). Accordingly, adenosine A_{2A} receptors are up-regulated in animal models of chronic stress and polymorphisms of A_{2A} receptors are associated with emotional disturbances (reviewed in Cunha et al., 2008), their over-expression triggers emotional dysfunction (Coelho et al., 2014) and their blockade prevents chronic stress-induced emotional dysfunction (Kaster et al., 2015). Additionally, there is a hypofunction of neuronal A_1 receptors due to decrease astrocyte-derived adenosine (Hines et al., 2013), which compensation with various antidepressant treatments can revert depressive-like behavior (Etiévant et al., 2015; Serchov et al., 2015).

This compilation of evidences illustrates the relevance of the purinergic signaling in the control of neuron-glia bidirectional communication and its therapeutic potential in the normalization of aberrant synaptic processing in frontolimbic circuits upon depression. Based on the available information, the most promising strategy is a multi-target approach, based on the increase of astrocytic release of purines (both ATP and adenosine to activate A_1 receptors) coupled to antagonists of P2X7 and of A_{2A} receptors (**Figure 2**).

AUTHOR CONTRIBUTIONS

RAC planned and organized the review; all others contributed with valuable suggestions, partial writting and editing of the review.

ACKNOWLEDGMENTS

The research activity was supported by funding from NARSAD, *Fundação para a Ciência e a Tecnologia* (PTDC/NEU-NMC/4154/2014, UID/NEU/04539/2013), COMPETE and FCT-CAPES.

REFERENCES

Achour, S., and Pascual, O. (2010). Glia: the many ways to modulate synaptic plasticity. *Neurochem. Int.* 57, 440–445. doi: 10.1016/j.neuint.2010.02.013

Agostinho, P., Cunha, R. A., and Oliveira, C. (2010). Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. *Curr. Pharm. Des.* 16, 2766–2778. doi: 10.2174/138161210793176572

Allen, N. J. (2014). Astrocyte regulation of synaptic behavior. *Annu. Rev. Cell Dev. Biol.* 30, 439–463. doi: 10.1146/annurev-cellbio-100913-013053

Altshuler, L. L., Abulseoud, O. A., Foland-Ross, L., Bartzokis, G., Chang, S., Mintz, J., et al. (2010). Amygdala astrocyte reduction in subjects with major depressive disorder but not bipolar disorder. *Bipolar Disord.* 12, 541–549. doi: 10.1111/j.1399-5618.2010.00838.x

Araque, A., Carmignoto, G., Haydon, P. G., Oliet, S. H., Robitaille, R., and Volterra, A. (2014). Gliotransmitters travel in time and space. *Neuron* 81, 728–739. doi: 10.1016/j.neuron.2014.02.007

Araque, A., Parpura, V., Sanzgiri, R. P., and Haydon, P. G. (1999). Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* 22, 208–215. doi: 10.1016/s0166-2236(98)01349-6

Arnth-Jensen, N., Jabaudon, D., and Scanziani, M. (2002). Cooperation between independent hippocampal synapses is controlled by glutamate uptake. *Nat. Neurosci.* 5, 325–331. doi: 10.1038/nn825

Asztely, F., Erdemli, G., and Kullmann, D. M. (1997). Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake. *Neuron* 18, 281–293. doi: 10.1016/s0896-6273(00)80268-8

Augusto, E., Matos, M., Sévigny, J., El-Tayeb, A., Bynoe, M. S., Müller, C. E., et al. (2013). Ecto-5′-nucleotidase (CD73)-mediated formation of adenosine is critical for the striatal adenosine A_{2A} receptor functions. *J. Neurosci.* 33, 11390–11399. doi: 10.1523/JNEUROSCI.5817-12.2013

Banasr, M., and Duman, R. S. (2008). Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol. Psychiatry* 64, 863–870. doi: 10.1016/j.biopsych.2008.06.008

Batalha, V. L., Pego, J. M., Fontinha, B. M., Costenla, A. R., Valadas, J. S., Baqi, Y., et al. (2013). Adenosine A_{2A} receptor blockade reverts hippocampal stress-induced deficits and restores corticosterone circadian oscillation. *Mol. Psychiatry* 18, 320–331. doi: 10.1038/mp.2012.8

Bechtholt-Gompf, A. J., Walther, H. V., Adams, M. A., Carlezon, W. A., Jr., Ongür, D., and Cohen, B. M. (2010). Blockade of astrocytic glutamate uptake in rats induces signs of anhedonia and impaired spatial memory. *Neuropsychopharmacology* 35, 2049–2059. doi: 10.1038/npp.2010.74

Bennett, M. R. (2007). Synaptic P2X7 receptor regenerative-loop hypothesis for depression. *Aust. N Z J Psychiatry* 41, 563–571. doi: 10.1080/00048670701399994

Berman, R. M., Cappiello, A., Anand, A., Oren, D. A., Heninger, G. R., Charney, D. S., et al. (2000). Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry* 47, 351–354. doi: 10.1016/s0006-3223(99)00230-9

Berton, O., Hahn, C. G., and Thase, M. E. (2012). Are we getting closer to valid translational models for major depression? *Science* 338, 75–79. doi: 10.1126/science.1222940

Bezzi, P., Gundersen, V., Galbete, J. L., Seifert, G., Steinhäuser, C., Pilati, E., et al. (2004). Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. *Nat. Neurosci.* 7, 613–620. doi: 10.1038/nn1246

Biber, K., Neumann, H., Inoue, K., and Boddeke, H. W. (2007). Neuronal 'On' and 'Off' signals control microglia. *Trends Neurosci.* 30, 596–602. doi: 10.1016/j.tins.2007.08.007

Bushong, E. A., Martone, M. E., Jones, Y. Z., and Ellisman, M. H. (2002). Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J. Neurosci.* 22, 183–192.

Cao, X., Li, L. P., Wang, Q., Wu, Q., Hu, H. H., Zhang, M., et al. (2013). Astrocyte-derived ATP modulates depressive-like behaviors. *Nat. Med.* 19, 773–777. doi: 10.1038/nm.3162

Castrén, E. (2014). Neurotrophins and psychiatric disorders. *Handb. Exp. Pharmacol.* 220, 461–479. doi: 10.1007/978-3-642-45106-5_17

Cherry, J. D., Olschowka, J. A., and O'Banion, M. K. (2014). Are "resting" microglia more "m2"? *Front. Immunol.* 5:594. doi: 10.3389/fimmu.2014.00594

Choudary, P. V., Molnar, M., Evans, S. J., Tomita, H., Li, J. Z., Vawter, M. P., et al. (2005). Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proc. Natl. Acad. Sci. U S A* 102, 15653–15658. doi: 10.1073/pnas.0507901102

Chung, Y. C., Kim, S. R., Park, J. Y., Chung, E. S., Park, K. W., Won, S. Y., et al. (2011). Fluoxetine prevents MPTP-induced loss of dopaminergic neurons by inhibiting microglial activation. *Neuropharmacology* 60, 963–974. doi: 10.1016/j.neuropharm.2011.01.043

Coelho, J. E., Alves, P., Canas, P. M., Valadas, J. S., Shmidt, T., Batalha, V. L., et al. (2014). Overexpression of adenosine A_{2A} receptors in rats: effects on depression, locomotion, and anxiety. *Front. Psychiatry* 5:67. doi: 10.3389/fpsyt.2014.00067

Cornell-Bell, A. H., Finkbeiner, S. M., Cooper, M. S., and Smith, S. J. (1990). Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science* 247, 470–473. doi: 10.1126/science.1967852

Corona, A. W., Huang, Y., O'Connor, J. C., Dantzer, R., Kelley, K. W., Popovich, P. G., et al. (2010). Fractalkine receptor (CX3CR1) deficiency sensitizes mice to the behavioral changes induced by lipopolysaccharide. *J. Neuroinflammation* 7:93. doi: 10.1186/1742-2094-7-93

Coryell, W., and Young, E. A. (2005). Clinical predictors of suicide in primary major depressive disorder. *J. Clin. Psychiatry* 66, 412–417. doi: 10.4088/jcp.v66n0401

Costello, D. A., Lyons, A., Denieffe, S., Browne, T. C., Cox, F. F., and Lynch, M. A. (2011). Long-term potentiation is impaired in membrane glycoprotein CD200-deficient mice: a role for Toll-like receptor activation. *J. Biol. Chem.* 286, 34722–34732. doi: 10.1074/jbc.M111.280826

Cotter, D., Mackay, D., Chana, G., Beasley, C., Landau, S., and Everall, I. P. (2002). Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. *Cereb. Cortex* 12, 386–394. doi: 10.1093/cercor/12.4.386

Cristovão, G., Pinto, M. J., Cunha, R. A., Almeida, R. D., and Gomes, C. A. (2014). Activation of microglia bolsters synapse formation. *Front. Cell. Neurosci.* 8:153. doi: 10.3389/fncel.2014.00153

Crunelli, V., Carmignoto, G., and Steinhäuser, C. (2015). Novel astrocyte targets: new avenues for the therapeutic treatment of epilepsy. *Neuroscientist* 21, 62–83. doi: 10.1177/1073858414523320

Cunha, R. A. (2001). Regulation of the ecto-nucleotidase pathway in rat hippocampal nerve terminals. *Neurochem. Res.* 26, 979–991. doi: 10.1046/j.1471-4159.2000.0740334.x

Cunha, R. A. (2008). Different cellular sources and different roles of adenosine: A_1 receptor-mediated inhibition through astrocytic-driven volume transmission and synapse-restricted A_{2A} receptor-mediated facilitation of plasticity. *Neurochem. Int.* 52, 65–72. doi: 10.1016/j.neuint.2007.06.026

Cunha, G. M., Canas, P. M., Oliveira, C. R., and Cunha, R. A. (2006). Increased density and synapto-protective effect of adenosine A_{2A} receptors upon sub-chronic restraint stress. *Neuroscience* 141, 1775–1781. doi: 10.1016/j.neuroscience.2006.05.024

Cunha, R. A., Ferré, S., Vaugeois, J. M., and Chen, J. F. (2008). Potential therapeutic interest of adenosine A_{2A} receptors in psychiatric disorders. *Curr. Pharm. Des.* 14, 1512–1524. doi: 10.2174/138161208784480090

Cunha, R. A., Vizi, E. S., Ribeiro, J. A., and Sebastião, A. M. (1996). Preferential release of ATP and its extracellular catabolism as a source of adenosine upon high- but not low-frequency stimulation of rat hippocampal slices. *J. Neurochem.* 67, 2180–2187. doi: 10.1046/j.1471-4159.1996.67052180.x

Czéh, B., Simon, M., Schmelting, B., Hiemke, C., and Fuchs, E. (2006). Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology* 31, 1616–1626. doi: 10.1038/sj.npp.1300982

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., and Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. doi: 10.1038/nrn2297

Decrock, E., De Bock, M., Wang, N., Bultynck, G., Giaume, C., Naus, C. C., et al. (2015). Connexin and pannexin signaling pathways, an architectural blueprint for CNS physiology and pathology? *Cell. Mol. Life Sci.* 72, 2823–2851. doi: 10.1007/s00018-015-1962-7

de Kloet, E. R., Joëls, M., and Holsboer, F. (2005). Stress and the brain: from adaptation to disease. *Nat. Rev. Neurosci.* 6, 463–475. doi: 10.1038/nrn1683

Diamond, J. S. (2001). Neuronal glutamate transporters limit activation of NMDA receptors by neurotransmitter spillover on CA1 pyramidal cells. *J. Neurosci.* 21, 8328–8338.

Duman, R. S., and Aghajanian, G. K. (2012). Synaptic dysfunction in depression: potential therapeutic targets. *Science* 338, 68–72. doi: 10.1126/science.1222939

Duric, V., Banasr, M., Stockmeier, C. A., Simen, A. A., Newton, S. S., Overholser, J. C., et al. (2013). Altered expression of synapse and glutamate related genes in post-mortem hippocampus of depressed subjects. *Int. J. Neuropsychopharmacol.* 16, 69–82. doi: 10.1017/S1461145712000016

Ernst, C., Nagy, C., Kim, S., Yang, J. P., Deng, X., Hellstrom, I. C., et al. (2011). Dysfunction of astrocyte connexins 30 and 43 in dorsal lateral prefrontal cortex of suicide completers. *Biol. Psychiatry* 70, 312–319. doi: 10.1016/j.biopsych.2011.03.038

Etiévant, A., Oosterhof, C., Bétry, C., Abrial, E., Novo-Perez, M., Rovera, R., et al. (2015). Astroglial control of the antidepressant-like effects of prefrontal cortex deep brain stimulation. *EBioMedicine* 2, 896–906. doi: 10.1016/j.ebiom.2015.06.023

Färber, K., Pannasch, U., and Kettenmann, H. (2005). Dopamine and noradrenaline control distinct functions in rodent microglial cells. *Mol. Cell. Neurosci.* 29, 128–138. doi: 10.1016/j.mcn.2005.01.003

Fellin, T., Pascual, O., Gobbo, S., Pozzan, T., Haydon, P. G., and Carmignoto, G. (2004). Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. *Neuron* 43, 729–743. doi: 10.1016/j.neuron.2004.08.011

Ferrari, A. J., Charlson, F. J., Norman, R. E., Patten, S. B., Freedman, G., Murray, C. J., et al. (2013). Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med.* 10:e1001547. doi: 10.1371/journal.pmed.1001547

Feyissa, A. M., Chandran, A., Stockmeier, C. A., and Karolewicz, B. (2009). Reduced levels of NR2A and NR2B subunits of NMDA receptor and PSD-95 in the prefrontal cortex in major depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 33, 70–75. doi: 10.1016/j.pnpbp.2008.10.005

Figley, C. R., and Stroman, P. W. (2011). The role(s) of astrocytes and astrocyte activity in neurometabolism, neurovascular coupling, and the production of functional neuroimaging signals. *Eur. J. Neurosci.* 33, 577–588. doi: 10.1111/j.1460-9568.2010.07584.x

Fontainhas, A. M., Wang, M., Liang, K. J., Chen, S., Mettu, P., Damani, M., et al. (2011). Microglial morphology and dynamic behavior is regulated by ionotropic glutamatergic and GABAergic neurotransmission. *PLoS One* 6:e15973. doi: 10.1371/journal.pone.0015973

Fredholm, B. B., Chen, J. F., Cunha, R. A., Svenningsson, P., and Vaugeois, J. M. (2005). Adenosine and brain function. *Int. Rev. Neurobiol.* 63, 191–270. doi: 10.1016/S0074-7742(05)63007-3

Genoud, C., Quairiaux, C., Steiner, P., Hirling, H., Welker, E., and Knott, G. W. (2006). Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. *PLoS Biol.* 4:e343. doi: 10.1371/journal.pbio.0040343

George, J., Gonçalves, F. Q., Cristóvão, G., Rodrigues, L., Meyer Fernandes, J. R., Gonçalves, T., et al. (2015). Different danger signals differently impact on microglial proliferation through alterations of ATP release and extracellular metabolism. *Glia* 63, 1636–1645. doi: 10.1002/glia.22833

Gilabert-Juan, J., Varea, E., Guirado, R., Blasco-Ibáñez, J. M., Crespo, C., and Nácher, J. (2012). Alterations in the expression of PSA-NCAM and synaptic proteins in the dorsolateral prefrontal cortex of psychiatric disorder patients. *Neurosci. Lett.* 530, 97–102. doi: 10.1016/j.neulet.2012.09.032

Gittins, R. A., and Harrison, P. J. (2011). A morphometric study of glia and neurons in the anterior cingulate cortex in mood disorder. *J. Affect. Disord.* 133, 328–332. doi: 10.1016/j.jad.2011.03.042

Gomes, C., Ferreira, R., George, J., Sanches, R., Rodrigues, D. I., Goncalves, N., et al. (2013). Activation of microglial cells triggers a release of brain-derived neurotrophic factor (BDNF) inducing their proliferation in an adenosine A_{2A} receptor-dependent manner: A_{2A} receptor blockade prevents BDNF release and proliferation of microglia. *J. Neuroinflammation* 10:16. doi: 10.1186/1742-2094-10-16

Grabe, H. J., Ahrens, N., Rose, H. J., Kessler, C., and Freyberger, H. J. (2001). Neurotrophic factor S100 beta in major depression. *Neuropsychobiology* 44, 88–90. doi: 10.1159/000054922

Gray, J. D., Milner, T. A., and McEwen, B. S. (2013). Dynamic plasticity: the role of glucocorticoids, brain-derived neurotrophic factor and other trophic factors. *Neuroscience* 239, 214–227. doi: 10.1016/j.neuroscience.2012.08.034

Gyekis, J. P., Yu, W., Dong, S., Wang, H., Qian, J., Kota, P., et al. (2013). No association of genetic variants in BDNF with major depression: a meta- and gene-based analysis. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 162B, 61–70. doi: 10.1002/ajmg.b.32122

Haber, M., Zhou, L., and Murai, K. K. (2006). Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. *J. Neurosci.* 26, 8881–8891. doi: 10.1523/jneurosci.1302-06.2006

Halassa, M. M., Fellin, T., and Haydon, P. G. (2007). The tripartite synapse: roles for gliotransmission in health and disease. *Trends Mol. Med.* 13, 54–63. doi: 10.1016/j.molmed.2006.12.005

Halassa, M. M., Florian, C., Fellin, T., Munoz, J. R., Lee, S. Y., Abel, T., et al. (2009). Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron* 61, 213–219. doi: 10.1016/j.neuron.2008.11.024

Hanisch, U. K., and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat. Neurosci.* 10, 1387–1394. doi: 10.1038/nn1997

Haydon, P. G., and Carmignoto, G. (2006). Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol. Rev.* 86, 1009–1031. doi: 10.1152/physrev.00049.2005

Henneberger, C., Papouin, T., Oliet, S. H., and Rusakov, D. A. (2010). Long-term potentiation depends on release of D-serine from astrocytes. *Nature* 463, 232–236. doi: 10.1038/nature08673

Hertz, L., Rothman, D. L., Li, B., and Peng, L. (2015). Chronic SSRI stimulation of astrocytic 5-HT2B receptors change multiple gene expressions/editings and metabolism of glutamate, glucose and glycogen: a potential paradigm shift. *Front. Behav. Neurosci.* 9:25. doi: 10.3389/fnbeh.2015.00025

Hines, D. J., Schmitt, L. I., Hines, R. M., Moss, S. J., and Haydon, P. G. (2013). Antidepressant effects of sleep deprivation require astrocyte-dependent adenosine mediated signaling. *Transl. Psychiatry* 3:e212. doi: 10.1038/tp.2012.136

Hinwood, M., Tynan, R. J., Charnley, J. L., Beynon, S. B., Day, T. A., and Walker, F. R. (2013). Chronic stress induced remodeling of the prefrontal cortex: structural re-organization of microglia and the inhibitory effect of minocycline. *Cereb. Cortex* 23, 1784–1797. doi: 10.1093/cercor/bhs151

Hoshiko, M., Arnoux, I., Avignone, E., Yamamoto, N., and Audinat, E. (2012). Deficiency of the microglial receptor CX3CR1 impairs postnatal functional development of thalamocortical synapses in the barrel cortex. *J. Neurosci.* 32, 15106–15111. doi: 10.1523/JNEUROSCI.1167-12.2012

Howren, M. B., Lamkin, D. M., and Suls, J. (2009). Associations of depression with C-reactive protein, IL-1 and IL-6: a meta-analysis. *Psychosom. Med.* 71, 171–186. doi: 10.1097/PSY.0b013e3181907c1b

Iwata, M., Shirayama, Y., Ishida, H., Hazama, G. I., and Nakagome, K. (2011). Hippocampal astrocytes are necessary for antidepressant treatment of learned helplessness rats. *Hippocampus* 21, 877–884. doi: 10.1002/hipo.20803

Ji, K., Akgul, G., Wollmuth, L. P., and Tsirka, S. E. (2013). Microglia actively regulate the number of functional synapses. *PLoS One* 8:e56293. doi: 10.1371/journal.pone.0056293

John, C. S., Smith, K. L., Van't Veer, A., Gompf, H. S., Carlezon, W. A., Jr., Cohen, B. M., et al. (2012). Blockade of astrocytic glutamate uptake in the prefrontal cortex induces anhedonia. *Neuropsychopharmacology* 37, 2467–2475. doi: 10.1038/npp.2012.105

Jourdain, P., Bergersen, L. H., Bhaukaurally, K., Bezzi, P., Santello, M., Domercq, M., et al. (2007). Glutamate exocytosis from astrocytes controls synaptic strength. *Nat. Neurosci.* 10, 331–339. doi: 10.1038/nn1849

Kang, H. J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C. A., Licznerski, P., et al. (2012). Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. *Nat. Med.* 18, 1413–1417. doi: 10.1038/nm.2886

Kaster, M. P., Machado, N. J., Silva, H. B., Nunes, A., Ardais, A. P., Santana, M., et al. (2015). Caffeine acts through neuronal adenosine A_{2A} receptors to prevent mood and memory dysfunction triggered by chronic stress. *Proc. Natl. Acad. Sci. U S A* 112, 7833–7838. doi: 10.1073/pnas.1423088112

Kessler, R. C., Berglund, P., Demler, O., Jin, R., Koretz, D., Merikangas, K. R., et al. (2003). The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). *JAMA.* 289, 3095–3105. doi: 10.1001/jama.289.23.3095

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553. doi: 10.1152/physrev.00011.2010

Kettenmann, H., Kirchhoff, F., and Verkhratsky, A. (2013). Microglia: new roles for the synaptic stripper. *Neuron* 77, 10–18. doi: 10.1016/j.neuron.2012.12.023

Kiecolt-Glaser, J. K., Derry, H. M., and Fagundes, C. P. (2015). Inflammation: depression fans the flames and feasts on the heat. *Am. J. Psychiatry* 172, 1075–1091. doi: 10.1176/appi.ajp.2015.15020152

Koizumi, S., Fujishita, K., Tsuda, M., Shigemoto-Mogami, Y., and Inoue, K. (2003). Dynamic inhibition of excitatory synaptic transmission by astrocyte-derived ATP in hippocampal cultures. *Proc. Natl. Acad. Sci. U S A* 100, 11023–11028. doi: 10.1073/pnas.1834448100

Kong, H., Sha, L. L., Fan, Y., Xiao, M., Ding, J. H., Wu, J., et al. (2009). Requirement of AQP4 for antidepressive efficiency of fluoxetine: implication in adult hippocampal neurogenesis. *Neuropsychopharmacology* 34, 1263–1276. doi: 10.1038/npp.2008.185

Kong, H., Zeng, X. N., Fan, Y., Yuan, S. T., Ge, S., Xie, W. P., et al. (2014). Aquaporin-4 knockout exacerbates corticosterone-induced depression by inhibiting astrocyte function and hippocampal neurogenesis. *CNS Neurosci. Ther.* 20, 391–402. doi: 10.1111/cns.12222

Krabbe, G., Matyash, V., Pannasch, U., Mamer, L., Boddeke, H. W., and Kettenmann, H. (2012). Activation of serotonin receptors promotes microglial injury-induced motility but attenuates phagocytic activity. *Brain Behav. Immun.* 26, 419–428. doi: 10.1016/j.bbi.2011.12.002

Kreisel, T., Frank, M. G., Licht, T., Reshef, R., Ben-Menachem-Zidon, O., Baratta, M. V., et al. (2014). Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. *Mol. Psychiatry* 19, 699–709. doi: 10.1038/mp.2013.155

Krishnan, V., and Nestler, E. J. (2008). The molecular neurobiology of depression. *Nature* 455, 894–902. doi: 10.1038/nature07455

Lesch, K. P., and Waider, J. (2012). Serotonin in the modulation of neural plasticity and networks: implications for neurodevelopmental disorders. *Neuron* 76, 175–191. doi: 10.1016/j.neuron.2012.09.013

Li, N., Lee, B., Liu, R. J., Banasr, M., Dwyer, J. M., Iwata, M., et al. (2010). mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science* 329, 959–964. doi: 10.1126/science.1190287

Li, N., Liu, R. J., Dwyer, J. M., Banasr, M., Lee, B., Son, H., et al. (2011). Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol. Psychiatry* 69, 754–761. doi: 10.1016/j.biopsych.2010.12.015

Li, P., Rial, D., Canas, P. M., Yoo, J. H., Li, W., Zhou, X., et al. (2015). Optogenetic activation of intracellular adenosine A_{2A} receptor signaling in the hippocampus is sufficient to trigger CREB phosphorylation and impair memory. *Mol. Psychiatry* 20, 1339–1349. doi: 10.1038/mp.2014.182

Lima, A., Sardinha, V. M., Oliveira, A. F., Reis, M., Mota, C., Silva, M. A., et al. (2014). Astrocyte pathology in the prefrontal cortex impairs the cognitive function of rats. *Mol. Psychiatry* 19, 834–841. doi: 10.1038/mp.2013.182

Liu, W. X., Wang, J., Xie, Z. M., Xu, N., Zhang, G. F., Jia, M., et al. (2015). Regulation of glutamate transporter 1 via BDNF-TrkB signaling plays a role in the anti-apoptotic and antidepressant effects of ketamine in chronic unpredictable stress model of depression. *Psychopharmacology (Berl)* doi: 10.1007/s00213-015-4128-2 [Epub ahead of print].

Lucas, M., Mirzaei, F., Pan, A., Okereke, O. I., Willett, W. C., O'Reilly, É. J., et al. (2011). Coffee, caffeine and risk of depression among women. *Arch. Intern. Med.* 171, 1571–1578. doi: 10.1001/archinternmed.2011.427

Lucas, M., O'Reilly, E. J., Pan, A., Mirzaei, F., Willett, W. C., Okereke, O. I., et al. (2014). Coffee, caffeine and risk of completed suicide: results from three prospective cohorts of American adults. *World J. Biol. Psychiatry* 15, 377–386. doi: 10.3109/15622975.2013.795243

Luongo, L., Guida, F., Imperatore, R., Napolitano, F., Gatta, L., Cristino, L., et al. (2014). The A_1 adenosine receptor as a new player in microglia physiology. *Glia* 62, 122–132. doi: 10.1002/glia.22592

Lynch, M. A. (2009). The multifaceted profile of activated microglia. *Mol. Neurobiol.* 40, 139–156. doi: 10.1007/s12035-009-8077-9

Machado-Vieira, R., Manji, H. K., and Zarate, C. A. (2009). The role of the tripartite glutamatergic synapse in the pathophysiology and therapeutics of mood disorders. *Neuroscientist* 15, 525–539. doi: 10.1177/1073858409336093

Madrigal, J. L., Leza, J. C., Polak, P., Kalinin, S., and Feinstein, D. L. (2009). Astrocyte-derived MCP-1 mediates neuroprotective effects of noradrenaline. *J. Neurosci.* 29, 263–267. doi: 10.1523/JNEUROSCI.4926-08.2009

Magariños, A. M., Verdugo, J. M., and McEwen, B. S. (1997). Chronic stress alters synaptic terminal structure in hippocampus. *Proc. Natl. Acad. Sci. U S A* 94, 14002–14008. doi: 10.1073/pnas.94.25.14002

Magistretti, P. J., Pellerin, L., Rothman, D. L., and Shulman, R. G. (1999). Energy on demand. *Science* 283, 496–497. doi: 10.1126/science.283.5401.496

Marzo, A., Bai, J., and Otani, S. (2009). Neuroplasticity regulation by noradrenaline in mammalian brain. *Curr. Neuropharmacol.* 7, 286–295. doi: 10.2174/157015909790031193

Matos, M., Augusto, E., Agostinho, P., Cunha, R. A., and Chen, J. F. (2013). Antagonistic interaction between adenosine A_{2A} receptors and Na^+/K^+-ATPase-α2 controlling glutamate uptake in astrocytes. *J. Neurosci.* 33, 18492–18502. doi: 10.1523/JNEUROSCI.1828-13.2013

Matos, M., Shen, H. Y., Augusto, E., Wang, Y., Wei, C. J., Wang, Y. T., et al. (2015). Deletion of adenosine A_{2A} receptors from astrocytes disrupts glutamate homeostasis leading to psychomotor and cognitive impairment: relevance to schizophrenia. *Biol. Psychiatry* 78, 763–774. doi: 10.1016/j.biopsych.2015.02.026

McEwen, B. S., Eiland, L., Hunter, R. G., and Miller, M. M. (2012). Stress and anxiety: structural plasticity and epigenetic regulation as a consequence of stress. *Neuropharmacology* 62, 3–12. doi: 10.1016/j.neuropharm.2011.07.014

Medina, A., Watson, S. J., Bunney, W., Jr., Myers, R. M., Schatzberg, A., Barchas, J., et al. (2015). Evidence for alterations of the glial syncytial function in major depressive disorder. *J. Psychiatr. Res.* 72, 15–21. doi: 10.1016/j.jpsychires.2015.10.010

Meyer, R. M., Burgos-Robles, A., Liu, E., Correia, S. S., and Goosens, K. A. (2014). A ghrelin-growth hormone axis drives stress-induced vulnerability to enhanced fear. *Mol. Psychiatry* 19, 1284–1294. doi: 10.1038/mp.2013.135

Miguel-Hidalgo, J. J., Baucom, C., Dilley, G., Overholser, J. C., Meltzer, H. Y., Stockmeier, C. A., et al. (2000). Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. *Biol. Psychiatry* 48, 861–873. doi: 10.1016/s0006-3223(00)00999-9

Miguel-Hidalgo, J. J., Waltzer, R., Whittom, A. A., Austin, M. C., Rajkowska, G., and Stockmeier, C. A. (2010). Glial and glutamatergic markers in depression, alcoholism and their comorbidity. *J. Affect. Disord.* 127, 230–240. doi: 10.1016/j.jad.2010.06.003

Miguel-Hidalgo, J. J., Wilson, B. A., Hussain, S., Meshram, A., Rajkowska, G., and Stockmeier, C. A. (2014). Reduced connexin 43 immunolabeling in the orbitofrontal cortex in alcohol dependence and depression. *J. Psychiatr. Res.* 55, 101–109. doi: 10.1016/j.jpsychires.2014.04.007

Milior, G., Lecours, C., Samson, L., Bisht, K., Poggini, S., Pagani, F., et al. (2015). Fractalkine receptor deficiency impairs microglial and neuronal responsiveness to chronic stress. *Brain Behav. Immun.* doi: 10.1016/j.bbi.2015.07.024 [Epub ahead of print].

Müller, N., and Schwarz, M. J. (2007). The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. *Mol. Psychiatry* 12, 988–1000. doi: 10.1038/sj.mp.4002006

Murphy-Royal, C., Dupuis, J. P., Varela, J. A., Panatier, A., Pinson, B., Baufreton, J., et al. (2015). Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission. *Nat. Neurosci.* 18, 219–226. doi: 10.1038/nn.3901

Nagy, C., Suderman, M., Yang, J., Szyf, M., Mechawar, N., Ernst, C., et al. (2015). Astrocytic abnormalities and global DNA methylation patterns in depression and suicide. *Mol. Psychiatry* 20, 320–328. doi: 10.1038/mp.2014.21

Nedergaard, M. (1994). Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* 263, 1768–1771. doi: 10.1126/science.8134839

Newman, E. A. (2003). Glial cell inhibition of neurons by release of ATP. *J. Neurosci.* 23, 1659–1666.

Oberheim, N. A., Wang, X., Goldman, S., and Nedergaard, M. (2006). Astrocytic complexity distinguishes the human brain. *Trends Neurosci.* 29, 547–553. doi: 10.1016/j.tins.2006.08.004

Ogata, K., and Kosaka, T. (2002). Structural and quantitative analysis of astrocytes in the mouse hippocampus. *Neuroscience* 113, 221–233. doi: 10.1016/s0306-4522(02)00041-6

Oliveira, J. F., Sardinha, V. M., Guerra-Gomes, S., Araque, A., and Sousa, N. (2015). Do stars govern our actions? Astrocyte involvement in rodent behavior. *Trends Neurosci.* 38, 535–549. doi: 10.1016/j.tins.2015.07.006

Omrani, A., Melone, M., Bellesi, M., Safiulina, V., Aida, T., Tanaka, K., et al. (2009). Up-regulation of GLT-1 severely impairs LTD at mossy fibre-CA3 synapses. *J. Physiol. Lond.* 587, 4575–4588. doi: 10.1113/jphysiol.2009.177881

Ongür, D., Drevets, W. C., and Price, J. L. (1998). Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc. Natl. Acad. Sci. U S A* 95, 13290–13295. doi: 10.1073/pnas.95.22.13290

Panatier, A., Theodosis, D. T., Mothet, J. P., Touquet, B., Pollegioni, L., Poulain, D. A., et al. (2006). Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* 125, 775–784. doi: 10.1016/j.cell.2006.02.051

Pangrsic, T., Potokar, M., Stenovec, M., Kreft, M., Fabbretti, E., Nistri, A., et al. (2007). Exocytotic release of ATP from cultured astrocytes. *J. Biol. Chem.* 282, 28749–28758. doi: 10.1074/jbc.m700290200

Pankratov, Y., and Lalo, U. (2015). Role for astroglial α1-adrenoreceptors in gliotransmission and control of synaptic plasticity in the neocortex. *Front. Cell. Neurosci.* 9:230. doi: 10.3389/fncel.2015.00230

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458. doi: 10.1126/science.1202529

Parkhurst, C. N., Yang, G., Ninan, I., Savas, J. N., Yates, J. R., III, Lafaille, J. J., et al. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* 155, 1596–1609. doi: 10.1016/j.cell.2013.11.030

Parpura, V., Basarsky, T. A., Liu, F., Jeftinija, K., Jeftinija, S., and Haydon, P. G. (1994). Glutamate-mediated astrocyte-neuron signalling. *Nature* 369, 744–747. doi: 10.1038/369744a0

Pascual, O., Ben Achour, S., Rostaing, P., Triller, A., and Bessis, A. (2012). Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. *Proc. Natl. Acad. Sci. U S A* 109, 197–205. doi: 10.1073/pnas.1111098109

Pascual, O., Casper, K. B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J. Y., et al. (2005). Astrocytic purinergic signaling coordinates synaptic networks. *Science* 310, 113–116. doi: 10.1126/science.1116916

Pellerin, L., Bouzier-Sore, A. K., Aubert, A., Serres, S., Merle, M., Costalat, R., et al. (2007). Activity-dependent regulation of energy metabolism by astrocytes: an update. *Glia* 55, 1251–1262. doi: 10.1002/glia.20528

Peng, L., Verkhratsky, A., Gu, L., and Li, B. (2015). Targeting astrocytes in major depression. *Expert. Rev. Neurother.* 15, 1299–1306. doi: 10.1586/14737175.2015.1095094

Perea, G., and Araque, A. (2007). Astrocytes potentiate transmitter release at single hippocampal synapses. *Science* 317, 1083–1086. doi: 10.1126/science.1144640

Perea, G., Yang, A., Boyden, E. S., and Sur, M. (2014). Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons *in vivo*. *Nat. Commun.* 5:3262. doi: 10.1038/ncomms4262

Perry, V. H., and Gordon, S. (1988). Macrophages and microglia in the nervous system. *Trends Neurosci.* 11, 273–277. doi: 10.1016/0166-2236(88)90110-5

Petzold, G. C., Albeanu, D. F., Sato, T. F., and Murthy, V. N. (2008). Coupling of neural activity to blood flow in olfactory glomeruli is mediated by astrocytic pathways. *Neuron* 58, 897–910. doi: 10.1016/j.neuron.2008.04.029

Pilc, A., Wierońska, J. M., and Skolnick, P. (2013). Glutamate-based antidepressants: preclinical psychopharmacology. *Biol. Psychiatry* 73, 1125–1132. doi: 10.1016/j.biopsych.2013.01.021

Pinto-Duarte, A., Coelho, J. E., Cunha, R. A., Ribeiro, J. A., and Sebastião, A. M. (2005). Adenosine A_{2A} receptors control the extracellular levels of adenosine through modulation of nucleoside transporters activity in the rat hippocampus. *J. Neurochem.* 93, 595–604. doi: 10.1111/j.1471-4159.2005.03071.x

Pocock, J. M., and Kettenmann, H. (2007). Neurotransmitter receptors on microglia. *Trends Neurosci.* 30, 527–535. doi: 10.1016/j.tins.2007.07.007

Radley, J. J., Rocher, A. B., Miller, M., Janssen, W. G., Liston, C., Hof, P. R., et al. (2006). Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cereb. Cortex* 16, 313–320. doi: 10.1093/cercor/bhi104

Raison, C. L., Capuron, L., and Miller, A. H. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol.* 27, 24–31. doi: 10.1016/j.it.2005.11.006

Raivich, G. (2005). Like cops on the beat: the active role of resting microglia. *Trends Neurosci.* 28, 571–573. doi: 10.1016/j.tins.2005.09.001

Rajkowska, G., Hughes, J., Stockmeier, C. A., Javier Miguel-Hidalgo, J., and Maciag, D. (2013). Coverage of blood vessels by astrocytic endfeet is reduced in major depressive disorder. *Biol. Psychiatry* 73, 613–621. doi: 10.1016/j.biopsych.2012.09.024

Rajkowska, G., Miguel-Hidalgo, J. J., Wei, J., Dilley, G., Pittman, S. D., Meltzer, H. Y., et al. (1999). Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol. Psychiatry* 45, 1085–1098. doi: 10.1016/s0006-3223(99)00041-4

Rajkowska, G., and Stockmeier, C. A. (2013). Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Curr. Drug Targets* 14, 1225–1236. doi: 10.2174/1389450111314990156

Rebola, N., Simões, A. P., Canas, P. M., Tomé, A. R., Andrade, G. M., Barry, C. E., et al. (2011). Adenosine A_{2A} receptors control neuroinflammation and consequent hippocampal neuronal dysfunction. *J. Neurochem.* 117, 100–111. doi: 10.1111/j.1471-4159.2011.07178.x

Rodrigues, R. J., Tomé, A. R., and Cunha, R. A. (2015). ATP as a multi-target danger signal in the brain. *Front. Neurosci.* 9:148. doi: 10.3389/fnins.2015.00148

Ros-Bernal, F., Hunot, S., Herrero, M. T., Parnadeau, S., Corvol, J. C., Lu, L., et al. (2011). Microglial glucocorticoid receptors play a pivotal role in regulating dopaminergic neurodegeneration in parkinsonism. *Proc. Natl. Acad. Sci. U S A* 108, 6632–6637. doi: 10.1073/pnas.1017820108

Rose, C. F., Verkhratsky, A., and Parpura, V. (2013). Astrocyte glutamine synthetase: pivotal in health and disease. *Biochem. Soc. Trans.* 41, 1518–1524. doi: 10.1042/BST20130237

Rouach, N., Koulakoff, A., Abudara, V., Willecke, K., and Giaume, C. (2008). Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science* 322, 1551–1555. doi: 10.1126/science.1164022

Roumier, A., Béchade, C., Poncer, J. C., Smalla, K. H., Tomasello, E., Vivier, E., et al. (2004). Impaired synaptic function in the microglial KARAP/DAP12-deficient mouse. *J. Neurosci.* 24, 11421–11428. doi: 10.1523/jneurosci.2251-04.2004

Sanacora, G., and Banasr, M. (2013). From pathophysiology to novel antidepressant drugs: glial contributions to the pathology and treatment of mood disorders. *Biol. Psychiatry* 73, 1172–1179. doi: 10.1016/j.biopsych.2013.03.032

Schafer, D. P., Lehrman, E. K., Kautzman, A. G., Koyama, R., Mardinly, A. R., Yamasaki, R., et al. (2012). Microglia sculpt postnatal neural circuits in an activity and complement-dependent manner. *Neuron* 74, 691–705. doi: 10.1016/j.neuron.2012.03.026

Schafer, D. P., Lehrman, E. K., and Stevens, B. (2013). The "quad-partite" synapse: microglia-synapse interactions in the developing and mature CNS. *Glia* 61, 24–36. doi: 10.1002/glia.22389

Schipke, C. G., Heuser, I., and Peters, O. (2011). Antidepressants act on glial cells: SSRIs and serotonin elicit astrocyte calcium signaling in the mouse prefrontal cortex. *J. Psychiatr. Res.* 45, 242–248. doi: 10.1016/j.jpsychires.2010.06.005

Schlicht, K., Büttner, A., Siedler, F., Scheffer, B., Zill, P., Eisenmenger, W., et al. (2007). Comparative proteomic analysis with postmortem prefrontal cortex tissues of suicide victims versus controls. *J. Psychiatr. Res.* 41, 493–501. doi: 10.1016/j.jpsychires.2006.04.006

Schnieder, T. P., Trencevska, I., Rosoklija, G., Stankov, A., Mann, J. J., Smiley, J., et al. (2014). Microglia of prefrontal white matter in suicide. *J. Neuropathol. Exp. Neurol.* 73, 880–890. doi: 10.1097/NEN.0000000000000107

Schroeter, M. L., Abdul-Khaliq, H., Krebs, M., Diefenbacher, A., and Blasig, I. E. (2008). Serum markers support disease-specific glial pathology in major depression. *J. Affect. Disord.* 111, 271–280. doi: 10.1016/j.jad.2008.03.005

Scianni, M., Antonilli, L., Chece, G., Cristalli, G., Di Castro, M. A., Limatola, C., et al. (2013). Fractalkine (CX3CL1) enhances hippocampal N-methyl-D-aspartate receptor (NMDAR) function via D-serine and adenosine receptor type A2 (A2AR) activity. *J. Neuroinflammation* 10:108. doi: 10.1186/1742-2094-10-108

Sequeira, A., Mamdani, F., Ernst, C., Vawter, M. P., Bunney, W. E., Lebel, V., et al. (2009). Global brain gene expression analysis links glutamatergic and GABAergic alterations to suicide and major depression. *PLoS One* 4:e6585. doi: 10.1371/journal.pone.0006585

Serchov, T., Clement, H. W., Schwarz, M. K., Iasevoli, F., Tosh, D. K., Idzko, M., et al. (2015). Increased signaling via adenosine a1 receptors, sleep deprivation, imipramine and ketamine inhibit depressive-like behavior via induction of homer1a. *Neuron* 87, 549–562. doi: 10.1016/j.neuron.2015.07.010

Serrano, A., Haddjeri, N., Lacaille, J. C., and Robitaille, R. (2006). GABAergic network activation of glial cells underlies hippocampal heterosynaptic depression. *J. Neurosci.* 26, 5370–5382. doi: 10.1523/jneurosci.5255-05.2006

Setiawan, E., Wilson, A. A., Mizrahi, R., Rusjan, P. M., Miler, L., Rajkowska, G., et al. (2015). Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA Psychiatry* 72, 268–275. doi: 10.1001/jamapsychiatry.2014.2427

Shao, W., Zhang, S. Z., Tang, M., Zhang, X. H., Zhou, Z., Yin, Y. Q., et al. (2013). Suppression of neuroinflammation by astrocytic dopamine D2 receptors via αB-crystallin. *Nature* 494, 90–94. doi: 10.1038/nature11748

Si, X., Miguel-Hidalgo, J. J., O'Dwyer, G., Stockmeier, C. A., and Rajkowska, G. (2004). Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. *Neuropsychopharmacology* 29, 2088–2096. doi: 10.1038/sj.npp.1300525

Ślusarczyk, J., Trojan, E., Glombik, K., Budziszewska, B., Kubera, M., Lasoń, W., et al. (2015). Prenatal stress is a vulnerability factor for altered morphology and biological activity of microglia cells. *Front. Cell. Neurosci.* 9:82. doi: 10.3389/fncel.2015.00082

Sousa, N., Cerqueira, J. J., and Almeida, O. F. (2008). Corticosteroid receptors and neuroplasticity. *Brain Res. Rev.* 57, 561–570. doi: 10.1016/j.brainresrev.2007.06.007

Sousa, N., Lukoyanov, N. V., Madeira, M. D., Almeida, O. F., and Paula-Barbosa, M. M. (2000). Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience* 97, 253–266. doi: 10.1016/s0306-4522(00)00050-6

Steiner, J., Bielau, H., Brisch, R., Danos, P., Ullrich, O., Mawrin, C., et al. (2008). Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J. Psychiatr. Res.* 42, 151–157. doi: 10.1016/j.jpsychires.2006.10.013

Stockmeier, C. A., Mahajan, G. J., Konick, L. C., Overholser, J. C., Jurjus, G. J., Meltzer, H. Y., et al. (2004). Cellular changes in the postmortem hippocampus in major depression. *Biol. Psychiatry* 56, 640–650. doi: 10.1016/j.biopsych.2004.08.022

Stokes, L., Spencer, S. J., and Jenkins, T. A. (2015). Understanding the role of P2X7 in affective disorders-are glial cells the major players? *Front. Cell. Neurosci.* 9:258. doi: 10.3389/fncel.2015.00258

Su, F., Yi, H., Xu, L., and Zhang, Z. (2015). Fluoxetine and S-citalopram inhibit M1 activation and promote M2 activation of microglia *in vitro*. *Neuroscience* 294, 60–68. doi: 10.1016/j.neuroscience.2015.02.028

Sun, J. D., Liu, Y., Yuan, Y. H., Li, J., and Chen, N. H. (2012). Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. *Neuropsychopharmacology* 37, 1305–1320. doi: 10.1038/npp.2011.319

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., et al. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810–823. doi: 10.1016/j.cell.2011.02.018

Theis, M., and Giaume, C. (2012). Connexin-based intercellular communication and astrocyte heterogeneity. *Brain Res.* 1487, 88–98. doi: 10.1016/j.brainres.2012.06.045

Torres-Platas, S. G., Cruceanu, C., Chen, G. G., Turecki, G., and Mechawar, N. (2014). Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. *Brain Behav. Immun.* 42, 50–59. doi: 10.1016/j.bbi.2014.05.007

Torres-Platas, S. G., Hercher, C., Davoli, M. A., Maussion, G., Labonté, B., Turecki, G., et al. (2011). Astrocytic hypertrophy in anterior cingulate white matter of depressed suicides. *Neuropsychopharmacology* 36, 2650–2658. doi: 10.1038/npp.2011.154

Torres-Platas, S. G., Nagy, C., Wakid, M., Turecki, G., and Mechawar, N. (2015). Glial fibrillary acidic protein is differentially expressed across cortical and subcortical regions in healthy brains and downregulated in the thalamus and caudate nucleus of depressed suicides. *Mol. Psychiatry* doi: 10.1038/mp.2015.65 [Epub ahead of print].

Trang, T., Beggs, S., and Salter, M. W. (2012). ATP receptors gate microglia signaling in neuropathic pain. *Exp. Neurol.* 234, 354–361. doi: 10.1016/j.expneurol.2011.11.012

Tritsch, N. X., and Sabatini, B. L. (2012). Dopaminergic modulation of synaptic transmission in cortex and striatum. *Neuron* 76, 33–50. doi: 10.1016/j.neuron.2012.09.023

Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M. W., et al. (2003). P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 424, 778–783. doi: 10.1038/nature01786

Tsvetkov, E., Shin, R. M., and Bolshakov, V. Y. (2004). Glutamate uptake determines pathway specificity of long-term potentiation in the neural circuitry of fear conditioning. *Neuron* 41, 139–151. doi: 10.1016/s0896-6273(03)00800-6

Tzanoulinou, S., Riccio, O., de Boer, M. W., and Sandi, C. (2014). Peripubertal stress-induced behavioral changes are associated with altered expression of genes involved in excitation and inhibition in the amygdala. *Transl. Psychiatry* 4:e410. doi: 10.1038/tp.2014.54

van Calker, D., and Biber, K. (2005). The role of glial adenosine receptors in neural resilience and the neurobiology of mood disorders. *Neurochem. Res.* 30, 1205–1217. doi: 10.1007/s11064-005-8792-1

Verkhratsky, A., Steardo, L., Parpura, V., and Montana, V. (2015). Translational potential of astrocytes in brain disorders. *Prog. Neurobiol.* doi: 10.1016/j.pneurobio.2015.09.003 [Epub ahead of print].

Viswanathan, A., and Freeman, R. D. (2007). Neurometabolic coupling in cerebral cortex reflects synaptic more than spiking activity. *Nat. Neurosci.* 10, 1308–1312. doi: 10.1038/nn1977

Volterra, A., and Meldolesi, J. (2005). Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci.* 6, 626–640. doi: 10.1038/nrn1722

Wake, H., Moorhouse, A. J., Miyamoto, A., and Nabekura, J. (2013). Microglia: actively surveying and shaping neuronal circuit structure and function. *Trends Neurosci.* 36, 209–217. doi: 10.1016/j.tins.2012.11.007

Walker, A. K., Budac, D. P., Bisulco, S., Lee, A. W., Smith, R. A., Beenders, B., et al. (2013). NMDA receptor blockade by ketamine abrogates lipopolysaccharide-induced depressive-like behavior in C57BL/6J mice. *Neuropsychopharmacology* 38, 1609–1616. doi: 10.1038/npp.2013.71

Wallraff, A., Köhling, R., Heinemann, U., Theis, M., Willecke, K., and Steinhäuser, C. (2006). The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. *J. Neurosci.* 26, 5438–5447. doi: 10.1523/jneurosci.0037-06.2006

Wallraff, A., Odermatt, B., Willecke, K., and Steinhäuser, C. (2004). Distinct types of astroglial cells in the hippocampus differ in gap junction coupling. *Glia* 48, 36–43. doi: 10.1002/glia.20040

Wong, W. T., Wang, M., and Li, W. (2011). Regulation of microglia by ionotropic glutamatergic and GABAergic neurotransmission. *Neuron Glia Biol.* 7, 41–46. doi: 10.1017/S1740925X11000123

Yang, Y., Ge, W., Chen, Y., Zhang, Z., Shen, W., Wu, C., et al. (2003). Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. *Proc. Natl. Acad. Sci. U S A* 100, 15194–15199. doi: 10.1073/pnas.2431073100

Ye, Y., Wang, G., Wang, H., and Wang, X. (2011). Brain-derived neurotrophic factor (BDNF) infusion restored astrocytic plasticity in the hippocampus of a rat model of depression. *Neurosci. Lett.* 503, 15–19. doi: 10.1016/j.neulet.2011.07.055

Yin, Y., Zhang, X., Li, Z., Deng, L., Jiao, G., Zhang, B., et al. (2013). Glucocorticoid receptor β regulates injury-mediated astrocyte activation and contributes to glioma pathogenesis via modulation of β-catenin/TCF transcriptional activity. *Neurobiol. Dis.* 59, 165–176. doi: 10.1016/j.nbd.2013.07.013

Yirmiya, R., Rimmerman, N., and Reshef, R. (2015). Depression as a microglial disease. *Trends Neurosci.* 38, 637–658. doi: 10.1016/j.tins.2015.08.001

Zarate, C. A., Jr., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., et al. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry* 63, 856–864. doi: 10.1001/archpsyc.63.8.856

Zhan, Y., Paolicelli, R. C., Sforazzini, F., Weinhard, L., Bolasco, G., Pagani, F., et al. (2014). Deficient neuron-microglia signaling results in impaired functional brain connectivity and social behavior. *Nat. Neurosci.* 17, 400–406. doi: 10.1038/nn.3641

Zhang, J. M., Wang, H. K., Ye, C. Q., Ge, W., Chen, Y., Jiang, Z. L., et al. (2003). ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron* 40, 971–982. doi: 10.1016/s0896-6273(03)00717-7

Zhao, J., Bao, A. M., Qi, X. R., Kamphuis, W., Luchetti, S., Lou, J. S., et al. (2012). Gene expression of GABA and glutamate pathway markers in the prefrontal cortex of non-suicidal elderly depressed patients. *J. Affect. Disord.* 138, 494–502. doi: 10.1016/j.jad.2012.01.013

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Rial, Lemos, Pinheiro, Duarte, Gonçalves, Real, Prediger, Gonçalves, Gomes, Canas, Agostinho and Cunha. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution and reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Cellular Neuroscience

REVIEW
published: 10 July 2015
doi: 10.3389/fncel.2015.00268

Glia in the cytokine-mediated onset of depression: fine tuning the immune response

Wendy K. Jo[1], Yuanyuan Zhang[2], Hinderk M. Emrich[1,2] and Detlef E. Dietrich[1,2,3]*

[1] Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, Hannover, Germany, [2] Clinic for Mental Health, Hannover Medical School, Hannover, Germany, [3] Burghof-Klinik, Rinteln, Germany

Major depressive disorder (MDD) is a mood disorder of multifactorial origin affecting millions of people worldwide. The alarming estimated rates of prevalence and relapse make it a global public health concern. Moreover, the current setback of available antidepressants in the clinical setting is discouraging. Therefore, efforts to eradicate depression should be directed towards understanding the pathomechanisms involved in the hope of finding cost-effective treatment alternatives. The pathophysiology of MDD comprises the breakdown of different pathways, including the hypothalamus-pituitary-adrenal (HPA) axis, the glutamatergic system, and monoaminergic neurotransmission, affecting cognition and emotional behavior. Inflammatory cytokines have been postulated to be the possible link and culprit in the disruption of these systems. In addition, evidence from different studies suggests that impairment of glial functions appears to be a major contributor as well. Thus, the intricate role between glia, namely microglia and astrocytes, and the central nervous system's (CNSs) immune response is briefly discussed, highlighting the kynurenine pathway as a pivotal player. Moreover, evaluations of different treatment strategies targeting the inflammatory response are considered. The immuno-modulatory properties of vitamin D receptor (VDR) suggest that vitamin D is an attractive and plausible candidate in spite of controversial findings. Further research investigating the role of VDR in mood disorders is warranted.

Keywords: depression, glia, inflammation, KYN pathway, vitamin D, VDR

OPEN ACCESS

Edited by:
Luisa Pinto,
University of Minho, Portugal

Reviewed by:
Rheinallt Parri,
Aston University, UK
Edo Ronald De Kloet,
Leiden University, Netherlands

****Correspondence:***
Wendy K. Jo,
Research Center for Emerging Infections and Zoonoses (RIZ),
University of Veterinary Medicine Hannover, Bünteweg 17,
30559 Hannover, Germany
wendy.karen.j.lei@tiho-hannover.de

Received: *22 May 2015*
Accepted: *29 June 2015*
Published: *10 July 2015*

Citation:
Jo WK, Zhang Y, Emrich HM and Dietrich DE (2015) Glia in the cytokine-mediated onset of depression: fine tuning the immune response.
Front. Cell. Neurosci. 9:268.
doi: 10.3389/fncel.2015.00268

Introduction

Depression is a common neuropsychiatric disorder constituting one of the leading causes of disability worldwide, estimated to affect 350 million people, and projected to be the leading cause of disease burden by 2030 (World-Health-Organization, 2008; World-Federation-for-Mental-Health, 2012). The comorbid state of depression together with a number of other physical and psychiatric disorders results in increased disability and mortality rates. Among some of the most frequently associated disorders are cardiovascular disease, cancer, diabetes, obesity, attention deficit hyperactivity disorder, and Alzheimer's disease (Schillani et al., 2012; Hannon et al., 2013; Mavrides and Nemeroff, 2013; Wuwongse et al., 2013; Di Trani et al., 2014).

Although major depressive disorder (MDD) has been extensively studied, rates of depression have not decreased over the past decades. According to a review comparing cases of

mental disorders between 2004 and 2010 in the European Union, rates in this period remained more or less stable with the increase in better detection and health care systems. However, in a previous study the prevalence of persons affected each year was estimated to be 38.2%, depression being the second most prevalent form of mental disorders (6.9%; Wittchen et al., 2011). Moreover, with the currently prescribed pharmacological agents, relapse rates are as high as 50%, only one-third of patients achieving complete remission. Therefore, there is a clear necessity to increase the amount of research investigating the molecular mechanisms pertaining depression. The aim of this review is to connect the different pathophysiological pathways of depression in an effort to find suitable treatments.

Major Depressive Disorder

MDD along with bipolar disorder are categorized under mood disorders, also referred to as affective disorders. MDD is a condition characterized by one or more depressive episodes along with pathophysiological changes in the brain (Muller and Schwarz, 2007). These depressive episodes appear without a history of manic or hypomanic episodes, as in the case of bipolar disorder. Typical symptoms include low mood, anhedonia, appetite alterations, sleep disturbances, fatigue, poor concentration, and feelings of worthlessness, among others (World-Federation-for-Mental-Health, 2012). Moreover, MDD is attributed as a risk factor for suicide attempts and drug abuse (Davis et al., 2008). Furthermore, depressive episodes are often recurrent, incrementing individual and social burden (World-Federation-for-Mental-Health, 2012).

Characteristic pathophysiological hallmark features include monoamine depletion, glucocorticoid receptor (GR) resistance, and excess of glutamate, corticotrophin-releasing hormone and cortisol levels, therefore compromising the monoaminergic and glutamatergic neurotransmission along with the hypothalamus-pituitary-adrenal (HPA) axis activity (Muller and Schwarz, 2007).

The onset of MDD is complex and can be triggered by several different factors. While genetic factors have been estimated to contribute 30–40% of the etiology of MDD, the remaining factors are accounted for by environmental risk factors. Negative traumatic events during lifetime (e.g., child abuse, parental divorce, death of a loved one, etc.) can lead to MDD. However, this depends on the genetic susceptibility, gender, family history, and personality traits of the person affected (Heim and Binder, 2012). Family risk factors are estimated to increase its incidence two to three times (Wilde et al., 2014). Development of MDD is suggested to be the outcome of the interplay between both genetic and environmental risk factors rather than the result of one factor. For instance, it was demonstrated in one study that interactions between experiences of childhood abuse and genetic variations of both serotonin transporter and corticotrophin receptor 1 genes lead to the development of MDD (Ressler et al., 2010).

The trend towards the study of MDD from an epigenetic approach is increasing with the aid of new available technologies. These studies allude to environmental stressors that can modify epigenetically the expressions of susceptible genes, thereby resulting in the dysfunction of relevant mechanisms in MDD (Mill and Petronis, 2007; Fass et al., 2014). For example, histone acetylation and methylation were reported in animal models of depression (Tsankova et al., 2006; Fuchikami et al., 2009). Similar mechanisms were proposed to occur in depressed patients as evidenced by post-mortem brain and serological studies (Cruceanu et al., 2013; Sun et al., 2013). Moreover, exposure to early negative experiences was shown to alter methylation of GR and brain derived neurotropic factor (BDNF; Elliott et al., 2010; Perroud et al., 2013). Furthermore, epigenetic modifications in the gene promoter region of BDNF in mood disorder patients have been shown by several studies to affect the patient's response to the treatment received (D'Addario et al., 2012; Lopez et al., 2013; Tadić et al., 2014). These studies suggest the evaluation of epigenetic changes in BDNF as a potent biomarker for treatment response in mood disorder patients.

Crosstalk Between the Monoaminergic and Glutamatergic Systems

Over the past half-century, comprehensive studies concentrating on the monoamine hypothesis have led to the development of a number of treatments, whose target is to increase the availability of monoamines, such as serotonin and norepinephrine (Charney, 1998). Depletion of serotonin is commonly attributed to the shift from the serotonin pathway to the kynurenine (KYN) pathway in the catabolism of the essential amino acid tryptophan. The KYN pathway can be mediated by a variety of cell types in the body. However, the resulting downstream product depends on the kind of enzymes that each cell type has for the processing of tryptophan. In the central nervous system (CNS), the KYN pathway appears to be mainly mediated by astrocytes, microglia, and infiltrating macrophages. It is initiated by activation of indoleamine 2, 3-dioxygenase (IDO) catabolizing tryptophan into KYN, which can be further converted to kynurenic acid (KA) or quinolinic acid (QUIN). KA and QUIN have contrasting roles influencing the glutamatergic system, the first acting as antagonist and the latter as agonist of the glutamate N-methyl-D-aspartate receptor (NMDAr; Campbell et al., 2014). Microglia are the main producers of QUIN in the brain, whereas astrocytes are the CNS-key cells involved in KA synthesis. This is explained by the fact that microglia express kynurenine 3-monooxygenase (KMO), the rate-limiting enzyme in the production of QUIN. Conversely, astrocytes exclusively express kynurenine aminotransferases, which are essential in the conversion of KYN to KA (Guillemin et al., 2001, 2005). IDO, the initiator of the KYN pathway, has been reported to be induced by a number of different pro-inflammatory cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor α (TNF-α), and interleukin-6 (IL-6). Therefore, the activation of the KYN pathway is attributed to be cytokine-mediated (O'Connor et al., 2009a; Kim et al., 2012; Campbell et al., 2014).

MDD also compromises the glutamatergic system characterized by the increased activation of NMDAr (Trullas and Skolnick, 1990). QUIN and glutamate are agonists of

NMDAr, levels of both being found to be elevated in MDD. Inflammation can induce the synthesis of QUIN through the activation of the KYN pathway in microglia and macrophages in the CNS (Guillemin et al., 2003). QUIN acts not only as an agonist of NMDAr at the glycine-binding site, but has also been shown to stimulate the release of glutamate from neurons, inhibiting its re-uptake by astrocytes (Tavares et al., 2002, 2005). Furthermore, several studies indicate that QUIN toxicity can also lead to lipid peroxidation and nitrosative stress (Behan et al., 1999; Aguilera et al., 2007). On the other hand, KA is an antagonist of the NMDAr with antioxidant activity found to induce neuro-protective effects in ischemic and oxidative stress rodent models (Nozaki and Beal, 1992; Lugo-Huitrón et al., 2011). Nevertheless, the role of KA in relation to depression is not yet clear. A recent longitudinal study of suicide attempters with depression showed that cerebrospinal fluid (CSF) QUIN levels are increased, while CSF KA levels are decreased over a 2-year period along with an increase in IL-6 and worsening of depressive and suicidal symptoms (Bay-Richter et al., 2015). However, treatment of the pro-inflammatory cytokine IFN-α in patients with hepatitis C virus (HCV) was shown to elevate both QUIN and KA concentrations in the CSF, with only QUIN levels found to be correlated to depression scores (Raison et al., 2010). IFN-α therapy has been widely used to treat HCV, though it is reported to induce depressive and manic symptoms in 30–50% of patients (Bonaccorso et al., 2002; Constant et al., 2005). Moreover, studies evaluating the KYN pathway in the periphery have reported an increased KYN/KA ratio associated with depressive symptoms in individuals with HCV treated with IFN-α, and a reduced KA/QUIN ratio in MDD patients compared with controls (Wichers et al., 2005; Savitz et al., 2015). Of note, new findings using skeletal muscle-specific peroxisome proliferator-activated receptor gamma coactivator 1-alpha1 (PGC-1α1) transgenic mouse model suggest that physical exercise is beneficial in stress-induced depression due to its modulation of the KYN pathway. Enhanced PGC-1α1 in the skeletal muscle stimulated KA synthesis, thus reducing peripheral KYN, which, contrastingly to KA, can readily cross the BBB (Agudelo et al., 2014). Interestingly, contrary to the observations in depression, elevated levels of KA have been associated with the cognitive deficits in schizophrenic patients (Erhardt et al., 2004; Linderholm et al., 2012). Augmented inhibition of the NMDAr by KA appears to cause changes in the glutamatergic system affecting the dopaminergic neurotransmission, a hallmark feature of schizophrenia (Wu et al., 2007; Pocivavsek et al., 2011).

Glial Pathology in Relation to Neuro-Pathophysiological Alterations

Accumulating evidence suggests that glial pathology is a prominent feature in MDD. Glial cells are the non-neuronal cells in the CNS, comprised of astrocytes, microglia and oligodendrocytes. Human post-mortem studies of mood disorder patients consistently demonstrate a significant decrease in the number of glia in the pre-frontal cortex and limbic structures of the brain (Rajkowska et al., 1999; Cotter et al., 2001; Bowley et al., 2002; Hamidi et al., 2004; Altshuler et al., 2010). Moreover, it was reported that glial ablation in the pre-frontal cortex of mice induces anhedonia, anxiety and helplessness behavior after exposure to chronic unpredictable stress procedures. The depressive-like behaviors generated were assessed by sucrose preference test, novelty suppressed feeding test, forced swim test, and two-way active avoidance test (Banasr and Duman, 2008). Furthermore, it was shown in another study by the same group that exposure to chronic unpredictable stress can likewise induce glial dysfunction resulting in depressive-like behavior (Banasr et al., 2010). It was proposed that initial glial impairment by different stressors leads to neuronal damage in the progression of the disorder (Rajkowska and Miguel-Hidalgo, 2007).

Astrocytes have multifaceted roles in the CNS, participating actively in synaptic information transmission, secretion of synaptogenic molecules, such as BDNF, modulation of the BBB, mediation of the immune system, and uptake of cytotoxic molecules from the extracellular space, such as glutamate (Jo et al., 2014). Astrocytes express the excitatory amino acid transporters 1 and 2 (EAAT1 and EAAT2), which enables them to transport glutamate to the intracellular compartment. Once inside, glutamate is further converted to glutamine by the enzyme glutamine synthase (Anderson and Swanson, 2000). Astrocytes also take part in the KYN pathway synthesizing KA but not QUIN (Guillemin et al., 2001). Accumulating data from human post-mortem studies of depressed patients reveal a significant decrease in the number of astroglial cells and less coverage of blood vessels by astrocytic end-feet (Johnston-Wilson et al., 2000; Gittins and Harrison, 2011; Rajkowska et al., 2013). Loss of astrocytes has been demonstrated by studies reporting decreases of the astrocytic marker glial fibrillary acidic protein and both enzymes EAAT1 and EAAT2 (Johnston-Wilson et al., 2000; Miguel-Hidalgo et al., 2010; Gittins and Harrison, 2011). Moreover, morphometric analyses in the anterior cingulate cortex region of depressed suicides revealed the presence of hypertrophic astrocytes, indicating possible astrocytic activation in the area (Torres-Platas et al., 2011). Furthermore, significantly increased genome-wide DNA methylation patterns were reported in the pre-frontal cortex of depressed patients (Nagy et al., 2014). This was found alongside the down-regulation of astrocytic markers in the same study, implying possible epigenetic regulations associated with astrocytic pathology (Nagy et al., 2014). The evidence reported suggests that in the event of the activation of the KYN pathway by pro-inflammatory cytokines, without functional astrocytes, QUIN/KA ratio would be increased. Moreover, glutamate clearance from the extracellular space would also result disturbed. Hence, elevated levels of QUIN and glutamate would promote NMDAr agonism disrupting the glutamatergic system.

Microglia are professional phagocytes regarded as CNS-resident immune cells. They are the primary sentinels patrolling the CNS, alert to any potential harmful event including pathogen invasion. Moreover, their role is also extended to debris clearance, trophic support to neurons, and synaptic

pruning during neurogenesis (Ousman and Kubes, 2012). In human post-mortem studies, higher microglial activation was observed in different regions including anterior cingulate cortex, pre-frontal cortex and hippocampus of depressed suicides (Steiner et al., 2008; Torres-Platas et al., 2014). QUIN levels were also found to be dysregulated in different regions of the brain. Elevated microglial QUIN expression was reported in the cingulate cortex, whereas a decrease or no change in microglial QUIN immunoreactivity was observed in the hippocampus of acutely depressed patients (Steiner et al., 2011; Busse et al., 2014). Supporting this notion, it was shown in parallel that chronic unpredictable stress in mice led to depressive-like behavior such as decrease in sucrose consumption and social exploration along with microglial proliferation and activation, ending in subsequent microglial apoptosis (Kreisel et al., 2014). However, blockade of microglial initial activation minimized the aforementioned detrimental effects (Kreisel et al., 2014). Moreover, microglial cell culture experiments showed that adding IFN-α induced microglia activation and production of pro-inflammatory cytokines, such as IL-1β, IL-6 and TNF-α (Zheng et al., 2015). Furthermore, in another study, IFN-α treated microglia stimulated the expression of inducible nitric oxide synthase (iNOS) and secretion of NO, alongside the down-regulation of heme oxygenase-1 (HO-1), a potent anti-inflammatory and neuro-protective protein (Lu et al., 2013). Hence, it was concluded that IFN-α may contribute to the pathogenesis of depression by triggering inflammation, oxidative stress, and abrogating anti-inflammatory and neuro-protective responses (Lu et al., 2013; Zheng et al., 2015). It is therefore suggested that with the activation of microglia, the secreted pro-inflammatory cytokines would activate the KYN pathway. Tryptophan degradation is then shifted to the KYN pathway, resulting in depletion of monoamines and production of QUIN, compromising the monoaminergic and glutamatergic systems.

Oligodendrocytes are the myelin-cell producers in the CNS enveloping neuronal axons, allowing communication through action potentials. Among the glia, oligodendrocytes are particularly vulnerable to stress-related insults (Edgar and Sibille, 2012). Oligodendrocytic dysfucntion in mood disorders is evidenced by human post-mortem studies reporting reduced numbers of oligodendrocytes in the pre-frontal cortex and amygdala (Hamidi et al., 2004; Uranova et al., 2004). Perineuronal oligodendrocytes have been identified in later studies as the subtype of oligodendrocytic cell affected. Perineuronal oligodendrocytes are non-myelin producer cells that undergo functional changes following an injury allowing them to synthesize myelin and possibly rescue neuronal axons from de-myelination. The loss of perineuronal oligodendrocytes was reported in the pre-frontal cortex of mood disorders and schizophrenia (Vostrikov et al., 2007; Szuchet et al., 2011). Moreover, this decrease in the number of perineuronal oligodendrocytes was correlated to cytoarchitectural abnormalities in genome-wide association analyses (Kim and Webster, 2010).

Despite the differences discussed regarding the type of glia affected and its state (resting or activated) in the different neuropsychiatric disorders, the evidence presented so far suggests that dysfunctional glia play an active role in the abrogation of the monoaminergic and glutamatergic neurotransmission systems, contributing to cognitive deficits and behavioral changes in mood disorders.

Depression as an Inflammatory Disease

The inflammatory hypothesis of depression has gained momentum since it first appeared two decades ago, proposing MDD as a cytokine-mediated disorder (Maes et al., 1995; Raison et al., 2006; Dantzer et al., 2008). An increasing amount of clinical evidence has shown the presence of augmented levels of pro-inflammatory markers in the periphery and in the CNS (Maes et al., 1993; Rotter et al., 2013; Dahl et al., 2014). Moreover, high rates of comorbidity have been reported between depression and immune-associated disorders, such as cardiovascular disease, multiple sclerosis, and autoimmune disorders (Maes et al., 1993; Bachen et al., 2009; Byatt et al., 2011). Meta-analyses of studies associating inflammatory markers with MDD reported significantly higher levels of TNF-α, IL-6, and C-reactive protein (CRP) in depressed patients (Dowlati et al., 2010; Valkanova et al., 2013). A recent longitudinal study conducted in England revealed that children with elevated serum IL-6 and CRP levels were more at risk to develop depression and psychosis later in life (Khandaker et al., 2014). Furthermore, several studies report that cytokine therapy, such as IFN-α, can generate depressive and manic symptoms in HCV and cancer patients (Bonaccorso et al., 2002; Capuron et al., 2002; Constant et al., 2005). In one study, serum levels of TNF-α, IL-6 and soluble IL-2 receptor were shown to be upregulated after IFN-α treatment (Wichers et al., 2007). In another study, elevated levels of QUIN and KA were found in the CSF of patients treated with peripheral administration of IFN-α (Raison et al., 2010). In further support of the inflammatory hypothesis of depression, studies using animal models have demonstrated that administering lipopolysaccharides (LPS) as well as pro-inflammatory cytokines induce depressive-like behaviors such as anhedonia, assessed by sucrose/saccharine preference tests (Yirmiya, 1996; De La Garza, 2005).

It is important to point out that despite this overwhelming evidence of an association between inflammation and depression, failed attempts to prove this correlation have also been reported (Haack et al., 1999; Steptoe et al., 2003). Moreover, the profile of cytokines in suicidal and non-suicidal patients is distinct (Kim et al., 2008). Cytokine level changes in response to antidepressant treatment are also varied (Hannestad et al., 2011). The heterogeneous results indicate that MDD is a rather complex disorder with multiple variants involving the inflammatory response participation to a great extent, but not in all cases. Nevertheless, cytokines can mediate several pathways which are crucial in the pathophysiology of depression. As discussed earlier, pro-inflammatory cytokines can induce IDO, therefore activating the KYN pathway. This event can lead to the dysregulation of the monoaminergic and

glutamatergic neurotransmissions. Moreover, the GR resistance has been attributed to be cytokine mediated. A number of cytokines, such as IL-1α, TNF-α and IFN-α, have been reported to inhibit GR function (Wang et al., 2004; Hu et al., 2009; Van Bogaert et al., 2011). Without competent GRs, glucocorticoids such as cortisol cannot exert its anti-inflammatory effects, thus ending in the exacerbation of the inflammatory response (Pace et al., 2007). In addition, GR resistance promotes hyperactivation of the HPA axis leading to the overproduction of glucocorticoids, hallmark features of MDD (Anacker et al., 2011). On the other hand, enhanced activation of the immune system can also be mediated by inadequate levels of glucocorticoids, resulting in the development of depressive symptoms (Raison and Miller, 2003). For example, in the case of atypical depression, corticotrophin-releasing hormone deficiency, hypoactivity of the HPA axis, and increased inflammatory response are often found in these patients (Gold and Chrousos, 2002). It is proposed that insufficient glucocorticoid signaling, either by low levels of cortisol or GR resistance, can lead to a cytokine-mediated onset of depression (Raison and Miller, 2003).

Different hypotheses have been postulated in order to explain the chronic low-grade inflammatory status in mood disorders. A number of lifestyle factors have been proposed to affect the immune status in depressed individuals, such as non-healthy dietary habits (e.g., high saturated fats, processed meats, refined carbohydrates, etc.), physical inactivity, smoking habits, and vitamin D deficiency (Berk et al., 2013). Chronic psychological stress is a well-studied risk factor suggested to induce GR resistance and trigger the inflammatory cascade (Miller et al., 2002; Raison et al., 2006; Cohen et al., 2012). From the genetic perspective, polymorphisms in the genes *PSMB4* (proteasome β4 subunit) and *TBX21* (T bet) that result in T-cell dysfunction, were reported to contribute to the pathology of MDD (Wong et al., 2008; Berk et al., 2013). A recent meta-analysis of 28 studies identified significant associations between depression and infections in Borna disease virus (BDV), herpes simplex virus-1, varicella zoster virus, Epstein-Bar virus (EBV), and *Chlamydophila trachomatis*. Results indicated that patients with depression are 3.25 times more likely to be infected with BDV (Wang et al., 2014). However, negative findings have also been reported (Bennett et al., 2012; Hornig et al., 2012; Pearce et al., 2012). Despite controversies aroused concerning direct virus association and mood disorders, several studies demonstrated that there is a higher risk of developing mood disorders later in life among individuals who have had a severe infection (Goodwin, 2011; Benros et al., 2013). In accordance with these findings, it was previously suggested that stress could alter the immune system, thereby resulting in reactivation of persistent viruses in the CNS, further enhancing the inflammatory reaction (Dietrich et al., 1998). Experimental evidence demonstrated that rats exposed to stress developed intestinal permeability and bacterial translocation. LPS from bacterial translocation activated toll-like receptor 4 (TLR4) in the brain triggering the neuroinflammatory response (Garate et al., 2011).

The systemic inflammatory response generates circulating cytokines and other mediators that can gain access to the brain through different pathways. The mechanisms involve cytokine signal propagation, activation of vagal afferents, active transport across the BBB, and diffusion at sites where the BBB is leaky or absent. It is important to indicate that this communication is bi-directional (Maier, 2003). Response within the CNS is consequently triggered. Microglia and astroglia, important immune-regulators, become activated and further enhance the inflammatory response (Biesmans et al., 2013). In addition, pro-inflammatory cytokines can activate the KYN pathway in both glial cell types resulting in the dysregulation of the monoaminergic and glutamatergic systems. On account of the evidence linking inflammation and depression it was proposed that depression is a byproduct of the immune systems in its attempt to fight infection (Raison and Miller, 2013).

Therapeutic Intervention

Among the many types of antidepressants that exist to date, tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin re-uptake inhibitors (SSRIs), and serotonin-norepinephrine re-uptake inhibitors are some of the most common types to be prescribed (Anderson et al., 2012; Klomp et al., 2014). Tricyclic antidepressants were the most widely prescribed pharmacological agent. However, its supplementation was found to lead to severer and lethal (overdoses) side effects compared with the other types of antidepressants (Boyce and Judd, 1999). Administering monoamine oxidase inhibitors was found to carry dangerous side effects as well, and these inhibitors are now used mainly in treatment-resistant depression (Finberg, 2014). SSRIs have become the first choice of antidepressants over the last two decades. Although their efficacy is comparable to the tricyclic antidepressants, the preferred choice for SSRIs arises from the fact that they are safer, have better tolerability, and lower rates of treatment discontinuation (Anderson, 2000). However, response rates are about 50%, full remission is achieved only in one-third of patients responding to treatment, and relapse is more often than not the case (Trivedi et al., 2006). Therefore, the current drawbacks of presently available antidepressants in a clinical setting lead to the search for better cost-effective treatments and the consideration of alternative therapies. For instance, the triple reuptake inhibitors, which tackle the monoaminergic system by inhibiting serotonin, norepinephrine, and dopamine transporters appear to be a promising antidepressant agent (Chen and Skolnick, 2007). However, their efficacy and side-effects are currently under investigation (Tran et al., 2012; Risinger et al., 2014). The use of the NMDAr antagonist ketamine as an antidepressant has gained some validity in the last years. Despite its status as a popular abusive drug causing hallucinations and psychosis, its use in treatment-resistant depressive patients by targeting the glutamatergic system has shown faster and greater improvements (Zarate et al., 2006; Murrough et al., 2013). However, safety and effectiveness of ketamine usage in the long term needs to be further evaluated (Rush, 2013).

Targeting Inflammation in Depression

On account of increasing evidence pointing towards the consideration of MDD as a cytokine-mediated disorder, a number of studies have investigated the implication of current available agents on the immune response. The outcome suggests that pharmacological antidepressants can exert anti-inflammatory effects as a mechanism of action. Studies assessing fluoxetine and the tricyclic antidepressants clomipramine and imipramine demonstrated that these agents are capable of modulating the immune response by inhibiting the activation of glial cells in cell culture models (Hwang et al., 2008; Obuchowicz et al., 2014). Moreover, although the meta-analysis of 22 studies indicated that SSRIs appeared to decrease IL-6 and TNF-α cytokine levels to some extent, it also demonstrated that despite the reduced depressive symptoms found in the studies, an overall effect in the decline of IL-6 and TNF-α after treatment was not found. Noteworthy, IL-1β levels were found to be significantly reduced (Hannestad et al., 2011).

Other treatment alternatives aiming to target the chronic low-grade inflammatory response present in a subgroup of depressive patients have also been sought. These include non-steroidal anti-inflammatory drugs (NSAIDs), minocycline, omega-3 fatty acids, and vitamin D, among others. Usage of selective cyclooxygenase (COX)-2 and non-selective COX inhibitors NSAIDs, namely celecoxib and aspirin, to treat MDD has yielded varied results. A recent meta-analysis of 11 studies evaluating the use of selective COX-2 or non-selective COX inhibitors, including seven randomized clinical trials (RCTs) and four cohort studies, found no overall significant changes in the response to either type of NSAIDs (Eyre et al., 2015). However, in the studies evaluated, effectiveness to reduce depressive symptoms had better outcomes when celecoxib was used as an add-on therapy (Muller et al., 2006; Akhondzadeh et al., 2009). On the other hand, the usage of the tetracycline derivative minocycline to treat mood disorders is currently undergoing clinical trials (Savitz et al., 2012; Dean et al., 2014). Minocycline has been suggested to regulate neuroplasticity by exerting anti-apoptotic, anti-inflammatory and anti-oxidative properties (Soczynska et al., 2012). Moreover, it has been shown to modulate the glutamatergic and monoaminergic systems (O'Connor et al., 2009b; Wixey et al., 2011). Minocycline has been demonstrated to inhibit activation and proliferation of microglia, therefore regulating the immune response (Tikka et al., 2001; Henry et al., 2008). Furthermore, minocycline has been also shown to facilitate the recovery of sickness behavior and down-regulate pro-inflammatory cytokines in mice treated with either LPS or TNF-α (Henry et al., 2008; Zheng et al., 2015).

In regards to omega-3 fatty acids, although their supplementation has been reported to exert beneficial effects in mood disorders (presumably due to its anti-inflammatory properties), a recent meta-analysis of 13 separate RCTs demonstrated no significant effect on MDD (Nemets et al., 2002; Su et al., 2003; Bloch and Hannestad, 2012).

In the case of vitamin D, a meta-analysis of 14 observational studies falling in the category of case-control, cross-sectional and cohort demonstrates that low levels of vitamin D are significantly associated with depression (Anglin et al., 2013). However, the effectiveness of its supplementation in reducing depressive symptoms remains controversial (Jaddou et al., 2012; Kjaergaard et al., 2012). In a recent meta-analysis of seven RCTs, it was shown that supplementation of vitamin D had no overall effect on depressive symptoms. Interestingly, the meta-analysis also points out that better outcomes were achieved with vitamin D supplementation only in cases where clinically significant depressed patients participated in the study. In addition, the largest effect was achieved when vitamin D was administered as an adjuvant (Shaffer et al., 2014). It is noteworthy to mention that although vitamin D intake is broadly considered to induce no adverse effects, overdoses of vitamin D have proven to cause hypercalcemia (Hathcock et al., 2007). Moreover, a study conducted in older community-dwelling women reported that annual high doses of vitamin D (500,000 IU) in a period of 3–5 years increased the risk of falls and fractures, contrary to what was expected (Sanders et al., 2010).

Vitamin D—A Potential Therapeutic Agent?

Vitamin D is regarded as the sunshine vitamin due to the fact that the main natural source of vitamin D is UV-mediated in the epidermal layer of the skin through sunlight stimulation. UV-light converts 7-dehydrocholesterol to pre-vitamin D_3 form, which is later hydroxylated to the major circulating form vitamin D_3 by the enzyme vitamin D 25-hydroxylase. A final hydroxylation is conducted by 1-α-hydroxylase generating the bioactive form $1,25(OH)_2D_3$, also known as calcitriol. However, other natural sources include cod-liver oil, oily fish, butter, cream, and egg yolk (Baeke et al., 2010). This light-dependent source and the fact that vitamin D was demonstrated to act on the brain, spinal cord and several endocrine tissues led to suggest a linkage between vitamin D and neuropsychiatric disorders in individuals less exposed to sunlight (Stumpf and Privette, 1989). Evidence of vitamin D deficiency was later shown in seasonal affective disorder patients (Lansdowne and Provost, 1998). Since then, a number of studies on vitamin D and its effect on MDD have been conducted, yielding highly controversial results, as discussed above. Moreover, vitamin D deficiencies are also reported in the general population (Holick, 2006). Nevertheless, the importance of contemplating vitamin D as a plausible alternative to treat MDD arises not only from its cost-effectiveness and low adverse effects, but is also due to its influence in the immune response.

Vitamin D is a steroid hormone with pleiotropic effects. Aside from its long-recognized role in regulating calcium and phosphorous balance, vitamin D can also influence cell differentiation and proliferation, as well as modulate the immune system. In the CNS, vitamin D can act as an immune-regulator, and as stimulator of neurotrophic factors and neurotransmitters expression (Di Rosa et al., 2011; Eyles et al., 2011; Gezen-Ak et al., 2011). Vitamin D has two main receptors, membrane-associated rapid response steroid-binding (MARRS) and vitamin D receptor (VDR), which are critical for its different regulatory properties (Khanal and Nemere, 2007; Meyer et al., 2010).

MARRS is a membrane receptor that when bound to vitamin D induces rapid non-genomic responses, such as modulation of calcium concentrations and activity of protein kinase C (Khanal and Nemere, 2007). VDR, on the other hand, is a transcription factor that regulates the expression of multiple genes and is responsible for the non-classical responses of vitamin D (Meyer et al., 2010). Upon binding to vitamin D, VDR translocates to the nucleus and heterodimerizes with the retinoid X receptor (RXR). Subsequently, the VDR-RXR complex formed binds to vitamin D responsive elements in the DNA to activate or repress the expression of vitamin D target genes (Fetahu et al., 2014). Moreover, the VDR gene has large cytosine/guanine dinucleotide (CpG) repeats at the promoter region that are susceptible to epigenetic modifications. Conversely, VDR can also modulate the epigenome, inducing DNA methylation and chromatin modulation (Fetahu et al., 2014).

VDR is expressed in more than 38 types of cells, including immune cells (e.g., monocytes, dendritic cells, activated B and T cells) and CNS cells (e.g., neurons, astrocytes, and microglia; Di Rosa et al., 2011; Cui et al., 2013; Smolders et al., 2013). VDR activation was reported to regulate the innate immune response by inducing tolerogenic dendritic cells, inhibiting type 1 T helper (Th1) cell responses, as well as downregulating TLR2, TLR4, and TLR9, inducing decreased expressions of IL-6 (Dickie et al., 2010). Studies with neurons also showed that VDR can regulate the expressions of nerve growth factor and iNOS (Gezen-Ak et al., 2011; Dursun et al., 2013). In addition, the VDR gene has been reported to be associated with viral and bacterial infections. In one study, Epstein-Barr virus nuclear antigen 3 (EBNA-3; produced by EBV) was demonstrated to bind to VDR inducing blockage of VDR-dependent genes, thus protecting cells from VDR-induced growth arrest and/or apoptosis (Yenamandra et al., 2010). Another study showed downregulation of VDR expression in monocytes by *Borrelia burgdorferi* infection (Salazar et al., 2009). In addition, human immunodeficiency virus (HIV) was proved to downregulate VDR expression by inducing its hypermethylation in T cells. This event resulted in the activation of the renin angiotensin system and generation of reactive oxidative species, consequently leading to T cell apoptosis (Chandel et al., 2013). Moreover, vitamin D elicits the expression of the anti-microbial peptides cathelicidin and defensin, important for counteracting infection (Gombart, 2009).

Despite the substantial amount of evidence linking VDR and the immune response, its role in the regulation of the inflammatory response in mood disorders remains to be elucidated. Only two studies to date have reported an association between VDR gene variant and susceptibility to develop depressive symptoms in old age (Kuningas et al., 2009; Glocke et al., 2013). Moreover, studies evaluating the relationship of vitamin D and the cytokine network in mood disorders fall short of expectations. In a recent study, it was reported that suicide attempters had significantly lower levels of vitamin D and higher levels of the pro-inflammatory cytokine IL-1β circulating the blood in comparison to non-suicidal depressed participants and healthy controls. In another study evaluating obese women with polycystic ovary syndrome, vitamin D deficiency was found to be associated with higher depressive symptoms and higher CRP, independent of polycystic ovary syndrome presence (Moran et al., 2015). Furthermore, studies regarding vitamin D immuno-modulatory effects on microglia and astrocytes in depression models are limited in spite of the considerable amount of evidence showing the relevance of these glial cells in the mediation of the CNS immune system. Microarray analysis of primary mixed CNS glia cultures showed that when challenged by a mixed group of Th1 or Th2 cytokines (pro-inflammatory and anti-inflammatory cytokines, respectively) the dopaminergic receptor and enzymes involved in vitamin D metabolism were affected. The expression of 25-hydroxylase (CPY27B1), which generates calcitriol, became upregulated when stimulated with pro-inflammatory cytokines of the Th1 group, whereas the expression of 24-hydroxylase (CPY24A1), the enzyme that catabolizes calcitriol, became downregulated when stimulated with anti-inflammatory cytokines of the Th2 group (Lisak et al., 2009). In line with these results are findings from another study, in which the addition of IFN-γ and TNF to primary human microglia and astrocytic cultures showed upregulation of CYP27B1 mRNA expression, which was reduced in the presence of active vitamin D (Smolders et al., 2013). In a recent report, vitamin D deficient microglia cultures stimulated by TLR agonists showed a decrease of TNF-α and IL-6. Moreover, lower phagocytosis and intracellular killing rates of *Escherichia coli* were also observed (Djukic et al., 2014). In an Alzheimer's disease model, human primary microglia triggered by β-amyloid appear to influence the expression of various inflammatory-related proteins, as well as the upregulation of IDO and VDR (Walker et al., 2006).

Hence, the potential role of VDR influencing the peripheral and/or CNS immune response makes it an attractive target of study in the cytokine-mediated model of MDD (**Figure 1**). In addition, VDR susceptibility to epigenetic changes and likewise its role as epigenetic modulator raises the possibility of considering VDR as a potential biomarker in MDD. For instance, novel biomarkers, such as BDNF promoter methylation, are found to predict mood disorder patient's response to treatment in some studies (D'Addario et al., 2012; Lopez et al., 2013; Tadić et al., 2014). Moreover, relations of VDR epigenetic modifications are already being widely investigated in cancer studies (Fetahu et al., 2014).

It is noteworthy that vitamin D and omega-3 fatty acids also take part in pathways other than the inflammatory network influencing the development of neuropsychiatric illnesses. It was proposed that deficiencies of vitamin D and omega-3 fatty acids in the brain affect serotonin-related mechanisms (Patrick and Ames, 2015). The heterodimer VDR-RXR stimulates serotonin synthesis by activating the tryptophan hydroxylase-2 transcription (Patrick and Ames, 2014). In addition, the omega-3 fatty acid eicosapentaenoic acid was suggested to facilitate serotonin release from neurons (Patrick and Ames, 2015). Moreover, omega-3 fatty acids were

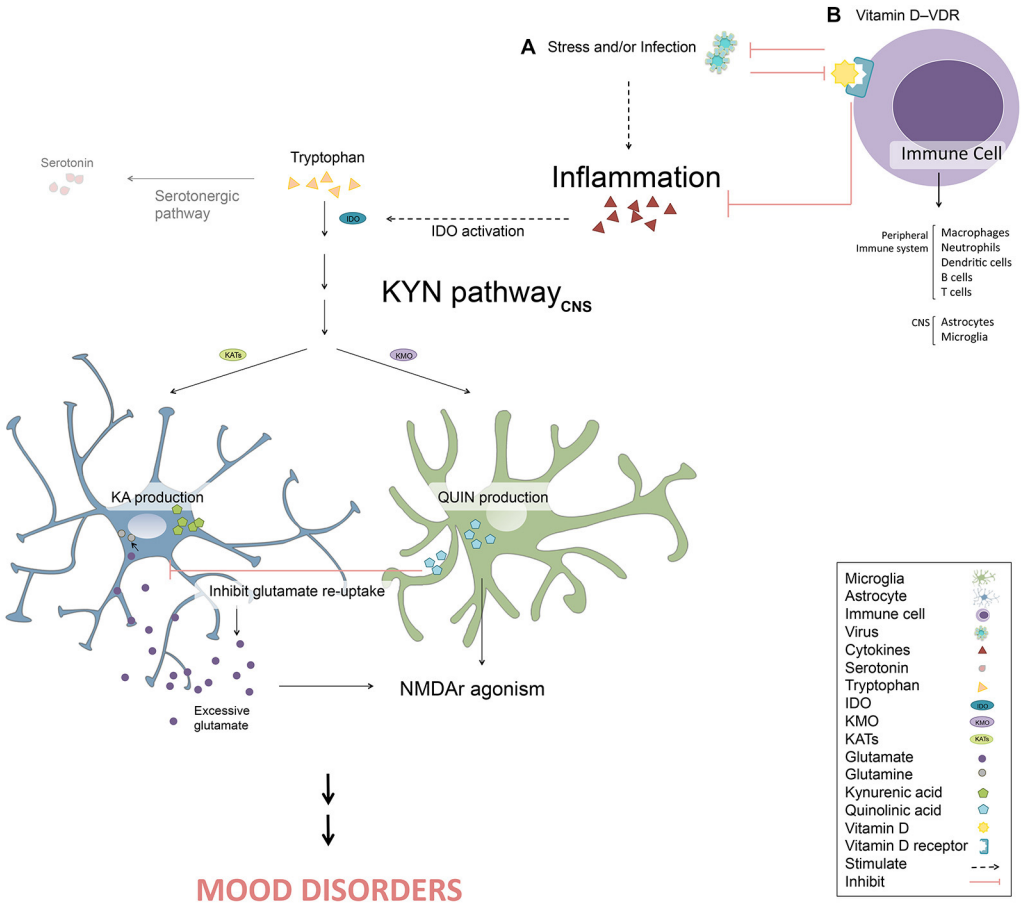


FIGURE 1 | Vitamin D and its receptor (VDR) in the cytokine-mediated onset of depression. (A) Stress and/or infection activate the inflammatory response resulting in inflammatory cytokines production. These cytokines can trigger the kynurenine (KYN) pathway by stimulating indoleamine 2, 3-dioxygenase (IDO). Therefore, tryptophan enters the KYN pathway instead of the serotonergic pathway, resulting in the depletion of monoamines. Once the KYN pathway is triggered, kynurenic acid (KA) in astrocytes and quinolinic acid (QUIN) in microglia are synthesized. QUIN contributes to the activation of N-methyl-D-aspartate receptor (NMDAr) itself and by inhibiting glutamate re-uptake, resulting in excessive extracellular glutamate, inducing further NMDAr agonism. Excessive NMDAr activation leads to the disruption of the glutamatergic system, a key pathological feature in mood disorders. **(B)** The binding of vitamin D to VDR in a number of immune cells (e.g., macrophages, neutrophils, dendritic cells, B cells, T cells, astrocytes and microglia) leads to attenuation of the inflammatory response and stimulation of anti-microbial peptides production, potentially ameliorating symptoms of mood disorders. However, certain pathogens can also block or modulate epigenetic VDR activity.

also found as a low-affinity ligand of VDR (Jurutka et al., 2007).

Conclusions and Future Directions

Depression constitutes a distressing health concern topic. In the last decades, the importance of treating MDD has been accentuated, attracting public interest due to its increasing worldwide prevalence, particularly in high-income countries, threatening to become the leading cause of global disability. Moreover, currently available pharmacological antidepressants do not achieve desired results, producing severe side effects with high relapse rates. Increasing evidence suggests the involvement of the immune response. Cytokine-induced sickness behavior is proposed to culminate in MDD by promoting the activation of the KYN pathway and GR resistance, hence compromising the monoaminergic and glutamatergic neurotransmission along with the HPA axis hyperactivation. Therefore, new alternative options targeting the inflammatory response in mood disorders are being tested. Vitamin D appears to be a plausible candidate although current results from RCTs do not provide sufficient evidence to encourage its supplementation in MDD. It is, however, arguable that the intervention of vitamin D-VDR in different molecular mechanisms affecting the immune system is not fully understood. Research directed towards investigating the role of VDR in regulating the periphery and CNS immune response in relation to mood disorders is suggested. Moreover, another important issue raised in this review is the active participation of glial cells in the dysregulation of relevant mechanisms in the pathology of depression. Microglia and astrocytes are important CNS-immune mediators. However, interactions between VDR and glia have hardly been studied.

In view of the global importance of MDD and the gaps in knowledge that still exist concerning its

pathophysiology, further pursuit of knowledge in regards to the glio-pathogenesis of the inflammatory system activation aiming to find better treatment options is highly warranted.

Acknowledgments

We wish to thank Mrs. F. Sherwood-Brock for proof-reading the manuscript.

References

Agudelo, L. Z., Femenía, T., Orhan, F., Porsmyr-Palmertz, M., Goiny, M., Martinez-Redondo, V., et al. (2014). Skeletal muscle PGC-1alpha1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. *Cell* 159, 33–45. doi: 10.1016/j.cell.2014.07.051

Aguilera, P., Chánez-Cárdenas, M. E., Floriano-Sánchez, E., Barrera, D., Santamaría, A., Sanchez-González, D. J., et al. (2007). Time-related changes in constitutive and inducible nitric oxide synthases in the rat striatum in a model of Huntington's disease. *Neurotoxicology* 28, 1200–1207. doi: 10.1016/j.neuro.2007.07.010

Akhondzadeh, S., Jafari, S., Raisi, F., Nasehi, A. A., Ghoreishi, A., Salehi, B., et al. (2009). Clinical trial of adjunctive celecoxib treatment in patients with major depression: a double blind and placebo controlled trial. *Depress. Anxiety* 26, 607–611. doi: 10.1002/da.20589

Altshuler, L. L., Abulseoud, O. A., Foland-Ross, L., Bartzokis, G., Chang, S., Mintz, J., et al. (2010). Amygdala astrocyte reduction in subjects with major depressive disorder but not bipolar disorder. *Bipolar Disord.* 12, 541–549. doi: 10.1111/j.1399-5618.2010.00838.x

Anacker, C., Zunszain, P. A., Carvalho, L. A., and Pariante, C. M. (2011). The glucocorticoid receptor: pivot of depression and of antidepressant treatment? *Psychoneuroendocrinology* 36, 415–425. doi: 10.1016/j.psyneuen.2010.03.007

Anderson, C. M., and Swanson, R. A. (2000). Astrocyte glutamate transport: review of properties, regulation and physiological functions. *Glia* 32, 1–14. doi: 10.1002/1098-1136(200010)32:1<1::aid-glia10>3.0.co;2-w

Anderson, H. D., Pace, W. D., Libby, A. M., West, D. R., and Valuck, R. J. (2012). Rates of 5 common antidepressant side effects among new adult and adolescent cases of depression: a retrospective US claims study. *Clin. Ther.* 34, 113–123. doi: 10.1016/j.clinthera.2011.11.024

Anderson, I. M. (2000). Selective serotonin reuptake inhibitors versus tricyclic antidepressants: a meta-analysis of efficacy and tolerability. *J. Affect. Disord.* 58, 19–36. doi: 10.1016/s0165-0327(99)00092-0

Anglin, R. E., Samaan, Z., Walter, S. D., and McDonald, S. D. (2013). Vitamin D deficiency and depression in adults: systematic review and meta-analysis. *Br. J. Psychiatry* 202, 100–107. doi: 10.1192/bjp.bp.111.106666

Bachen, E. A., Chesney, M. A., and Criswell, L. A. (2009). Prevalence of mood and anxiety disorders in women with systemic lupus erythematosus. *Arthritis Rheum.* 61, 822–829. doi: 10.1002/art.24519

Baeke, F., Takiishi, T., Korf, H., Gysemans, C., and Mathieu, C. (2010). Vitamin D: modulator of the immune system. *Curr. Opin. Pharmacol.* 10, 482–496. doi: 10.1016/j.coph.2010.04.001

Banasr, M., Chowdhury, G. M., Terwilliger, R., Newton, S. S., Duman, R. S., Behar, K. L., et al. (2010). Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Mol. Psychiatry* 15, 501–511. doi: 10.1038/mp.2008.106

Banasr, M., and Duman, R. S. (2008). Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol. Psychiatry* 64, 863–870. doi: 10.1016/j.biopsych.2008.06.008

Bay-Richter, C., Linderholm, K. R., Lim, C. K., Samuelsson, M., Träskman-Bendz, L., Guillemin, G. J., et al. (2015). A role for inflammatory metabolites as modulators of the glutamate N-methyl-D-aspartate receptor in depression and suicidality. *Brain Behav. Immun.* 43, 110–117. doi: 10.1016/j.bbi.2014.07.012

Behan, W. M., McDonald, M., Darlington, L. G., and Stone, T. W. (1999). Oxidative stress as a mechanism for quinolinic acid-induced hippocampal damage: protection by melatonin and deprenyl. *Br. J. Pharmacol.* 128, 1754–1760. doi: 10.1038/sj.bjp.0702940

Bennett, J. M., Glaser, R., Malarkey, W. B., Beversdorf, D. Q., Peng, J., and Kiecolt-Glaser, J. K. (2012). Inflammation and reactivation of latent herpesviruses in older adults. *Brain Behav. Immun.* 26, 739–746. doi: 10.1016/j.bbi.2011.11.007

Benros, M. E., Waltoft, B. L., Nordentoft, M., Ostergaard, S. D., Eaton, W. W., Krogh, J., et al. (2013). Autoimmune diseases and severe infections as risk factors for mood disorders: a nationwide study. *JAMA Psychiatry* 70, 812–820. doi: 10.1001/jamapsychiatry.2013.1111

Berk, M., Williams, L. J., Jacka, F. N., O'Neil, A., Pasco, J. A., Moylan, S., et al. (2013). So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med.* 11:200. doi: 10.1186/1741-7015-11-200

Biesmans, S., Meert, T. F., Bouwknecht, J. A., Acton, P. D., Davoodi, N., De Haes, P., et al. (2013). Systemic immune activation leads to neuroinflammation and sickness behavior in mice. *Mediators Inflamm.* 2013:271359. doi: 10.1155/2013/271359

Bloch, M. H., and Hannestad, J. (2012). Omega-3 fatty acids for the treatment of depression: systematic review and meta-analysis. *Mol. Psychiatry* 17, 1272–1282. doi: 10.1038/mp.2011.100

Bonaccorso, S., Marino, V., Biondi, M., Grimaldi, F., Ippoliti, F., and Maes, M. (2002). Depression induced by treatment with interferon-alpha in patients affected by hepatitis C virus. *J. Affect. Disord.* 72, 237–241. doi: 10.1016/s0165-0327(02)00264-1

Bowley, M. P., Drevets, W. C., Ongür, D., and Price, J. L. (2002). Low glial numbers in the amygdala in major depressive disorder. *Biol. Psychiatry* 52, 404–412. doi: 10.1016/s0006-3223(02)01404-x

Boyce, P., and Judd, F. (1999). The place for the tricyclic antidepressants in the treatment of depression. *Aust. N. Z. J. Psychiatry* 33, 323–327. doi: 10.1046/j.1440-1614.1999.00580.x

Busse, M., Busse, S., Myint, A. M., Gos, T., Dobrowolny, H., Müller, U. J., et al. (2014). Decreased quinolinic acid in the hippocampus of depressive patients: evidence for local anti-inflammatory and neuroprotective responses? *Eur. Arch. Psychiatry Clin. Neurosci.* 265, 321–329. doi: 10.1007/s00406-014-0562-0

Byatt, N., Rothschild, A. J., Riskind, P., Ionete, C., and Hunt, A. T. (2011). Relationships between multiple sclerosis and depression. *J. Neuropsychiatry Clin. Neurosci.* 23, 198–200. doi: 10.1176/appi.neuropsych.23.2.198

Campbell, B. M., Charych, E., Lee, A. W., and Möller, T. (2014). Kynurenines in CNS disease: regulation by inflammatory cytokines. *Front. Neurosci.* 8:12. doi: 10.3389/fnins.2014.00012

Capuron, L., Ravaud, A., Neveu, P. J., Miller, A. H., Maes, M., and Dantzer, R. (2002). Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol. Psychiatry* 7, 468–473. doi: 10.1038/sj.mp.4000995

Chandel, N., Husain, M., Goel, H., Salhan, D., Lan, X., Malhotra, A., et al. (2013). VDR hypermethylation and HIV-induced T cell loss. *J. Leukoc. Biol.* 93, 623–631. doi: 10.1189/jlb.0812383

Charney, D. S. (1998). Monoamine dysfunction and the pathophysiology and treatment of depression. *J. Clin. Psychiatry* 59(Suppl. 14), 11–14. doi: 10.5772/21140

Chen, Z., and Skolnick, P. (2007). Triple uptake inhibitors: therapeutic potential in depression and beyond. *Expert Opin. Investig. Drugs* 16, 1365–1377. doi: 10.1517/13543784.16.9.1365

Cohen, S., Janicki-Deverts, D., Doyle, W. J., Miller, G. E., Frank, E., Rabin, B. S., et al. (2012). Chronic stress, glucocorticoid receptor resistance, inflammation and disease risk. *Proc. Natl. Acad. Sci. U S A* 109, 5995–5999. doi: 10.1073/pnas.1118355109

Constant, A., Castera, L., Dantzer, R., Couzigou, P., de Ledinghen, V., Demotes-Mainard, J., et al. (2005). Mood alterations during interferon-alfa therapy in patients with chronic hepatitis C: evidence for an overlap between manic/hypomanic and depressive symptoms. *J. Clin. Psychiatry* 66, 1050–1057. doi: 10.4088/jcp.v66n0814

Cotter, D., Mackay, D., Landau, S., Kerwin, R., and Everall, I. (2001). Reduced glial cell density and neuronal size in the anterior cingulate cortex in major

depressive disorder. *Arch. Gen. Psychiatry* 58, 545–553. doi: 10.1001/archpsyc.58.6.545

Cruceanu, C., Alda, M., Nagy, C., Freemantle, E., Rouleau, G. A., and Turecki, G. (2013). H3K4 tri-methylation in synapsin genes leads to different expression patterns in bipolar disorder and major depression. *Int. J. Neuropsychopharmacol.* 16, 289–299. doi: 10.1017/s1461145712000363

Cui, X., Pelekanos, M., Liu, P. Y., Burne, T. H., McGrath, J. J., and Eyles, D. W. (2013). The vitamin D receptor in dopamine neurons; its presence in human substantia nigra and its ontogenesis in rat midbrain. *Neuroscience* 236, 77–87. doi: 10.1016/j.neuroscience.2013.01.035

D'Addario, C., Dell'Osso, B., Palazzo, M. C., Benatti, B., Lietti, L., Cattaneo, E., et al. (2012). Selective DNA methylation of BDNF promoter in bipolar disorder: differences among patients with BDI and BDII. *Neuropsychopharmacology* 37, 1647–1655. doi: 10.1038/npp.2012.10

Dahl, J., Ormstad, H., Aass, H. C., Malt, U. F., Bendz, L. T., Sandvik, L., et al. (2014). The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology* 45, 77–86. doi: 10.1016/j.psyneuen.2014.03.019

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., and Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. doi: 10.1038/nrn2297

Davis, L., Uezato, A., Newell, J. M., and Frazier, E. (2008). Major depression and comorbid substance use disorders. *Curr. Opin. Psychiatry* 21, 14–18. doi: 10.1097/YCO.0b013e3282f32408

De La Garza, R. II (2005). Endotoxin- or pro-inflammatory cytokine-induced sickness behavior as an animal model of depression: focus on anhedonia. *Neurosci. Biobehav. Rev.* 29, 761–770. doi: 10.1016/j.neubiorev.2005.03.016

Dean, O. M., Maes, M., Ashton, M., Berk, L., Kanchanatawan, B., Sughondhabirom, A., et al. (2014). Protocol and rationale-the efficacy of Minocycline as an adjunctive treatment for major depressive disorder: a double blind, randomised, Placebo controlled trial. *Clin. Psychopharmacol. Neurosci.* 12, 180–188. doi: 10.9758/cpn.2014.12.3.180

Di Rosa, M., Malaguarnera, M., Nicoletti, F., and Malaguarnera, L. (2011). Vitamin D3: a helpful immuno-modulator. *Immunology* 134, 123–139. doi: 10.1111/j.1365-2567.2011.03482.x

Di Trani, M., Di Roma, F., Andriola, E., Leone, D., Parisi, P., Miano, S., et al. (2014). Comorbid depressive disorders in ADHD: the role of ADHD severity, subtypes and familial psychiatric disorders. *Psychiatry Investig.* 11, 137–142. doi: 10.4306/pi.2014.11.2.137

Dickie, L. J., Church, L. D., Coulthard, L. R., Mathews, R. J., Emery, P., and McDermott, M. F. (2010). Vitamin D3 down-regulates intracellular toll-like receptor 9 expression and toll-like receptor 9-induced IL-6 production in human monocytes. *Rheumatology (Oxford)* 49, 1466–1471. doi: 10.1093/rheumatology/keq124

Dietrich, D. E., Schedlowski, M., Bode, L., Ludwig, H., and Emrich, H. M. (1998). A viro-psycho-immunological disease-model of a subtype affective disorder. *Pharmacopsychiatry* 31, 77–82. doi: 10.1055/s-2007-979305

Djukic, M., Onken, M. L., Schutze, S., Redlich, S., Götz, A., Hanisch, U. K., et al. (2014). Vitamin d deficiency reduces the immune response, phagocytosis rate and intracellular killing rate of microglial cells. *Infect. Immun.* 82, 2585–2594. doi: 10.1128/iai.01814-14

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., et al. (2010). A meta-analysis of cytokines in major depression. *Biol. Psychiatry* 67, 446–457. doi: 10.1016/j.biopsych.2009.09.033

Dursun, E., Gezen-Ak, D., and Yilmazer, S. (2013). A new mechanism for amyloid-beta induction of iNOS: vitamin D-VDR pathway disruption. *J. Alzheimers Dis.* 36, 459–474. doi: 10.3233/JAD-130416

Edgar, N., and Sibille, E. (2012). A putative functional role for oligodendrocytes in mood regulation. *Trans. Psychiatry* 2:e109. doi: 10.1038/tp.2012.34

Elliott, E., Ezra-Nevo, G., Regev, L., Neufeld-Cohen, A., and Chen, A. (2010). Resilience to social stress coincides with functional DNA methylation of the Crf gene in adult mice. *Nat. Neurosci.* 13, 1351–1353. doi: 10.1038/nn.2642

Erhardt, S., Schwieler, L., Emanuelsson, C., and Geyer, M. (2004). Endogenous kynurenic acid disrupts prepulse inhibition. *Biol. Psychiatry* 56, 255–260. doi: 10.1016/j.biopsych.2004.06.006

Eyles, D., Burne, T., and McGrath, J. (2011). Vitamin D in fetal brain development. *Semin. Cell Dev. Biol.* 22, 629–636. doi: 10.1016/j.semcdb.2011.05.004

Eyre, H. A., Air, T., Proctor, S., Rositano, S., and Baune, B. T. (2015). A critical review of the efficacy of non-steroidal anti-inflammatory drugs in depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 57, 11–16. doi: 10.1016/j.pnpbp.2014.10.003

Fass, D. M., Schroeder, F. A., Perlis, R. H., and Haggarty, S. J. (2014). Epigenetic mechanisms in mood disorders: targeting neuroplasticity. *Neuroscience* 264, 112–130. doi: 10.1016/j.neuroscience.2013.01.041

Fetahu, I. S., Höbaus, J., and Kállay, E. (2014). Vitamin D and the epigenome. *Front. Physiol.* 5:164. doi: 10.3389/fphys.2014.00164

Finberg, J. P. (2014). Update on the pharmacology of selective inhibitors of MAO-A and MAO-B: focus on modulation of CNS monoamine neurotransmitter release. *Pharmacol. Ther.* 143, 133–152. doi: 10.1016/j.pharmthera.2014.02.010

Fuchikami, M., Morinobu, S., Kurata, A., Yamamoto, S., and Yamawaki, S. (2009). Single immobilization stress differentially alters the expression profile of transcripts of the brain-derived neurotrophic factor (BDNF) gene and histone acetylation at its promoters in the rat hippocampus. *Int. J. Neuropsychopharmacol.* 12, 73–82. doi: 10.1017/s1461145708008997

Garate, I., García-Bueno, B., Madrigal, J. L., Bravo, L., Berrocoso, E., Caso, J. R., et al. (2011). Origin and consequences of brain toll-like receptor 4 pathway stimulation in an experimental model of depression. *J. Neuroinflammation* 8:151. doi: 10.1186/1742-2094-8-151

Gezen-Ak, D., Dursun, E., and Yilmazer, S. (2011). The effects of vitamin D receptor silencing on the expression of LVSCC-A1C and LVSCC-A1D and the release of NGF in cortical neurons. *PLoS One* 6:e17553. doi: 10.1371/journal.pone.0017553

Gittins, R. A., and Harrison, P. J. (2011). A morphometric study of glia and neurons in the anterior cingulate cortex in mood disorder. *J. Affect. Disord.* 133, 328–332. doi: 10.1016/j.jad.2011.03.042

Glocke, M., Lang, F., Schaeffeler, E., Lang, T., Schwab, M., and Lang, U. E. (2013). Impact of vitamin D receptor VDR rs2228570 polymorphism in oldest old. *Kidney Blood Press. Res.* 37, 311–322. doi: 10.1159/000350159

Gold, P. W., and Chrousos, G. P. (2002). Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs. low CRH/NE states. *Mol. Psychiatry* 7, 254–275. doi: 10.1038/sj.mp.4001032

Gombart, A. F. (2009). The vitamin D-antimicrobial peptide pathway and its role in protection against infection. *Future Microbiol.* 4, 1151–1165. doi: 10.2217/fmb.09.87

Goodwin, R. D. (2011). Association between infection early in life and mental disorders among youth in the community: a cross-sectional study. *BMC Public Health* 11:878. doi: 10.1186/1471-2458-11-878

Guillemin, G. J., Kerr, S. J., Smythe, G. A., Smith, D. G., Kapoor, V., Armati, P. J., et al. (2001). Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection. *J. Neurochem.* 78, 842–853. doi: 10.1046/j.1471-4159.2001.00498.x

Guillemin, G. J., Smith, D. G., Smythe, G. A., Armati, P. J., and Brew, B. J. (2003). Expression of the kynurenine pathway enzymes in human microglia and macrophages. *Adv. Exp. Med. Biol.* 527, 105–112. doi: 10.1007/978-1-4615-0135-0_12

Guillemin, G. J., Smythe, G., Takikawa, O., and Brew, B. J. (2005). Expression of indoleamine 2,3-dioxygenase and production of quinolinic acid by human microglia, astrocytes and neurons. *Glia* 49, 15–23. doi: 10.1002/glia.20090

Haack, M., Hinze-Selch, D., Fenzel, T., Kraus, T., Kühn, M., Schuld, A., et al. (1999). Plasma levels of cytokines and soluble cytokine receptors in psychiatric patients upon hospital admission: effects of confounding factors and diagnosis. *J. Psychiatr. Res.* 33, 407–418. doi: 10.1016/s0022-3956(99)00021-7

Hamidi, M., Drevets, W. C., and Price, J. L. (2004). Glial reduction in amygdala in major depressive disorder is due to oligodendrocytes. *Biol. Psychiatry* 55, 563–569. doi: 10.1016/j.biopsych.2003.11.006

Hannestad, J., DellaGioia, N., and Bloch, M. (2011). The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology* 36, 2452–2459. doi: 10.1038/npp.2011.132

Hannon, T. S., Rofey, D. L., Lee, S., and Arslanian, S. A. (2013). Depressive symptoms and metabolic markers of risk for type 2 diabetes in obese adolescents. *Pediat. Diabetes* 14, 497–503. doi: 10.1111/pedi.12035

Hathcock, J. N., Shao, A., Vieth, R., and Heaney, R. (2007). Risk assessment for vitamin D. *Am. J. Clin. Nutr.* 85, 6–18.

Heim, C., and Binder, E. B. (2012). Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions and epigenetics. *Exp. Neurol.* 233, 102–111. doi: 10.1016/j.expneurol.2011.10.032

Henry, C. J., Huang, Y., Wynne, A., Hanke, M., Himler, J., Bailey, M. T., et al. (2008). Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior and anhedonia. *J. Neuroinflammation* 5:15. doi: 10.1186/1742-2094-5-15

Holick, M. F. (2006). High prevalence of vitamin D inadequacy and implications for health. *Mayo Clinic Proc.* 81, 353–373. doi: 10.4065/81.3.353

Hornig, M., Briese, T., Licinio, J., Khabbaz, R. F., Altshuler, L. L., Potkin, S. G., et al. (2012). Absence of evidence for bornavirus infection in schizophrenia, bipolar disorder and major depressive disorder. *Mol. Psychiatry* 17, 486–493. doi: 10.1038/mp.2011.179

Hu, F., Pace, T. W., and Miller, A. H. (2009). Interferon-alpha inhibits glucocorticoid receptor-mediated gene transcription via STAT5 activation in mouse HT22 cells. *Brain Behav. Immun.* 23, 455–463. doi: 10.1016/j.bbi.2009.01.001

Hwang, J., Zheng, L. T., Ock, J., Lee, M. G., Kim, S. H., Lee, H. W., et al. (2008). Inhibition of glial inflammatory activation and neurotoxicity by tricyclic antidepressants. *Neuropharmacology* 55, 826–834. doi: 10.1016/j.neuropharm.2008.06.045

Jaddou, H. Y., Batieha, A. M., Khader, Y. S., Kanaan, S. H., El-Khateeb, M. S., and Ajlouni, K. M. (2012). Depression is associated with low levels of 25-hydroxyvitamin D among Jordanian adults: results from a national population survey. *Eur. Arch. Psychiatry Clin. Neurosci.* 262, 321–327. doi: 10.1007/s00406-011-0265-8

Jo, W. K., Law, A. C., and Chung, S. K. (2014). The neglected co-star in the dementia drama: the putative roles of astrocytes in the pathogeneses of major neurocognitive disorders. *Mol. Psychiatry* 19, 159–167. doi: 10.1038/mp.2013.171

Johnston-Wilson, N. L., Sims, C. D., Hofmann, J. P., Anderson, L., Shore, A. D., Torrey, E. F., et al. (2000). Disease-specific alterations in frontal cortex brain proteins in schizophrenia, bipolar disorder and major depressive disorder. The stanley neuropathology consortium. *Mol. Psychiatry* 5, 142–149. doi: 10.1038/sj.mp.4000696

Jurutka, P. W., Bartik, L., Whitfield, G. K., Mathern, D. R., Barthel, T. K., Gurevich, M., et al. (2007). Vitamin D receptor: key roles in bone mineral pathophysiology, molecular mechanism of action and novel nutritional ligands. *J. Bone Miner. Res.* 22(Suppl. 2), V2–V10. doi: 10.1359/jbmr.07s216

Khanal, R., and Nemere, I. (2007). Membrane receptors for vitamin D metabolites. *Crit. Rev. Eukaryot. Gene Expr.* 17, 31–47. doi: 10.1615/critreveukargeneexpr.v17.i1.30

Khandaker, G. M., Pearson, R. M., Zammit, S., Lewis, G., and Jones, P. B. (2014). Association of serum interleukin 6 and C-reactive protein in childhood with depression and psychosis in young adult life: a population-based longitudinal study. *JAMA Psychiatry* 71, 1121–1128. doi: 10.1001/jamapsychiatry.2014.1332

Kim, H., Chen, L., Lim, G., Sung, B., Wang, S., McCabe, M. F., et al. (2012). Brain indoleamine 2,3-dioxygenase contributes to the comorbidity of pain and depression. *J. Clin. Invest.* 122, 2940–2954. doi: 10.1172/JCI61884

Kim, Y. K., Lee, S. W., Kim, S. H., Shim, S. H., Han, S. W., Choi, S. H., et al. (2008). Differences in cytokines between non-suicidal patients and suicidal patients in major depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 32, 356–361. doi: 10.1016/j.pnpbp.2007.08.041

Kim, S., and Webster, M. J. (2010). Correlation analysis between genome-wide expression profiles and cytoarchitectural abnormalities in the prefrontal cortex of psychiatric disorders. *Mol. Psychiatry* 15, 326–336. doi: 10.1038/mp.2008.99

Kjaergaard, M., Waterloo, K., Wang, C. E., Almås, B., Figenschau, Y., Hutchinson, M. S., et al. (2012). Effect of vitamin D supplement on depression scores in people with low levels of serum 25-hydroxyvitamin D: nested case-control study and randomised clinical trial. *Br. J. Psychiatry* 201, 360–368. doi: 10.1192/bjp.bp.111.104349

Klomp, A., Václavu, L., Meerhoff, G. F., Reneman, L., and Lucassen, P. J. (2014). Effects of chronic fluoxetine treatment on neurogenesis and tryptophan hydroxylase expression in adolescent and adult rats. *PLoS One* 9:e97603. doi: 10.1371/journal.pone.0097603

Kreisel, T., Frank, M. G., Licht, T., Reshef, R., Ben-Menachem-Zidon, O., Baratta, M. V., et al. (2014). Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. *Mol. Psychiatry* 19, 699–709. doi: 10.1038/mp.2013.155

Kuningas, M., Mooijaart, S. P., Jolles, J., Slagboom, P. E., Westendorp, R. G., and van Heemst, D. (2009). VDR gene variants associate with cognitive function and depressive symptoms in old age. *Neurobiol. Aging* 30, 466–473. doi: 10.1016/j.neurobiolaging.2007.07.001

Lansdowne, A. T., and Provost, S. C. (1998). Vitamin D3 enhances mood in healthy subjects during winter. *Psychopharmacology (Berl)* 135, 319–323. doi: 10.1007/s002130050517

Linderholm, K. R., Skogh, E., Olsson, S. K., Dahl, M. L., Holtze, M., Engberg, G., et al. (2012). Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. *Schizophr. Bull.* 38, 426–432. doi: 10.1093/schbul/sbq086

Lisak, R. P., Benjamins, J. A., Bealmear, B., Nedelkoska, L., Studzinski, D., Retland, E., et al. (2009). Differential effects of Th1, monocyte/macrophage and Th2 cytokine mixtures on early gene expression for molecules associated with metabolism, signaling and regulation in central nervous system mixed glial cell cultures. *J. Neuroinflammation* 6:4. doi: 10.1186/1742-2094-6-4

Lopez, J. P., Mamdani, F., Labonte, B., Beaulieu, M. M., Yang, J. P., Berlim, M. T., et al. (2013). Epigenetic regulation of BDNF expression according to antidepressant response. *Mol. Psychiatry* 18, 398–399. doi: 10.1038/mp.2012.38

Lu, D. Y., Leung, Y. M., and Su, K. P. (2013). Interferon-alpha induces nitric oxide synthase expression and haem oxygenase-1 down-regulation in microglia: implications of cellular mechanism of IFN-alpha-induced depression. *Int. J. Neuropsychopharmacol.* 16, 433–444. doi: 10.1017/s1461145712000338

Lugo-Huitrón, R., Blanco-Ayala, T., Ugalde-Muñiz, P., Carrillo-Mora, P., Pedraza-Chaverrí, J., Silva-Adaya, D., et al. (2011). On the antioxidant properties of kynurenic acid: free radical scavenging activity and inhibition of oxidative stress. *Neurotoxicol. Teratol.* 33, 538–547. doi: 10.1016/j.ntt.2011.07.002

Maes, M., Scharpé, S., Meltzer, H. Y., Bosmans, E., Suy, E., Calabrese, J., et al. (1993). Relationships between interleukin-6 activity, acute phase proteins and function of the hypothalamic-pituitary-adrenal axis in severe depression. *Psychiatry Res.* 49, 11–27. doi: 10.1016/0165-1781(93)90027-e

Maes, M., Smith, R., and Scharpe, S. (1995). The monocyte-T-lymphocyte hypothesis of major depression. *Psychoneuroendocrinology* 20, 111–116. doi: 10.1016/0306-4530(94)00066-j

Maier, S. F. (2003). Bi-directional immune-brain communication: implications for understanding stress, pain and cognition. *Brain Behav. Immun.* 17, 69–85. doi: 10.1016/s0889-1591(03)00032-1

Mavrides, N., and Nemeroff, C. (2013). Treatment of depression in cardiovascular disease. *Depress. Anxiety* 30, 328–341. doi: 10.1002/da.22051

Meyer, M. B., Goetsch, P. D., and Pike, J. W. (2010). Genome-wide analysis of the VDR/RXR cistrome in osteoblast cells provides new mechanistic insight into the actions of the vitamin D hormone. *J. Steroid Biochem. Mol Biol.* 121, 136–141. doi: 10.1016/j.jsbmb.2010.02.011

Miguel-Hidalgo, J. J., Waltzer, R., Whittom, A. A., Austin, M. C., Rajkowska, G., and Stockmeier, C. A. (2010). Glial and glutamatergic markers in depression, alcoholism and their comorbidity. *J. Affect. Disord.* 127, 230–240. doi: 10.1016/j.jad.2010.06.003

Mill, J., and Petronis, A. (2007). Molecular studies of major depressive disorder: the epigenetic perspective. *Mol. Psychiatry* 12, 799–814. doi: 10.1038/sj.mp.4001992

Miller, G. E., Cohen, S., and Ritchey, A. K. (2002). Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid-resistance model. *Health Psychology* 21, 531–541. doi: 10.1037//0278-6133.21.6.531

Moran, L. J., Teede, H. J., and Vincent, A. J. (2015). Vitamin D is independently associated with depression in overweight women with and without PCOS. *Gynecol. Endocrinol.* 31, 179–182. doi: 10.3109/09513590.2014.975682

Muller, N., Schwarz, M. J., Dehning, S., Douhe, A., Cerovecki, A., Goldstein-Müller, B., et al. (2006). The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Mol. Psychiatry* 11, 680–684. doi: 10.1038/sj.mp.4001805

Muller, N., and Schwarz, M. J. (2007). The immune-mediated alteration of serotonin and glutamate: towards an integrated view of depression. *Mol. Psychiatry* 12, 988–1000. doi: 10.1038/sj.mp.4002006

Murrough, J. W., Iosifescu, D. V., Chang, L. C., Al Jurdi, R. K., Green, C. E., Perez, A. M., et al. (2013). Antidepressant efficacy of ketamine in treatment-resistant major depression: a two-site randomized controlled trial. *Am. J. Psychiatry* 170, 1134–1142. doi: 10.1176/appi.ajp.2013.13030392

Nagy, C., Suderman, M., Yang, J., Szyf, M., Mechawar, N., Ernst, C., et al. (2014). Astrocytic abnormalities and global DNA methylation patterns in depression and suicide. *Mol. Psychiatry* 20, 320–328. doi: 10.1038/mp.2014.21

Nemets, B., Stahl, Z., and Belmaker, R. H. (2002). Addition of omega-3 fatty acid to maintenance medication treatment for recurrent unipolar depressive disorder. *Am. J. Psychiatry* 159, 477–479. doi: 10.1176/appi.ajp.159.3.477

Nozaki, K., and Beal, M. F. (1992). Neuroprotective effects of L-kynurenine on hypoxia-ischemia and NMDA lesions in neonatal rats. *J. Cereb. Blood Flow Metab.* 12, 400–407. doi: 10.1038/jcbfm.1992.57

Obuchowicz, E., Bielecka, A. M., Paul-Samojedny, M., Pudelko, A., and Kowalski, J. (2014). Imipramine and fluoxetine inhibit LPS-induced activation and affect morphology of microglial cells in the rat glial culture. *Pharmacol. Rep.* 66, 34–43. doi: 10.1016/j.pharep.2013.08.002

O'Connor, J. C., André, C., Wang, Y., Lawson, M. A., Szegedi, S. S., Lestage, J., et al. (2009a). Interferon-gamma and tumor necrosis factor-alpha mediate the upregulation of indoleamine 2,3-dioxygenase and the induction of depressive-like behavior in mice in response to bacillus Calmette-Guerin. *J. Neurosci.* 29, 4200–4209. doi: 10.1523/jneurosci.5032-08.2009

O'Connor, J. C., Lawson, M. A., André, C., Moreau, M., Lestage, J., Castanon, N., et al. (2009b). Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol. Psychiatry* 14, 511–522. doi: 10.1038/sj.mp.4002148

Ousman, S. S., and Kubes, P. (2012). Immune surveillance in the central nervous system. *Nat. Neurosci.* 15, 1096–1101. doi: 10.1038/nn.3161

Pace, T. W., Hu, F., and Miller, A. H. (2007). Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. *Brain Behav. Immun.* 21, 9–19. doi: 10.1016/j.bbi.2006.08.009

Patrick, R. P., and Ames, B. N. (2014). Vitamin D hormone regulates serotonin synthesis. Part 1: relevance for autism. *FASEB J.* 28, 2398–2413. doi: 10.1096/fj.13-246546

Patrick, R. P., and Ames, B. N. (2015). Vitamin D and the omega-3 fatty acids control serotonin synthesis and action, part 2: relevance for ADHD, bipolar disorder, schizophrenia and impulsive behavior. *FASEB J.* 29, 2207–2222. doi: 10.1096/fj.14-268342

Pearce, B. D., Kruszon-Moran, D., and Jones, J. L. (2012). The relationship between Toxoplasma gondii infection and mood disorders in the third National Health and Nutrition Survey. *Biol. Psychiatry* 72, 290–295. doi: 10.1016/j.biopsych.2012.01.003

Perroud, N., Salzmann, A., Prada, P., Nicastro, R., Hoeppli, M. E., Furrer, S., et al. (2013). Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. *Trans. Psychiatry* 3:e207. doi: 10.1038/tp.2012.140

Pocivavsek, A., Wu, H. Q., Potter, M. C., Elmer, G. I., Pellicciari, R., and Schwarcz, R. (2011). Fluctuations in endogenous kynurenic acid control hippocampal glutamate and memory. *Neuropsychopharmacology* 36, 2357–2367. doi: 10.1038/npp.2011.127

Raison, C. L., and Miller, A. H. (2003). When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *Am. J. Psychiatry* 160, 1554–1565. doi: 10.1176/appi.ajp.160.9.1554

Raison, C. L., and Miller, A. H. (2013). The evolutionary significance of depression in Pathogen Host Defense (PATHOS-D). *Mol. Psychiatry* 18, 15–37. doi: 10.1038/mp.2012.2

Raison, C. L., Capuron, L., and Miller, A. H. (2006). Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol.* 27, 24–31. doi: 10.1016/j.it.2005.11.006

Raison, C. L., Dantzer, R., Kelley, K. W., Lawson, M. A., Woolwine, B. J., Vogt, G., et al. (2010). CSF concentrations of brain tryptophan and kynurenines during immune stimulation with IFN-alpha: relationship to CNS immune responses and depression. *Mol. Psychiatry* 15, 393–403. doi: 10.1038/mp.2009.116

Rajkowska, G., Hughes, J., Stockmeier, C. A., Javier Miguel-Hidalgo, J., and Maciag, D. (2013). Coverage of blood vessels by astrocytic endfeet is reduced in major depressive disorder. *Biol. Psychiatry* 73, 613–621. doi: 10.1016/j.biopsych.2012.09.024

Rajkowska, G., Miguel-Hidalgo, J. J., Wei, J., Dilley, G., Pittman, S. D., Meltzer, H. Y., et al. (1999). Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol. Psychiatry* 45, 1085–1098. doi: 10.1016/s0006-3223(99)00041-4

Rajkowska, G., and Miguel-Hidalgo, J. J. (2007). Gliogenesis and glial pathology in depression. *CNS Neurol. Disord. Drug Targets* 6, 219–233. doi: 10.2174/187152707780619326

Ressler, K. J., Bradley, B., Mercer, K. B., Deveau, T. C., Smith, A. K., Gillespie, C. F., et al. (2010). Polymorphisms in CRHR1 and the serotonin transporter loci: gene x gene x environment interactions on depressive symptoms. *Am. J. Med. Genet. B Neuropsychiat. Genet.* 153B, 812–824. doi: 10.1002/ajmg.b.31052

Risinger, R., Bhagwagar, Z., Luo, F., Cahir, M., Miler, L., Mendonza, A. E., et al. (2014). Evaluation of safety and tolerability, pharmacokinetics and pharmacodynamics of BMS-820836 in healthy subjects: a placebo-controlled, ascending single-dose study. *Psychopharmacology (Berl)* 231, 2299–2310. doi: 10.1007/s00213-013-3391-3

Rotter, A., Biermann, T., Stark, C., Decker, A., Demling, J., Zimmermann, R., et al. (2013). Changes of cytokine profiles during electroconvulsive therapy in patients with major depression. *J. ECT* 29, 162–169. doi: 10.1097/YCT.0b013e3182843942

Rush, A. J. (2013). Ketamine for treatment-resistant depression: ready or not for clinical use? *Am. J. Psychiatry* 170, 1079–1081. doi: 10.1176/appi.ajp.2013.13081034

Salazar, J. C., Duhnam-Ems, S., La Vake, C., Cruz, A. R., Moore, M. W., Caimano, M. J., et al. (2009). Activation of human monocytes by live Borrelia burgdorferi generates TLR2-dependent and -independent responses which include induction of IFN-beta. *PLoS Pathog.* 5:e1000444. doi: 10.1371/journal.ppat.1000444

Sanders, K. M., Stuart, A. L., Williamson, E. J., Simpson, J. A., Kotowicz, M. A., Young, D., et al. (2010). Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. *JAMA* 303, 1815–1822. doi: 10.1001/jama.2010.594

Savitz, J., Drevets, W. C., Wurfel, B. E., Ford, B. N., Bellgowan, P. S., Victor, T. A., et al. (2015). Reduction of kynurenic acid to quinolinic acid ratio in both the depressed and remitted phases of major depressive disorder. *Brain Behav. Immun.* 46, 55–59. doi: 10.1016/j.bbi.2015.02.007

Savitz, J., Preskorn, S., Teague, T. K., Drevets, D., Yates, W., and Drevets, W. (2012). Minocycline and aspirin in the treatment of bipolar depression: a protocol for a proof-of-concept, randomised, double-blind, placebo-controlled, 2x2 clinical trial. *BMJ Open* 2:e000643. doi: 10.1136/bmjopen-2011-000643

Schillani, G., Era, D., Cristante, T., Mustacchi, G., Richiardi, M., Grassi, L., et al. (2012). 5-HTTLPR polymorphism and anxious preoccupation in early breast cancer patients. *Radiol. Oncol.* 46, 321–327. doi: 10.2478/v10019-012-0024-0

Shaffer, J. A., Edmondson, D., Wasson, L. T., Falzon, L., Homma, K., Ezeokoli, N., et al. (2014). Vitamin D supplementation for depressive symptoms: a systematic review and meta-analysis of randomized controlled trials. *Psychosom. Med.* 76, 190–196. doi: 10.1097/psy.0000000000000044

Smolders, J., Schuurman, K. G., van Strien, M. E., Melief, J., Hendrickx, D., Hol, E. M., et al. (2013). Expression of vitamin D receptor and metabolizing enzymes in multiple sclerosis-affected brain tissue. *J. Neuropathol. Exp. Neurol.* 72, 91–105. doi: 10.1097/NEN.0b013e31827f4fcc

Soczynska, J. K., Mansur, R. B., Brietzke, E., Swardfager, W., Kennedy, S. H., Woldeyohannes, H. O., et al. (2012). Novel therapeutic targets in depression: minocycline as a candidate treatment. *Behav. Brain Res.* 235, 302–317. doi: 10.1016/j.bbr.2012.07.026

Steiner, J., Bielau, H., Brisch, R., Danos, P., Ullrich, O., Mawrin, C., et al. (2008). Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J. Psychiatr. Res.* 42, 151–157. doi: 10.1016/j.jpsychires.2006.10.013

Steiner, J., Walter, M., Gos, T., Guillemin, G. J., Bernstein, H. G., Sarnyai, Z., et al. (2011). Severe depression is associated with increased microglial quinolinic acid in subregions of the anterior cingulate gyrus: evidence for an immune-modulated glutamatergic neurotransmission? *J. Neuroinflammation* 8:94. doi: 10.1186/1742-2094-8-94

Steptoe, A., Kunz-Ebrecht, S. R., and Owen, N. (2003). Lack of association between depressive symptoms and markers of immune and vascular inflammation in middle-aged men and women. *Psychol. Med.* 33, 667–674. doi: 10.1017/s0033291702007250

Stumpf, W. E., and Privette, T. H. (1989). Light, vitamin D and psychiatry. Role of 1,25 dihydroxyvitamin D3 (soltriol) in etiology and therapy of seasonal affective disorder and other mental processes. *Psychopharmacology (Berl)* 97, 285–294. doi: 10.1007/bf00439440

Su, K. P., Huang, S. Y., Chiu, C. C., and Shen, W. W. (2003). Omega-3 fatty acids in major depressive disorder. A preliminary double-blind, placebo-controlled trial. *Eur. Neuropsychopharmacol.* 13, 267–271. doi: 10.1016/S0924-977X(03)00032-4

Sun, H., Kennedy, P. J., and Nestler, E. J. (2013). Epigenetics of the depressed brain: role of histone acetylation and methylation. *Neuropsychopharmacology* 38, 124–137. doi: 10.1038/npp.2012.73

Szuchet, S., Nielsen, J. A., Lovas, G., Domowicz, M. S., de Velasco, J. M., Maric, D., et al. (2011). The genetic signature of perineuronal oligodendrocytes reveals their unique phenotype. *Eur. J. Neurosci.* 34, 1906–1922. doi: 10.1111/j.1460-9568.2011.07922.x

Tadić, A., Muller-Engling, L., Schlicht, K. F., Kotsiari, A., Dreimüller, N., Kleimann, A., et al. (2014). Methylation of the promoter of brain-derived neurotrophic factor exon IV and antidepressant response in major depression. *Mol. Psychiatry* 19, 281–283. doi: 10.1038/mp.2013.58

Tavares, R. G., Schmidt, A. P., Abud, J., Tasca, C. I., and Souza, D. O. (2005). In vivo quinolinic acid increases synaptosomal glutamate release in rats: reversal by guanosine. *Neurochem. Res.* 30, 439–444. doi: 10.1007/s11064-005-2678-0

Tavares, R. G., Tasca, C. I., Santos, C. E., Alves, L. B., Porciúncula, L. O., Emanuelli, T., et al. (2002). Quinolinic acid stimulates synaptosomal glutamate release and inhibits glutamate uptake into astrocytes. *Neurochem. Int.* 40, 621–627. doi: 10.1016/s0197-0186(01)00133-4

Tikka, T., Fiebich, B. L., Goldsteins, G., Keinanen, R., and Koistinaho, J. (2001). Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J. Neurosci.* 21, 2580–2588.

Torres-Platas, S. G., Cruceanu, C., Chen, G. G., Turecki, G., and Mechawar, N. (2014). Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. *Brain Behav. Immun.* 42, 50–59. doi: 10.1016/j.bbi.2014.05.007

Torres-Platas, S. G., Hercher, C., Davoli, M. A., Maussion, G., Labonté, B., Turecki, G., et al. (2011). Astrocytic hypertrophy in anterior cingulate white matter of depressed suicides. *Neuropsychopharmacology* 36, 2650–2658. doi: 10.1038/npp.2011.154

Tran, P., Skolnick, P., Czobor, P., Huang, N. Y., Bradshaw, M., McKinney, A., et al. (2012). Efficacy and tolerability of the novel triple reuptake inhibitor amitifadine in the treatment of patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. *J. Psychiatr. Res.* 46, 64–71. doi: 10.1016/j.jpsychires.2011.09.003

Trivedi, M. H., Rush, A. J., Wisniewski, S. R., Nierenberg, A. A., Warden, D., Ritz, L., et al. (2006). Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am. J. Psychiatry* 163, 28–40. doi: 10.1176/appi.ajp.163.1.28

Trullas, R., and Skolnick, P. (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur. J. Pharmacol.* 185, 1–10. doi: 10.1016/0014-2999(90)90204-j

Tsankova, N. M., Berton, O., Renthal, W., Kumar, A., Neve, R. L., and Nestler, E. J. (2006). Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. *Nat. Neurosci.* 9, 519–525. doi: 10.1038/nn1659

Uranova, N. A., Vostrikov, V. M., Orlovskaya, D. D., and Rachmanova, V. I. (2004). Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the stanley neuropathology consortium. *Schizophr. Res.* 67, 269–275. doi: 10.1016/s0920-9964(03)00181-6

Valkanova, V., Ebmeier, K. P., and Allan, C. L. (2013). CRP, IL-6 and depression: a systematic review and meta-analysis of longitudinal studies. *J. Affect. Disord.* 150, 736–744. doi: 10.1016/j.jad.2013.06.004

Van Bogaert, T., Vandevyver, S., Dejager, L., Van Hauwermeiren, F., Pinheiro, I., Petta, I., et al. (2011). Tumor necrosis factor inhibits glucocorticoid receptor function in mice: a strong signal toward lethal shock. *J. Biol. Chem.* 286, 26555–26567. doi: 10.1074/jbc.m110.212365

Vostrikov, V. M., Uranova, N. A., and Orlovskaya, D. D. (2007). Deficit of perineuronal oligodendrocytes in the prefrontal cortex in schizophrenia and mood disorders. *Schizophr. Res.* 94, 273–280. doi: 10.1016/j.schres.2007.04.014

Walker, D. G., Link, J., Lue, L. F., Dalsing-Hernandez, J. E., and Boyes, B. E. (2006). Gene expression changes by amyloid beta peptide-stimulated human postmortem brain microglia identify activation of multiple inflammatory processes. *J. Leukoc. Biol.* 79, 596–610. doi: 10.1189/jlb.0705377

Wang, X., Wu, H., and Miller, A. H. (2004). Interleukin 1alpha (IL-1alpha) induced activation of p38 mitogen-activated protein kinase inhibits glucocorticoid receptor function. *Mol. Psychiatry* 9, 65–75. doi: 10.1038/sj.mp.4001339

Wang, X., Zhang, L., Lei, Y., Liu, X., Zhou, X., Liu, Y., et al. (2014). Meta-analysis of infectious agents and depression. *Sci. Rep.* 4:4530. doi: 10.1038/srep04530

Wichers, M. C., Kenis, G., Koek, G. H., Robaeys, G., Nicolson, N. A., and Maes, M. (2007). Interferon-alpha-induced depressive symptoms are related to changes in the cytokine network but not to cortisol. *J. Psychosom. Res.* 62, 207–214. doi: 10.1016/j.jpsychores.2006.09.007

Wichers, M. C., Koek, G. H., Robaeys, G., Verkerk, R., Scharpe, S., and Maes, M. (2005). IDO and interferon-alpha-induced depressive symptoms: a shift in hypothesis from tryptophan depletion to neurotoxicity. *Mol. Psychiatry* 10, 538–544. doi: 10.1038/sj.mp.4001600

Wilde, A., Chan, H. N., Rahman, B., Meiser, B., Mitchell, P. B., Schofield, P. R., et al. (2014). A meta-analysis of the risk of major affective disorder in relatives of individuals affected by major depressive disorder or bipolar disorder. *J. Affect. Disord.* 158, 37–47. doi: 10.1016/j.jad.2014.01.014

Wittchen, H. U., Jacobi, F., Rehm, J., Gustavsson, A., Svensson, M., Jönsson, B., et al. (2011). The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur. Neuropsychopharmacol.* 21, 655–679. doi: 10.1016/j.euroneuro.2011.07.018

Wixey, J. A., Reinebrant, H. E., and Buller, K. M. (2011). Inhibition of neuroinflammation prevents injury to the serotonergic network after hypoxia-ischemia in the immature rat brain. *J. Neuropathol. Exp. Neurol.* 70, 23–35. doi: 10.1097/NEN.0b013e3182020b7b

Wong, M. L., Dong, C., Maestre-Mesa, J., and Licinio, J. (2008). Polymorphisms in inflammation-related genes are associated with susceptibility to major depression and antidepressant response. *Mol. Psychiatry* 13, 800–812. doi: 10.1038/mp.2008.59

World-Federation-for-Mental-Health. (2012). *Depression: A Global Crisis.* Virginia, USA: WFMH.

World-Health-Organization. (2008). *The Global Burden of Disease: 2004 Update.* Geneva, Switzerland: WHO.

Wu, H. Q., Rassoulpour, A., and Schwarcz, R. (2007). Kynurenic acid leads, dopamine follows: a new case of volume transmission in the brain? *J. Neural Transm.* 114, 33–41. doi: 10.1007/s00702-006-0562-y

Wuwongse, S., Cheng, S. S., Wong, G. T., Hung, C. H., Zhang, N. Q., Ho, Y. S., et al. (2013). Effects of corticosterone and amyloid-beta on proteins essential for synaptic function: implications for depression and Alzheimer's disease. *Biochim. Biophys. Acta* 1832, 2245–2256. doi: 10.1016/j.bbadis.2013.07.022

Yenamandra, S. P., Hellman, U., Kempkes, B., Darekar, S. D., Petermann, S., Sculley, T., et al. (2010). Epstein-Barr virus encoded EBNA-3 binds to vitamin D receptor and blocks activation of its target genes. *Cell. Mol. Life Sci.* 67, 4249–4256. doi: 10.1007/s00018-010-0441-4

Yirmiya, R. (1996). Endotoxin produces a depressive-like episode in rats. *Brain Res.* 711, 163–174. doi: 10.1016/0006-8993(95)01415-2

Zarate, C. A. Jr., Singh, J. B., Carlson, P. J., Brutsche, N. E., Ameli, R., Luckenbaugh, D. A., et al. (2006). A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry* 63, 856–864. doi: 10.1001/archpsyc.63.8.856

Zheng, L. S., Kaneko, N., and Sawamoto, K. (2015). Minocycline treatment ameliorates interferon-alpha- induced neurogenic defects and depression-like behaviors in mice. *Front. Cell. Neurosci.* 9:5. doi: 10.3389/fncel.2015.00005

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Jo, Zhang, Emrich and Dietrich. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution and reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in
CELLULAR NEUROSCIENCE

GENERAL COMMENTARY
published: 18 November 2014
doi: 10.3389/fncel.2014.00390

The role of microglia in mediating the effect of the environment in brain plasticity and behavior

Igor Branchi[1], Silvia Alboni[2] and Laura Maggi[3]*

[1] *Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy*
[2] *Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy*
[3] *Department of Physiology and Pharmacology, Sapienza University, Rome, Italy*
**Correspondence: laura.maggi@uniroma1.it*

Edited by:
Luisa Pinto, University of Minho, Portugal

Reviewed by:
Hiroaki Wake, National Institute for Basic Biology, Japan
Catarina A. Gomes, University of Coimbra, Portugal

Keywords: microglia, plasticity, environment, depression, behavior, stress

A commentary on

Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis
by Kreisel, T., Frank, M. G., Licht, T., Reshef, R., Ben-Menachem-Zidon, O., Baratta, M. V., et al. (2014). Mol. Psychiatry 19, 699–709.

Major Depression (MD) affects 10–15% of the population worldwide and constitutes an enormous medical, societal, and economic burden. One of the prominent causes of such burden is the very limited understanding of the mechanisms underlying the psychopathology (Belmaker and Agam, 2008). Among the most relevant factors determining the onset of MD is the quality of the living environment, such as exposure to stressful life events (Cohen et al., 2007; Davidson and McEwen, 2012). In addition, increased inflammation, associated to conditions such as infection and immunotherapy, is reported to increase the likelihood to show depressive symptomatology (Dantzer et al., 2008; Han and Yu, 2014).

The nervous and the immune systems are engaged in an intense bidirectional interplay, along the ongoing changes in the living environment (Yirmiya and Goshen, 2011; McCusker and Kelley, 2013). In this perspective, microglia, the resident immune cells of the brain, have recently attracted a lot of interest in the biological psychiatry field (Muller, 2014). These cells can modify their features and function according to the inputs from the environment. Indeed, equipped with receptors for a plethora of molecules microglia cells can sense environmental changes over a time scale of minutes and respond performing diverse functions which, accordingly to the context, might result as either beneficial or harmful (Kierdorf and Prinz, 2013; Siskova and Tremblay, 2013), though factors regulating microglial transition across different functional states are not well-defined. For instance, these cells respond to sensory and behavioral experience (e.g., deprivation of visual stimuli, environmental enrichment) by modulating their interactions with neuronal circuits, notably regulating processes such as adult hippocampal neurogenesis (Ekdahl, 2012; Reshef et al., 2014) and elimination and formation of synapses (Paolicelli et al., 2011; Tremblay et al., 2011; Parkhurst et al., 2013; Sierra et al., 2014).

The role of microglia in interfacing environmental stimuli and changes in brain function has suggested that these cells may underlay the interplay between environmental stimuli and vulnerability to MD. In this context, the preclinical study performed by the group headed by Raz Yirmiya provides an interesting demonstration of the role played by the microglial cells in mediating the effects of stress on depression. Authors showed that stress exposure produced dynamic bi-directional alterations in microglia status that, in turn, are causally involved in stress-induced depressive-like behavior in rodents. They explored the effects of stress in three brain regions: the hippocampal dentate gyrus, the medial prefrontal cortex and the somatosensory cortex. They found that mice and rats exposed to unpredictable stress (US) show microglia modifications, mainly in the dentate gyrus of the hippocampal system, which are dependent on the duration of the US. Indeed, they demonstrated that a short-term exposure (1–2 days) to US results in a transient induction of microglia proliferation and activation (i.e., microglial cells assume an activated morphology and increased expression of activation markers) via IL-1\IL-1R mediated signaling. However, following 3–4 days of US, a microglia decline was observed in stressed animals compared to controls as indicated by high levels of caspase-3 (a protease that mediates the execution-phase of apoptosis) and DNA fragmentation (a key feature of apoptosis). Moreover, they found that chronic (5 weeks) US exposure induced depressive-like behavior (namely, decreased sucrose preference and social exploration) associated with a lowered number of microglia cells, reduction in length of microglial processes and soma area, and decreased expression of microglial markers (i.e., Iba-1 and CD11b). In addition, Yirmiya and collaborators showed, through pharmacological manipulations, that counteracting the bi-directional changes in microglial activity induced by stress can prevent or reverse the depressive-like phenotype. In particular, the block of microglial activation during the early phase of stress exposure through administration of minocycline (a commonly used drug to inhibit microglial

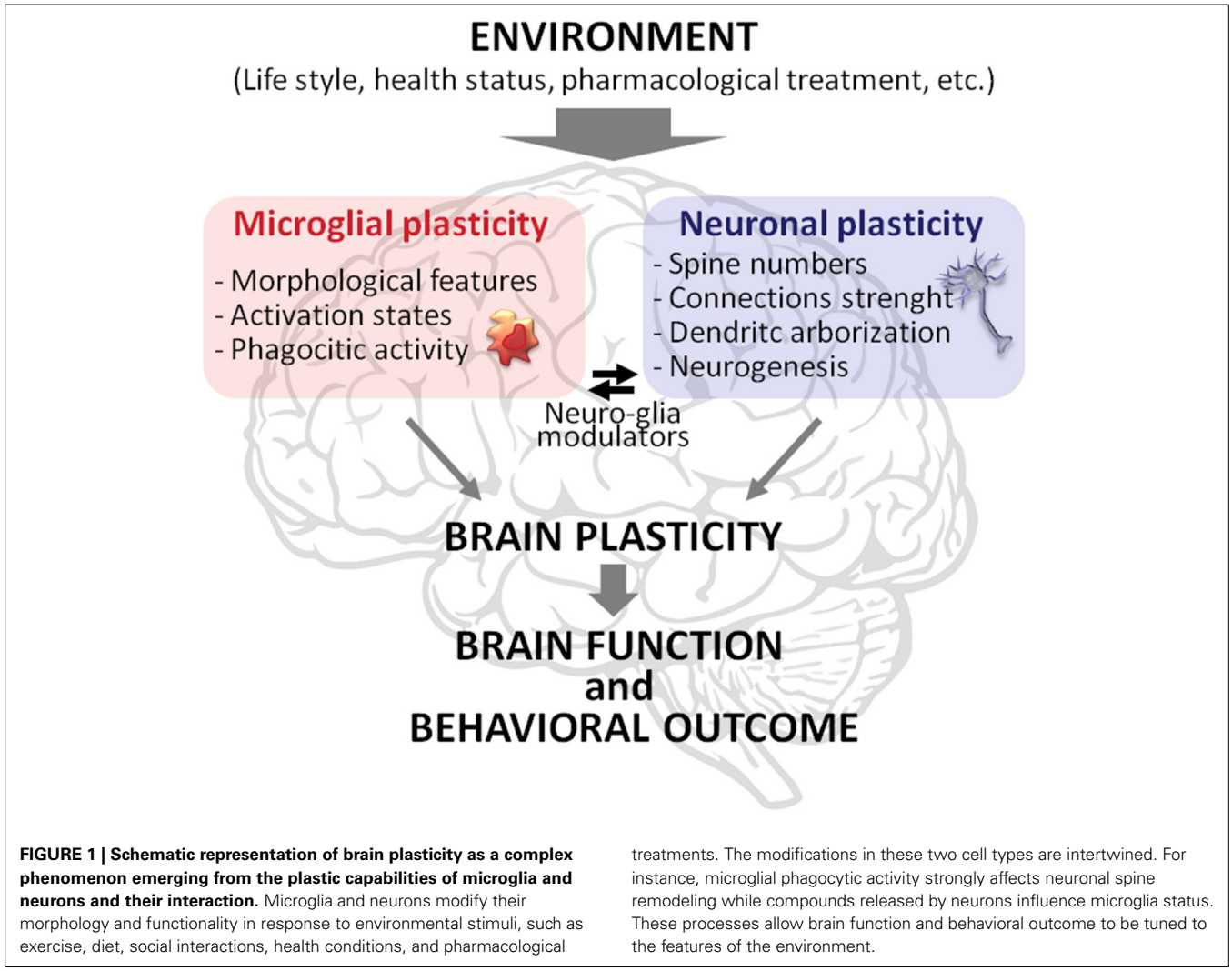


FIGURE 1 | Schematic representation of brain plasticity as a complex phenomenon emerging from the plastic capabilities of microglia and neurons and their interaction. Microglia and neurons modify their morphology and functionality in response to environmental stimuli, such as exercise, diet, social interactions, health conditions, and pharmacological treatments. The modifications in these two cell types are intertwined. For instance, microglial phagocytic activity strongly affects neuronal spine remodeling while compounds released by neurons influence microglia status. These processes allow brain function and behavioral outcome to be tuned to the features of the environment.

activation) or imipramine (an antidepressant with anti-inflammatory properties; Alboni et al., 2013) prevents, at long-term, specific stress-induced effects on microglia and behavior. Whereas, during the late phase of stress exposure, when microglial decline occurs, the stimulation of microglia through administration of lipopolysaccharide, macrophage colony-stimulating factor or granulocytes-macrophage colony-stimulating factor reverses the stress-induced depressive-like behaviors. These observations support a causal role for the dynamic microglial changes in triggering and maintaining depressive-like symptoms under stress conditions and suggest an inverted U-shaped relationship between time-dependent microglial status and behavioral outcome (Goshen et al., 2007; Kreisel et al., 2014).

Overall, the results of this paper open a new perspective about the role of microglia in the interplay between the quality of the environment and vulnerability to MD. We strengthen this finding, stressing the idea that microglia plays a key role in such interplay not only actively participating but being integral part of brain plasticity (**Figure 1**). Therefore, along with neuronal plasticity, microglial plasticity should be considered as a key component of the brain plasticity processes. In this perspective, neuronal plasticity, and brain plasticity are not synonyms, but the latter emerges from the plastic capabilities of both neurons and microglia, and glia in general, and from their interaction. Accordingly, it is warranted to explore brain plasticity in a comprehensive fashion, assessing neuronal plasticity (e.g., synaptic strength, spine, and dendritic modifications) in parallel with the dynamic changes in microglial status (e.g., morphological changes, phagocytic activity). This approach may lead to unravel novel molecular and cellular mechanisms underlying the onset and progression of psychopathologies to be exploited as targets for innovative therapeutic strategies.

REFERENCES

Alboni, S., Benatti, C., Montanari, C., Tascedda, F., and Brunello, N. (2013). Chronic antidepressant treatments resulted in altered expression of genes involved in inflammation in the rat hypothalamus. *Eur. J. Pharmacol.* 721, 158–167. doi: 10.1016/j.ejphar.2013.08.046

Belmaker, R. H., and Agam, G. (2008). Major depressive disorder. *N. Engl. J. Med.* 358, 55–68. doi: 10.1056/NEJMra073096

Cohen, S., Janicki-Deverts, D., and Miller, G. E. (2007). Psychological stress and disease. *JAMA* 298, 1685–1687. doi: 10.1001/jama.298.14.1685

Dantzer, R., O'connor, J. C., Freund, G. G., Johnson, R. W., and Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. doi: 10.1038/nrn2297

Davidson, R. J., and McEwen, B. S. (2012). Social influences on neuroplasticity: stress and interventions to promote well-being. *Nat. Neurosci.* 15, 689–695. doi: 10.1038/nn.3093

Ekdahl, C. T. (2012). Microglial activation - tuning and pruning adult neurogenesis. *Front. Pharmacol.* 3:41. doi: 10.3389/fphar.2012.00041

Goshen, I., Kreisel, T., Ounallah-Saad, H., Renbaum, P., Zalzstein, Y., Ben-Hur, T., et al. (2007). A dual role for interleukin-1 in hippocampal-dependent memory processes. *Psychoneuroendocrinology* 32, 1106–1115. doi: 10.1016/j.psyneuen.2007.09.004

Han, Q. Q., and Yu, J. (2014). Inflammation: a mechanism of depression? *Neurosci. Bull.* 30, 515–523. doi: 10.1007/s12264-013-1439-3

Kierdorf, K., and Prinz, M. (2013). Factors regulating microglia activation. *Front. Cell Neurosci.* 7:44. doi: 10.3389/fncel.2013.00044

Kreisel, T., Frank, M. G., Licht, T., Reshef, R., Ben-Menachem-Zidon, O., Baratta, M. V., et al. (2014). Dynamic microglial alterations underlie stress-induced depressive-like behavior and suppressed neurogenesis. *Mol. Psychiatry* 19, 699–709. doi: 10.1038/mp.2013.155

McCusker, R. H., and Kelley, K. W. (2013). Immune-neural connections: how the immune system's response to infectious agents influences behavior. *J. Exp. Biol.* 216, 84–98. doi: 10.1242/jeb.073411

Muller, N. (2014). Immunology of major depression. *Neuroimmunomodulation* 21, 123–130. doi: 10.1159/000356540

Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., et al. (2011). Synaptic pruning by microglia is necessary for normal brain development. *Science* 333, 1456–1458. doi: 10.1126/science.1202529

Parkhurst, C. N., Yang, G., Ninan, I., Savas, J. N., Yates, J. R. 3rd., Lafaille, J. J., et al. (2013). Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell* 155, 1596–1609. doi: 10.1016/j.cell.2013.11.030

Reshef, R., Kreisel, T., Beroukhim Kay, D., and Yirmiya, R. (2014). Microglia and their CX3CR1 signaling are involved in hippocampal- but not olfactory bulb-related memory and neurogenesis. *Brain Behav. Immun.* 41, 239–250. doi: 10.1016/j.bbi.2014.04.009

Sierra, A., Tremblay, M. E., and Wake, H. (2014). Never-resting microglia: physiological roles in the healthy brain and pathological implications. *Front. Cell. Neurosci.* 8:240. doi: 10.3389/fncel.2014.00240

Siskova, Z., and Tremblay, M. E. (2013). Microglia and synapse: interactions in health and neurodegeneration. *Neural Plast.* 2013:425845. doi: 10.1155/2013/425845

Tremblay, M. E., Stevens, B., Sierra, A., Wake, H., Bessis, A., and Nimmerjahn, A. (2011). The role of microglia in the healthy brain. *J. Neurosci.* 31, 16064–16069. doi: 10.1523/JNEUROSCI.4158-11.2011

Yirmiya, R., and Goshen, I. (2011). Immune modulation of learning, memory, neural plasticity and neurogenesis. *Brain Behav. Immun.* 25, 181–213. doi: 10.1016/j.bbi.2010.10.015

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 06 October 2014; accepted: 31 October 2014; published online: 18 November 2014.

*Citation: Branchi I, Alboni S and Maggi L (2014) The role of microglia in mediating the effect of the environment in brain plasticity and behavior. Front. Cell. Neurosci. **8**:390. doi: 10.3389/fncel.2014.00390*

This article was submitted to the journal Frontiers in Cellular Neuroscience.

Copyright © 2014 Branchi, Alboni and Maggi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Cellular Neuroscience

REVIEW
published: 17 December 2015
doi: 10.3389/fncel.2015.00476

Neuroinflammation and Depression: Microglia Activation, Extracellular Microvesicles and microRNA Dysregulation

Dora Brites[1,2]* *and Adelaide Fernandes*[1,2]

[1] Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal,
[2] Department of Biochemistry and Human Biology, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Patients with chronic inflammation are often associated with the emergence of depression symptoms, while diagnosed depressed patients show increased levels of circulating cytokines. Further studies revealed the activation of the brain immune cell microglia in depressed patients with a greater magnitude in individuals that committed suicide, indicating a crucial role for neuroinflammation in depression brain pathogenesis. Rapid advances in the understanding of microglial and astrocytic neurobiology were obtained in the past 15–20 years. Indeed, recent data reveal that microglia play an important role in managing neuronal cell death, neurogenesis, and synaptic interactions, besides their involvement in immune-response generating cytokines. The communication between microglia and neurons is essential to synchronize these diverse functions with brain activity. Evidence is accumulating that secreted extracellular vesicles (EVs), comprising ectosomes and exosomes with a size ranging from 0.1–1 μm, are key players in intercellular signaling. These EVs may carry specific proteins, mRNAs and microRNAs (miRNAs). Transfer of exosomes to neurons was shown to be mediated by oligodendrocytes, microglia and astrocytes that may either be supportive to neurons, or instead disseminate the disease. Interestingly, several recent reports have identified changes in miRNAs in depressed patients, which target not only crucial pathways associated with synaptic plasticity, learning and memory but also the production of neurotrophic factors and immune cell modulation. In this article, we discuss the role of neuroinflammation in the emergence of depression, namely dynamic alterations in the status of microglia response to stimulation, and how their activation phenotypes may have an etiological role in neurodegeneneration, in particular in depressive-like behavior. We will overview the involvement of miRNAs, exosomes, ectosomes and microglia in regulating critical pathways associated with depression and how they may contribute to other brain disorders including amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD) and Parkinson's disease (PD), which share several neuroinflammatory-associated processes. Specific reference will be made to EVs as potential biomarkers and disease monitoring approaches, focusing on their potentialities as drug delivery vehicles, and on putative therapeutic strategies using autologous exosome-based delivery systems to treat neurodegenerative and psychiatric disorders.

Keywords: astrocytes, exosomes, ectosomes, microglia, neurodegeneration, glia interplay, oligodendrocytes, microRNAs

OPEN ACCESS

Edited by:
Luisa Pinto,
University of Minho, Portugal

Reviewed by:
Andrew MacLean,
Tulane University School of Medicine, USA
Grzegorz Kreiner,
Polish Academy of Sciences, Poland

***Correspondence:**
Dora Brites
dbrites@ff.ulisboa.pt

Received: *01 October 2015*
Accepted: *23 November 2015*
Published: *17 December 2015*

Citation:
Brites D and Fernandes A (2015) Neuroinflammation and Depression: Microglia Activation, Extracellular Microvesicles and microRNA Dysregulation. Front. Cell. Neurosci. 9:476. doi: 10.3389/fncel.2015.00476

INTRODUCTION

Depression (major depressive disorder, MDD) is a mood disorder of multifactorial origin, including genetic and environmental factors, estimated to affect 350 million people worldwide (Jo et al., 2015). It is responsible for increased morbidity and mortality, adverse health behaviors, lost work productivity and increased health care utilization (Benton et al., 2007). Suicide accounts for almost 1 million lives lost each year, i.e., 3000 suicide deaths per day (World Health and Organization, 2012). About 25% of people diagnosed with MDD are under 19 years old and about 40% of patients do not adequately respond to current therapy (Dwivedi, 2014). To that it may account the poor understanding of the underlying mechanisms leading to MDD and suicidal behavior.

Stress, impaired neurogenesis and defects in synaptic plasticity represent three interconnected factors that are associated with depression (Dwivedi, 2011; Mouillet-Richard et al., 2012). Characteristic pathophysiological hallmark features include monoamine depletion, down-regulation of neurotrophin signaling, and glucocorticoid receptor (GR) resistance, as well as excess of glutamate, corticotrophin-releasing hormone and cortisol levels (Carvalho et al., 2015; Jo et al., 2015). Stress has been associated to the development of clinical depression, and evidence from preclinical studies suggests a role of microglia in depression and stress. Most curious, chronic stress has been known to promote microglial hyper-ramification and astroglial atrophy (Tynan et al., 2013), as well as lower immunoreactivity of myelin basic protein (MBP) and fewer mature oligodendrocytes (Yang et al., 2015) in the prefrontal cortex of rodents. Other molecules that are gaining attention in depressive pathophysiology are the cyclic adenosine monophosphate (cAMP) response element binding protein (CREB) involved in learning and memory, whose expression is decreased in depressed patients, and the vascular endothelial growth factor (VEGF), an angiogenic cytokine, with decreased mRNA levels in peripheral leukocytes of such individuals (Dwivedi, 2009).

As mentioned above, stress is a major risk factor for depression leading to the activation of hypothalamus-pituitary-adrenal (HPA) axis, as well as to a reduced hippocampal neurogenesis, together with impaired hippocampal synaptic plasticity and morphological neuroplasticity. Post-mortem studies revealed unique pathological alterations in neuronal and glial cells in the dorsolateral prefrontal cortex of patients with MDD, in particular a reduced cell size and density (Stockmeier and Rajkowska, 2004). Astrocyte deficits such as a decreased cell number of glial fibrillary acidic protein (GFAP)—immunoreactive cells and oligodendrocyte pathology, probably related to disruptions of white matter tracts, were found in depressed individuals (Rajkowska and Miguel-Hidalgo, 2007). Abnormal activation of microglia, the immunologic guardian cells of the brain, and increased microglial cell numbers were observed in depression and in anxiety disorders, although it is yet unclear how it relates with psychopathological conditions (Serafini et al., 2015). To note that reactive glial cells are sources and targets of various inflammatory cytokines, and as so are involved in the regulation of neuroinflammation and tissue repair (Rajkowska and Miguel-Hidalgo, 2007). Lately, depression has been described as a microglia-associated disorder, and besides the excessive cell activation and increased cell number, microglia decline and senescence was observed in some depressed patients as well (Yirmiya et al., 2015).

Recent evidences suggest that extracellular vesicles (EVs) secreted by neurons and glia, including endosome-derived exosomes and fragments of the cellular plasma membrane play a key role in intercellular communication and neuroinflammation by transporting messenger RNA (mRNA), microRNA (miRNA) and proteins (Pegtel et al., 2014). EVs are recognized as having a role in pathogenesis and dissemination of inflammatory diseases, besides their potential as biomarkers and therapeutic vehicles (Buzas et al., 2014). Reactive microglia were shown to release exosomes and microvesicles (MVs) carrying the pro-inflammatory cytokine interleukin-1β (IL-1β), the IL-1β-processing enzyme caspase-1, and the P2X7 receptor that may induce and propagate inflammatory reactions throughout the brain (Frühbeis et al., 2013).

A number of treatments were developed to increase the availability of monoamines, such as serotonin, due to a shift from serotonin to kynurenine pathway in tryptophan catabolism. In the central nervous system (CNS) the kynurenine pathway is mediated by astrocytes, microglia and infiltrating macrophages (Jo et al., 2015). Current therapies usually result in relapse rates of only 50%, reason why a better understanding of the pathomechanisms involved in MDD may help in the discovery of more effective and cost-effective treatment alternatives. Accumulating evidence suggests that glial pathology and the decrease in the number of glial cells are prominent features in MDD (Hamidi et al., 2004; Altshuler et al., 2010). Glial reduction in tissue samples from subjects diagnosed with MDD, at least in amygdala, was shown to be due to a loss of oligodendrocytes once no significant changes were observed on microglia or astrocytes (Hamidi et al., 2004). Patients with depression have been shown to evidence increased serum levels of pro-inflammatory cytokines that returned to normal by treatment with antidepressants for 3 months (Dahl et al., 2014). Some studies revealed that the reinforcement of neurotrophin expression and stimulation of neurogenesis by causing antidepressant-like effects may be of therapeutic relevance in chronically depressed patients (Van Buel et al., 2015). These Authors argue that targeted potentiation, instead of suppression of neuroinflammation, may be of therapeutic relevance in chronic depressed patients. Interestingly, electroconvulsive therapy of MDD was indicated to facilitate the action of antidepressants by inducing hippocampal neurogenesis through the modulation of microglial activation (Rotheneichner et al., 2014). In addition, minocycline, which is a suppressor of activated microglia, has been shown to exert protective effects by reducing microglial activation, oxidative stress and inflammation (Réus et al., 2015). Therefore, there are some controversial hypotheses on the best therapeutic approaches to MDD.

This review article aims to summarize data about the effects of immune system dysregulation and microglial activation on mood dysregulation and will also discuss the role of EVs and their specific cargo, namely miRNAs, as means by which these neuroinflammatory mechanisms take place and influence neighboring cells leading to the propagation of inflammation.

INFLAMMATION-ASSOCIATED DEPRESSION

Chronic inflammation in physically ill patients is often associated with the development of symptoms of depression (Benton et al., 2007; Goldberg, 2010). Activation of the peripheral immune system leads to increased cytokine levels that are actively transported into the CNS stimulating astrocytes and microglial cells, which in turn produce cytokines by a feedback mechanism (Müller and Ackenheil, 1998). In such condition there is sickness exacerbation and the development of symptoms of depression in susceptible patients (Dantzer et al., 2008). Although not completely clarified the intracellular molecular mechanisms linking inflammation and depression, it was demonstrated that microglia besides releasing inflammatory mediators also secrete glutamate and metabolize kynurenine transported to the CNS into quinolinic acid, a neurotoxic compound (Dantzer and Walker, 2014). Astrocytes seem not to be able to uptake the excess of glutamate that together with quinolinic acid will enhance glutamatergic neurotransmission leading to the development of symptoms of depression. Proinflammatory cytokines can additionally stimulate HPA axis to release glucocorticoids that supress neurogenesis (Liu et al., 2003). Recently, it was suggested that neuroinflammation may not only derive from pathological conditions, but also from enhanced neuronal activity denominated as neurogenic neuroinflammation that may aggravate stressful stimuli (Xanthos and Sandkuhler, 2014). How inflammation decreases neurogenesis and leads to dysfunction of neurotrophic system is scarcely understood as it is the cross-talk between microglia, mostly associated to neuroinflammation, and astrocytes that produce neurotrophins (Song and Wang, 2011).

It was observed that chronic unpredictable mild stress-exposed rats, a well-documented model of depression, produced increased IL-1β mRNA and protein levels in the prefrontal cortex, which were not reproduced in serum or cerebrospinal fluid (CSF). Nuclear factor kappa B (NF-κB) inflammatory pathway and nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3)-inflammasome activation in microglial cells revealed to be implicated (Pan et al., 2014). However, there are controversial data on IL-1β alterations in periphery and CSF between depressed animals and patients. In a meta-analysis study, no changes were found for IL-1β, though elevated levels of tumour necrosis factor (TNF)-α_and IL-6 were observed in depressed subjects compared with control subjects (Dowlati et al., 2010). In other studies, patients with depression showed increased serum levels of IL-1β, IL-6, IL-8, IL-12 and TNF-α, together with a decrease in IL-10 levels, an anti-inflammatory cytokine (Schiepers et al., 2005; O'Brien et al., 2007; Song et al., 2009). Actually, studies point to the activation of inflammatory responses and to microglial P2X7, a purinergic ion channel activated by ATP, as contributors to the pathogenesis of depression (for review, see Stokes et al., 2015). Microglia activation is particularly enhanced in individuals who committed suicide and in depressive patients (Steiner et al., 2008; Schnieder et al., 2014). In addition, suicide has been related not only to microglial activation, but also to an increase of perivascular macrophages around blood vessels (Torres-Platas et al., 2014). In fact, stress has been linked to the development of both depression and anxiety, with a key contribution of microglia activation, as well as of recruitment of peripheral macrophages into the brain to such events (Phillips et al., 2015; Réus et al., 2015). On the other hand, the neuroinflammatory status associated with depression-like symptoms may also result from the existence of peripheral or central chronic inflammatory processes that continuously activate peripheral macrophages, sending inflammatory signals to the brain (Roman et al., 2013).

Hippocampus is a region with a high density of microglial cells, especially in the CA1 region, and hippocampal microglial activation demonstrated to be originated by stress and suggested to be implicated in the pathophysiology of MDD, as well as in other psychiatric and stress-related disorders (Walker et al., 2013). Increased high mobility group box-1 (HMGB-1) protein was demonstrated to be increased in the hippocampus of male Sprague Dawley rats after tail shocks and to be responsible for microglia priming by acting on the NLRP3 inflammasome (Weber et al., 2015). In addition, low dose administration of lipopolysaccharide (LPS) to mice, as a model of depression, led to HMGB1 translocation from the nucleus to the cytoplasm, while blockage of HMGB1 abrogated the depressive-like behavior induced by LPS (Wu et al., 2015).

Magnetic resonance imaging findings in the chronic mild stress (CMS) rodent animal, a model of depression, showed demyelination signs at the bilateral frontal cortex, hippocampus and hypothalamus, which revealed to be associated with brain oedema and inflammation (El-Etr et al., 2015). Gene expression profiling studies from patients with MDD showed that genes involved in energy metabolism and mitochondrial function were downregulated (Konradi et al., 2012), that genes involved in immune response and inflammation were upregulated (Shelton et al., 2011), and that genes expressed in oligodendrocytes were downregulated (Aston et al., 2005). These studies further corroborate the association between energy impairment, inflammation and myelin loss during MDD.

Non-responders to depressive treatment have shown increased baseline inflammation and oxidative stress (Strawbridge et al., 2015; Vaváková et al., 2015). A recent study evidenced that the antidepressant drug venlafaxine, though not having any influence on the majority of microglia-related proinflammatory parameters, significantly reduced superoxide production while revealed a protective effect on mitochondrial membrane potential, suggesting ability to prevent the progression of depression (Dubovický et al., 2014).

Inflammation-associated depression is now considered a clinical entity that provides an understanding on the dynamics of interaction between peripheral and brain mechanisms (Dantzer et al., 2011). Experimentally, acute immumostimulation by

peripheral administration of LPS in mice caused sickness, increased immobility in the tail suspension test and depressive-like behavior in the forced swim test, together with a delayed cellular activity (Frenois et al., 2007; O'Connor et al., 2009). Some studies indicate that anti-inflammatory compounds in patients with inflammatory disorders and depressed patients may improve depressed mood, but conclusive data still waits confirmation (Miller et al., 2009; Song and Wang, 2011).

It is still a matter of debate whether a chronic inflammatory state may contribute to depression etiology or if inflammation occurs as a consequence of a depressive state. As mentioned above many stimuli related to depression may trigger microglia activation, namely: (i) peripheral or central inflammatory challenges; (ii) stress-related conditions derived from increase of glucocorticoids via HPA axis, reported to activate microglia (Sorrells and Sapolsky, 2007), from changes in gut microbiota, shown to control the maturation and functioning of microglia (Erny et al., 2015), or from psychological stress that promote microglial activation through the release of alarmins within the brain (Maslanik et al., 2013); and (iii) intense neuronal activity. This activation, may then promote the suppression of neurogenesis and neuroplasticity further enhancing the development of depression-like symptoms, suggesting that a prior inflammation may set the basis for the emergence of depression. Furthermore, continuous activation of microglia may concur to microglia function decline which have more recently been observed in depressive patients (Hannestad et al., 2013) and reported in several neurological conditions including aging, Alzheimer's disease (AD), and chronic stress which are associated with a higher prevalence of MDD.

The study of cellular and molecular mechanisms of inflammation-associated depression will open new possibilities for developing new antidepressant compounds targeting neuroinflammation or its downstream pathways. Nevertheless, we should be cautious in believing that depression can be treated by therapies targeting inflammation. Further studies are required to evaluate whether a combined therapy with anti-inflammatory compounds and antidepressants will result in additional clinical benefits.

ROLE OF microRNAs IN DEPRESSION

MiRNAs are a class of small noncoding RNAs that are key post-transcriptional regulators of gene expression, which may impair the translation of their target mRNA or promote its degradation, though they can on the contrary also act as translation activators or even impair transcription by binding to gene promoters (Krol et al., 2010; Younger and Corey, 2011). If we consider that more than 1400 miRNA genes were already identified, that each one may target a high number of different mRNAs, which individually can be suppressed by multiple mRNAs, there is no doubt on their role as robust determinants of cellular states (Krol et al., 2010; Rota et al., 2011; Lin and Gregory, 2015). Most of miRNAs are transcribed as pri-miRNAs by RNA polymerases II and III in the nucleus and cut into pre-miRNAs by the Drosha complex (Mouillet-Richard et al., 2012). Pre-miRNAs are transported to the cytoplasm by exportin 5 and Ran GTPase for final processing by the RNAse III enzyme Dicer generating 22 nt double-stranded mature miRNAs with a 5′ phosphate end (Gregory and Shiekhattar, 2005; Ksiazek-Winiarek et al., 2013). The mature miRNA is then incorporated into the RNA-induced silencing complex (RISC), which become able to repress translation (O'Connor et al., 2012).

Previous studies indicate that miR-124 and miR-128 are primarily expressed in neurons, whereas miR-23, miR-26, and miR-29 exist in large amount in astrocytes, supporting a differential nature of expression (Smirnova et al., 2005). Some miRNAs are associated with neurological functions such as learning and memory and miR-132, -134, and -let-7 are suggested to play a crucial role in the formation and plasticity of synapses, and miR-124a and miR-125b have been associated to the outgrowth of axons (Schratt et al., 2006; Le et al., 2009; see **Table 1**). MiR-124 is the most abundant in the brain and its dysregulation has been related with neurodegeneration, neuroimmune disorders and CNS stress among others (for review, see Sun et al., 2015). It was previously indicated to be a key regulator of adult neurogenesis (Cheng et al., 2009) and one of its targets is CREB (Rajasethupathy et al., 2009).

Also involved in neurogenesis is the miR-137 associated with the development of neural stem cells into mature neurons (Szulwach et al., 2010). Synaptic plasticity is a critical process in learning and memory and its disruption may trigger psychiatric disorders. Recent evidence suggests that neuronal plasticity plays an important role in the recovery from depression and brain derived-neurotrophic factor (BDNF) is a mediator of this plasticity (Castrén and Rantamäki, 2010). Actually, BDNF was found decreased in depressed patients and in stressed animals (Dwivedi, 2009). In this context, miRNAs are known to influence BDNF, which may in turn induce the synthesis of miR-132 to regulate neurogenesis (Castrén and Rantamäki, 2010; Yan et al., 2013). The mitogen-activated protein kinases (MAPK) superfamily, including the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase and p38 proteins, when activated in microglia trigger the release of pro-inflammatory mediators (Kaminska et al., 2009; see **Figure 1**), making MAPK signal transduction crucial in regulating gene expression and mediating a rapid response in stress-responsive microRNA expression (Biggar and Storey, 2011). Expression of miR-221 and miR-222 was shown to be related with ERK1/2 activation (Terasawa et al., 2009).

MiRNA expression was reported to be globally downregulated in the prefrontal cortex of depressed suicide victims (Smalheiser et al., 2012), which is consistent with the hypo-activation of the frontal cortex reported in depressed subjects (Covington et al., 2010). Those Authors observed that the 21 miRNAs found downregulated were related to cell growth and differentiation. However, miR-185 and miR-491-3p were increased in the frontal cortex of suicide completers (Serafini et al., 2014). Complementary studies using post-mortem human prefrontal cortex samples from MDD patients showed that miR-1202, a primate specific miRNA that is enriched in the human brain, was down-regulated in depressed individuals (Lopez et al., 2014). This miR-1202 targets the

TABLE 1 | Critical and dysregulated microRNA (miR) in conditions of stress/depression.

microRNA	Associated to depression-related pathways	Directly implicated in stress/depression	Reference
miR-1202	GRM4 as target. Modulator of glutamatergic, dopaminergic, GABAergic and serotonergic neurotransmission. Regulator of anxiety-related behaviors.	↓ in the MDD patient's brain	Davis et al. (2012), Lopez et al. (2014) and Rucker and McGuffin (2014)
Let-7a	Neuronal differentiation of embryonic neural progenitors. Formation and plasticity of synapses.	↑ in the frontal cortex following acute stress	Schratt et al. (2006), Schwamborn et al. (2009) and Rinaldi et al. (2010)
miR-124	Important for neurogenesis.	↑ in the medial pre-frontal cortex following maternal separation stress	Uchida et al. (2010)
miR-29a	Targets Voltage Dependent Anion Channel and ATP synthetase.	↑ in the medial pre-frontal cortex following maternal separation stress	Uchida et al. (2010) and Bargaje et al. (2012)
miR-26a	Important in neuronal development and morphogenesis.	↑ in the frontal cortex following acute stress	Rinaldi et al. (2010) and Li and Sun (2013)
miR-26b	Induces cell cycle in postmitotic neurons and apoptosis.	↑ in the frontal cortex following acute stress	Rinaldi et al. (2010) and Absalon et al. (2013)
miR-26b, miR-1972, miR-4485, miR-4498, and miR-4743	Target biological processed involved in brain development and function: axon guidance and extension, synaptic transmission, learning and memory.	↑ in peripheral blood mononuclear cells from MDD patients	Fan et al. (2014)
miR-221-3p, miR-34a-5p, and let-7d-3p	Target serotonin receptors, corticotrophin-releasing hormone receptor and glutamate transporters. Enrich pathways related to neuronal function in depression.	↑ in serum from MDD patients	Wan et al. (2015)
miR-451a		↑ in serum from MDD patients	
miR-132 and miR-182	BDNF as target.	↑ in serum from depressed patients. Polymorphism in the miR-182 gene is associated with MDD.	Li et al. (2013) and Saus et al. (2010)
miR-134	Can negatively regulate the size of dendritic spines.	↑ in amygdala after owing acute stress	Schratt et al. (2006) and Meerson et al. (2010)
miR-183	Regulates the circadian-clock period.	↑ in amygdala after owing acute stress	Xu et al. (2007) and Meerson et al. (2010)
miR-1302 and miR-625	P2XR7 as target. Neuronal receptor involved in synaptic transmission. Microglia scavenger receptor involved in phagocytosis.	SNP in putative miRNA target sites of miR-1302 and miR-625 of buccal epithelial cells from MDD patients	Sperlágh et al. (2006), Rahman et al. (2010) and Gu et al. (2011)
miR-9	Controls dendritic growth and synaptic transmission.	↑ in the frontal cortex following acute stress and in the medial pre-frontal cortex following maternal separation	Rinaldi et al. (2010), Uchida et al. (2010) and Giusti et al. (2014)
miR-144-5p	Targets PKC, Wnt/β-catenin, and PTEN pathways. Is involved in response to mood stabilizer treatment and stress responses.	↑ in the plasma of depressed patients	Zhou et al. (2009), Katsuura et al. (2012) and Wang et al. (2015)

BDNF, brain-derived neurotrophic factor; CSF, cerebrospinal fluid; GRM4, metabotropic glutamate receptor-4; PTEN, phosphatase and tensin homolog; PKC, protein kinase C; P2XR7, purinergic receptor P2x, ligand-gated ion channel 7; SNP, single nucleotide polymorphisms.

expression of the gene encoding metabotropic glutamate receptor-4 (GRM4), which was also up-regulated in MDD patient samples. This receptor is localized pre- and post-synaptically and functions as modulator of glutamatergic, dopaminergic, GABAergic and serotonergic neurotransmission (Pilc et al., 2008). More recently, it has been pointed as a regulator of anxiety-related behaviors (Davis et al., 2012).

A different study analyzed miRNAs in the peripheral blood mononuclear cells (PBMCs) of MDD patient and identified that 5 miRNAs were up-regulated, such as miRNA-26b, miRNA-1972, miRNA-4485, miRNA-4498, and miRNA-4743 (Fan et al., 2014; see **Table 1**). The Authors next looked at predicted targets of these miRNAs and identified genes with a wide variety of biological effects, including axon guidance and extension, synaptic transmission, learning and memory, which changes have been associated to the pathophysiology of MDD. In addition, another work evidenced that the plasma expression of miR-144-5p was inversely associated with depression and suggested its origin from the pathologic processes of depression (Wang et al., 2015). MiR-144-5p is involved in the response to mood stabilizer treatment (Zhou et al., 2009) and stress responses (Katsuura et al., 2012), and target the protein kinase C (PKC), Wnt/β-catenin, and phosphatase and tensin homolog (PTEN) pathways (Zhou et al., 2009).

Several altered miRNAs were also identified in the CSF of MDD patients but, notably, elevated miR-221-3p, miR-34a-5p and let-7d-3p, together with low miR-451a levels in

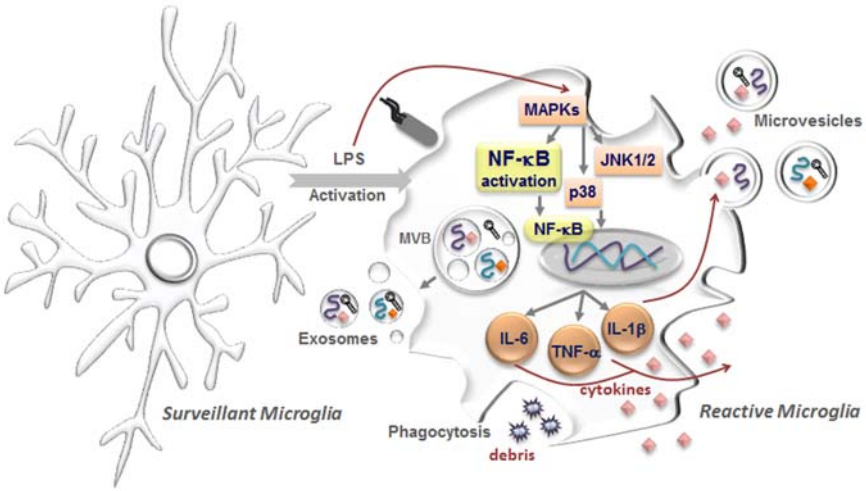


FIGURE 1 | Microglia activation with release of extracellular vesicles (EVs). In the healthy central nervous system, microglia have highly ramified morphology with thin processes, which constantly monitor brain parenchyma to maintain the homeostasis. These microglia are commonly designated as surveillant microglia. Upon stimuli, namely by the proinflammatory lipopolysaccharide (LPS), reactive or activated microglia acquire different morphologies from hypertrophic with enlarged processes to an amoeboid shape. Intracellularly, several pathways become activated including the mitogen-activated protein kinases (MAPKs) superfamily, comprising the c-Jun N-terminal kinase (JNK 1/2) and p38 proteins, which will trigger the activation of the nuclear factor-κB (NF-κB) and consequent induction of first-line cytokine production, such as interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α. In parallel, microglia are also able to sense cellular/molecular debris and intervene by phagocytosing them. Interestingly, messenger RNAs (mRNAs, curved symbols), microRNAs (miRNAs, black symbols) and cytokines (diamond-like symbols) are selectively incorporated into multivesicular bodies (MVBs) and may be released from activated microglia encapsulated in EVs, comprising exosomes and microvesicles. While exosomes are endocytic membrane-derived vesicles of small size (30–100 nm) that are contained in MVBs in the endosomal system and secreted upon MVB fusion with the plasma membrane, microvesicles/ectosomes are quite large vesicles (100–1000 nm) that bud directly from the plasma membrane. These vesicles will act as vehicles in cell-to-cell communication, being considered potential carriers of altered molecules promoting disease propagation.

serum, were suggested as potential biomarkers of MDD (Wan et al., 2015). Among the 16 miRNAs identified part of them were reported to be down-regulated during antidepressant medications (miR-30a-5p, miR-34a-5p and miR-221; Zhou et al., 2009). Analysis of their target genes showed that some important genes related to MDD, such as serotonin receptors (HTR2C), corticotrophin-releasing hormone receptor (CRHR1) and glutamate transporters (SCL1A2), were down-regulated. In contrast, diverse pathways linked to neuronal function in depression, including axon guidance, Wnt signaling pathway, neurotrophin signaling pathway, and long-term depression, were associated to alterations in miR-451a, let-7d-3p, miR-221-3p and miR-34a-5p in both serum and CSF. This study highlighted the existence of differentially altered miRNAs in CSF and serum of MDD patients and their modulation by antidepressant treatment suggesting their potential use as biomarkers for MDD. Curiously, overexpression of miR-34a, miR-30a-5p and let-7d were also shown to down-regulate BDNF expression (Croce et al., 2013).

Data from a mouse model with learned helplessness—an analog for depressive symptoms, demonstrated up-regulation of a polycistronic miRNA cluster (including miR-96, miR-182, and miR-183) that was shown to target genes in step with the circadian clock (Xu et al., 2007). In accordance, increased serum levels of miR-182 and also miR-132 were also found in patients with depression (Xu et al., 2007). Most attractive was the report that both miRNAs targeted BDNF, which have also been detected at lower serum levels in patients with depression (Pallavi et al., 2013). Recently, it was shown that genetic variations in microRNA processing genes, namely *DGR8* and *AG01* (variant AGOl rs636832), were associated with depression (He et al., 2012). Therefore, given the role of miRNAs as potential regulators of neurogenesis and neural plasticity, and the studies suggesting that miRNA processing polymorphisms may contribute to depression risk and response to treatment (Dwivedi, 2014), it is anticipated that miRNAs may constitute a diagnostic tool as well as targets to novel medicines.

EXOSOMES AND ECTOSOMES AS MEDIATORS OF NEUROINFLAMMATION

All neural cells, including neurons, astrocytes, oligodendrocytes and microglia, release EVs comprising exosomes and ectosomes, either in normal or pathological conditions (Gupta and Pulliam, 2014). For instance exosomes and ectosomes were shown to be implicated in amyloid-beta (Aβ) cell-to-cell spreading and neurotoxicity (Brites, 2015). In addition, proteins recruited to exosomes were suggested to be linked to overexpression of tau and associated to both toxicity and neurofibrillary lesion spreading in AD (Saman et al., 2014). Lately, it was suggested that increased incorporation of P-S396-tau, P-T181-tau, and Aβ1–42 in neurally derived blood exosomes predict the occurrence of AD up to 10 years before clinical onset (Fiandaca et al., 2015). Most curious tau dysfunction, in addition to the direct effects of Aβ, may drive alterations in microglial phenotypes and neuroinflammation (Metcalfe and Figueiredo-Pereira, 2010;

Wes et al., 2014). This may justify why minocycline, an anti-inflammatory agent, was shown to reduce neuroinflammation and to restore cognition in an AD mouse model (Parachikova et al., 2010).

Transfer of toxic proteins by exosomes include α-synuclein with a concomitant increase in recipient cells factors that is associated with Parkinson's disease (PD) pathology and progression, thus providing a suitable target for therapeutic intervention (Alvarez-Erviti et al., 2011a; Bellingham et al., 2012b). Moreover, it was demonstrated that α-synuclein can induce an increase of microglia secreted exosomes containing a high level of major histocompatibility complex (MHCs) class II molecules and membrane TNF-α (Chang et al., 2013). Exosomes derived from astrocytes and motor neurons also showed a key role in the amyotrophic lateral sclerosis (ALS) disease based on studies demonstrating the efficient transfer of mutant and misfolded copper-zinc superoxide dismutase 1 (SOD1) to other cells, reason why exosomes are now suggested as targets to modulate ALS disease (Basso et al., 2013; Grad et al., 2014).

Aberrantly expressed cellular miRNAs, selectively packaged and transported in exosomes, can lead to dysregulated gene expression in the recipient cell (Gupta and Pulliam, 2014). Exosomes released by prion-infected neuronal cells showed increased let-7b, let-7i, miR-128a, miR-21, miR-222, miR-29b, miR-342-3p and miR-424 levels, together with reduced miR-146a levels as compared to non-infected exosomes (Bellingham et al., 2012a). Exosomes were associated to the pathogenesis of infectious CNS diseases, prion disease, AD, PD and ALS (Brites and Vaz, 2014; Gupta and Pulliam, 2014; Brites, 2015).

BIOGENESIS OF EXOSOMES AND ECTOSOMES

Cells release into the extracellular environment several types of membrane vesicles from endosomal and plasma membrane origin designated by exosomes and MVs or ectosomes, respectively (Raposo and Stoorvogel, 2013). These EVs, a designation that was recommended for the two classes of vesicles (Cocucci and Meldolesi, 2015), represent an important mode of intercellular communication by serving as vehicles for transfer and delivery of membrane and cytosolic proteins, lipids, mRNAs, and microRNAs (miRNAs) between cells (Lin et al., 2015). EVs are present in many if not all bodily fluids, including blood, urine, saliva, amniotic fluid, breast milk and culture medium of cell cultures (Théry et al., 2002, 2006). Since they contain biologically active proteins and regulatory RNAs, they are suggested to be associated with the propagation of a disease and to create a microenvironment that may favor disease progression (Vingtdeux et al., 2012; Kahlert and Kalluri, 2013).

EVs also include other denominations besides exosomes and ectosomes, as shedding vesicles, nanoparticles, microparticles and oncomes among others (Cocucci and Meldolesi, 2015). Exosomes are endocytic membrane-derived vesicles of small size (30–100 nm) that are contained in multivesicular bodies (MVBs) in the endosomal system and secreted upon MVB fusion with the plasma membrane (Prada et al., 2013; see **Figure 1**). MVs observed by transmission electron microscopy pictures exhibit characteristic cup-shaped or ellipsoid morphology (Momen-Heravi et al., 2012; Fertig et al., 2014). Secretion of exosomes derived from motor neuron-like NSC-34 cells overexpressing mutant hSOD1G93A was proposed as a mechanism of cell-to-cell transfer of mutant SOD1 toxicity (Gomes et al., 2007). These cells indeed release with the characteristic cup-shape morphology by transmission electron microscopy.

Although little is known about MVB fusion with the plasma membrane, several Rab GTPases, including Rab5, Rab27, and Rab35, were suggested to be involved (Shifrin et al., 2013). Exosomal membranes are enriched in cholesterol and sphingomyelin and certain membrane proteins, such as tetraspanins and integrins (Frühbeis et al., 2012). Exosome production may be inhibited by targeting neutral sphingomyelinase-2 with GW4869 (Yuyama et al., 2012) or manumycin-A that also showed to impair transfer of miRNAs to other cells (Mittelbrunn et al., 2011). MVs/ectosomes are quite large vesicles (100–1000 nm) that bud directly from the plasma membrane (Turola et al., 2012; see **Figure 1**). The rate of ectosome shedding is variable, with cells showing elevated formation and release, and others a low rate of ectosome formation (Cocucci and Meldolesi, 2011). Although there are differences on the biogenesis of exosomes and ectosomes, the two EVs display a similar function when released (Cocucci and Meldolesi, 2015). CD63 and CD61 are indicated as markers of exosomes and TyA and C1q of ectosomes, considering the uncertainty of many others that have been appointed. The time of release varies from delayed in exosomes to seconds in ectosomes. Moreover, only 5% of exosomes have externalized phosphatidylserine while MVs/ectosomes evidence a major representation (Prada et al., 2013). The removal of plasma membrane by ectocytosis is compensated by the fusion of MVBs as a compensatory exocytic process of preformed intracellular vesicles (Sadallah et al., 2011; Cocucci and Meldolesi, 2015).

ROLE OF VESICLES IN NEURON—GLIA COMMUNICATION

Brain function depends on coordinated interactions between neurons and glial cells that include microglia, astrocytes, and oligodendrocytes. These cells release vesicles in a way regulated by glutamate (Chivet et al., 2014), which participate in the communication between those cells. EVs released from primary cortical astrocytes and microglial cells appear to be triggered by ATP-mediated activation of P2X7 receptors, after exposure of phosphatidylserine at the cell surface, followed by downstream stimulation of acid sphingomyelinase (Bianco et al., 2009).

Immature and reactive astrocytes in primary cultures were shown to release large vesicles (up to 8 μm) from the cell surface containing functional mitochondria and lipid droplets, probably as a consequence of repetitive ATP stimulation (Falchi et al., 2013). Vesicle delivery to the plasma membrane involves interaction with the cytoskeletal microtubules and actin filaments (Kreft et al., 2009). Astrocytes also release smaller EVs with approximately 100 nm in size (exosomes; Gosselin et al., 2013) containing both neuroprotective and neurotoxic molecules. Astrocyte-derived MVs and exosomes

were described to present neuroprotective proteins including synapsin 1 (Wang et al., 2011), molecules implicated in angiogenesis such as VEGF (Proia et al., 2008), and matrix metalloproteinases involved in extracellular matrix proteolysis (Sbai et al., 2010). Stressful events in astrocytes, like heat or oxidative stress, were shown to induce the release of exosomes carrying the heat shock protein 70 (Hsp70) and synapsin 1 with a pro-survival effect on neurons (Taylor et al., 2007). Upon pathological conditions it has been suggested a detrimental role of astrocyte-derived EVs in the propagation of pathogenic proteins in the course of neurodegenerative disorders. Astrocytes expressing SOD1 mutant, involved in familiar ALS, release a higher amount of exosomes carrying this mutant protein, which were shown to promote *in vitro* motor neuron death following SOD1 efficient transfer (Basso et al., 2013). Further, astrocytes exposed to Aβ species evidenced to secrete pro-apoptotic exosomes which may be taken up by astrocytes themselves or by other neighboring cells contributing to AD neurodegeneration (Wang et al., 2012). Most attractive, it was recently showed that the functional astrocytic excitatory amino-acid transporter (EAAT)-1 is present in secreted EVs, increasing its concentration upon astrocyte activation (Gosselin et al., 2013), suggesting a possible role of this microvesicular transporters in reducing excitotoxicity at an extracellular level.

Oligodendrocyte-axon interaction constitutes a functional unit allowing neuronal integrity (Nave, 2010). Oligodendrocytes secrete exosomes in a calcium-dependent fashion containing myelin proteins and lipids (Frühbeis et al., 2012). These exosomes seem to convey autocrine signals (Bakhti et al., 2011), but can also be internalized by microglia by micropinocytosis to be degraded, thus avoiding an immune reaction (Fitzner et al., 2011). Early studies suggest that oligodendrocyte exosomes may play a crucial role in allowing the supply of myelin proteins (i.e., proteolipid protein and myelin oligodendrocyte glycoprotein), heat-shock proteins, glycolytic enzymes and more recently glycolytic substrates including lactate (Krämer-Albers et al., 2007; Lee et al., 2012). Oligodendroglial exosomes may be also internalized by neurons through endocytosis and their cargo may contribute to neuroprotection and long-term axonal maintenance (Frühbeis et al., 2012). Moreover, the release of oligodendrocyte-derived exosomes seems to be modulated through neuronal signaling (Frühbeis et al., 2013). Glutamate release from neurons activates oligodendrocytes through N-methyl-D-aspartate receptor (NMDA) engagement leading to an increase of intracellular calcium and exosome secretion. These exosomes are then internalized specifically by neurons, and increased firing rate and altered gene expression upon oligodendroglial exosome exposure was observed (Frohlich et al., 2014), confirming that oligodendrocytes influence neuronal physiology, either by induction of signaling cascades or by transfer of mRNAs and miRNAs. Oligodendrocyte-derived MVs have also been described to inhibit both the morphological differentiation of oligodendrocytes and myelin formation through neuronal modulation (Bakhti et al., 2011), suggesting a sophisticated control of myelin membrane biogenesis via MVs. As a physiological degradation of oligodendroglial membrane, part of oligodendrocyte-derived exosomes may be internalized by MHC class II negative microglia through macropinocytosis being subsequently cleared via the lysosomal pathway with no inflammatory response (Fitzner et al., 2011). However, in pathological situations such as AD, where activated microglia are associated with an intense inflammatory milieu, oligodendrocytes were reported to secrete EVs containing degraded myelin proteins (Zhan et al., 2014). Upon stressful conditions, oligodendrocytes may also release immune mediators and express known chemoattractants, signifying an active role on microglia activation and recruitment to the injured area (Peferoen et al., 2014). Microglia activation and MV release will be the subject of the next section.

Recent evidences indicate that exosomes from neuroblastoma cells bind to neurons and glial cells, despite being preferentially endocytosed by glia, while those released upon synaptic activation bind selectively to other neurons (Chivet et al., 2014). On the other hand, the release of serotonin from neurons was evidenced to regulate microglia-derived exosomes involving the elevation of intracellular calcium, at least under physiological conditions, what suggests a neurotransmitter dependent release (Glebov et al., 2015). Most curious, it was also demonstrated that exosomes secreted by Schwann cells, the peripheral glial cell type, are internalized by neurons increasing neurite growth substantially and axonal regeneration (Lopez-Verrilli et al., 2013). Most relevant, intercellular chaperone transmission mediated by exosomes was demonstrated to contribute to maintenance of protein homeostasis (proteostasis; Takeuchi et al., 2015). Moreover, the secretion of harmful or unwanted material in exosomes together with the autophagy-lysosomal pathway also contribute to preserve intracellular protein and RNA homeostasis (Baixauli et al., 2014).

Besides the biological roles of EVs in the CNS, these EVs are also correlated with several neurological diseases, as already indicated. To be noted that alterations in exosome composition in some pathological conditions may switch the immunologically inert exosomes into active ones triggering inflammatory reactions in the CNS. EVs from oligodendroglioma cells revealed to induce astrocyte cell death in primary cultures, which was suggested to be mediated by the pro-apoptotic effects of Fas ligand (FAS-L) and TNF-related apoptosis-inducing ligand (TRAIL; Lo Cicero et al., 2011). Moreover, it was mentioned that exosome-associated amyloids can act as seeds for plaque formation contributing to AD (Rajendran et al., 2014). Intriguingly, the transfer of miR-1 in EVs from the glioblastoma cells was shown to induce multiple changes in the glioblastoma multiform (GBM) surrounding cells (Bronisz et al., 2014). Involvement of exosomes in pathological processes is also associated with their role as potential carriers of misfolded proteins (Bellingham et al., 2012b; Russo et al., 2012; Schneider and Simons, 2013). Cell-to-cell communication by exosomes has been related to the PD progression. Neuronal exosomes containing α-synuclein were suggested to be transmitted from neuron-to-neuron and from neuron-to-glia, while those from activated glial cells to be transferred in a glia-to-glia process, leading to disease spread and

propagation of the inflammatory response, respectively (Russo et al., 2012).

EXOSOMAL microRNA SIGNATURE

Cumulating data suggest that miRNAs are potential biomarkers for the diagnosis and prognosis of a variety of diseases such as cancer and neurological diseases (Mishra, 2014; Warnecke-Eberz et al., 2015). Although no references associate exosomes with depression syndromes, several reports highlight the involvement of miRNAs and posttranscriptional dysregulation in psychiatric disorders (see **Table 1**). One of the first association of depression with altered miRNAs was described for P2RX7 (purinergic receptor P2x, ligand-gated ion channel 7) gene, where single nucleotide polymorphisms (SNPs) were identified in putative miRNA target sites of miR-1302 and miR-625 within the P2RX7 3′-untranslated region (Rahman et al., 2010). P2RX7 channel is involved in synaptic neurotransmitter regulation (Sperlágh et al., 2006) and is present in microglia acting as a scavenger receptor in the absence of its ligand ATP, mediating phagocytosis of apoptotic cells and insoluble debris (Gu et al., 2011).

Recently, mRNAs and miRNAs were identified in exosomes and showed to derive from an active sorting mechanism since the miRNA profiling revealed that it may differ from that of the cells of origin (Zhang et al., 2015). The latest exosome content database identified 4563 proteins, 194 lipids, 1639 mRNAs, and 764 miRNAs in exosomes from multiple organisms (Mathivanan et al., 2012). Electron microscopic analysis revealed that microglia release exosomes with proteins already identified in B cell- and dendritic cell (DC)3-derived exosomes, but with unique proteins such as the aminopeptidase CD13 and the lactate transporter MCT1, which may supply supplementary energy substrate to neurons during synaptic activity (Potolicchio et al., 2005; see **Figure 2** for the typical protein composition of exosomes derived from microglia). The Authors used the N9 microglial cell line and analysis by mass spectroscopic peptide mapping, Western blotting, and enzymatic analysis. They found that N9-derived exosomes express specific markers of late endosomes corroborating their organelle origin, as well as MHC class II molecules and cathepsin S indicative of their function as antigen presenting cells. Integrins involved in antigen presentation and pattern recognition receptors important for innate immunity were also detected.

Recent evidence suggests that miRNAs are transferred by exosomes to recipient cells, while altering gene expression and mediating functional properties (Valadi et al., 2007; Mittelbrunn et al., 2011; Turchinovich et al., 2013; Weber, 2013). Exosomal miRNA was indicated to be released via ceramide-dependent secretory pathways controled by neutral sphingomyelinase that is crucial for budding of intracellular vesicles into MVBs (Trajkovic et al., 2008; Kosaka et al., 2010b; Kogure et al., 2011; Mittelbrunn et al., 2011). However, the precise mechanisms of vesicular miRNAs sorting and secretion are still to be clarified.

Since the majority of current reports describing isolation of MVs-associated extracellular miRNA only rely on ultracentrifugation, one should understand that such experiments inevitably characterize miRNAs in a mixed

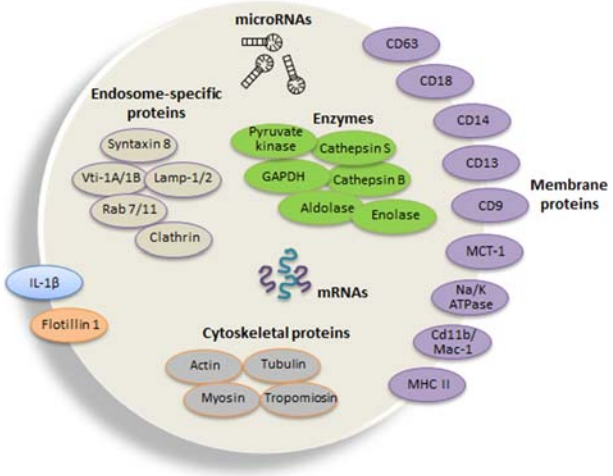


FIGURE 2 | Composition of typical microglial exosomes. Exosomes are packed with several cellular components including messenger RNAs (mRNAs, curved symbols), microRNAs (miRNAs, black symbols) and proteins. Microglia-derived exosomes express specific markers of late endosomes corroborating their organelle origin, as well as major histocompatibility complexes (MHCs) class II molecules and enzymes (i.e., cathepsin S) indicative of their function as antigen presenting cells, and integrins involved in antigen presentation and pattern recognition receptors important for innate immunity. Flotillin 1 is a membrane-associated protein that is enriched in exosomes and thus commonly used as an exosomal marker. Tetraspanins are a family of transmembrane proteins that become integrated in the membrane of EVs and among them are CD9, CD63 and CD81, which are particularly enriched in microglia-derived exosome. The surface-bound aminopeptidase CD13 that degrades enkephalins and the lactate transporter monocarboxylate transporter 1MCT-1 are also highly expressed in exosomes released from microglial cells. CD14 is a monocyte/macrophage marker, regularly used to characterize exosomal preparations. Extrusion of Na, K-ATPase is likewise frequent in different populations of exosomes. Microvesicles also carry the proinflammatory cytokine interleukin (IL-1β) that is shed from the plasma membrane of the microglial cell upon ATP stimulation. In addition, exosomes may additionally contain a distinct set of proteins, such as cytoskeletal proteins and the glycolytic protein glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The illustration is based on proteomic analysis of exosome preparations derived from N9 microglial cells (Potolicchio et al., 2005).

population of MVs and exosomes (Turchinovich et al., 2013). This is the case of a recent study identifying the presence of miR-155 and miR-146a, two critical inflammation-related miRNAs that showed to modulate microglia phenotype (Cardoso et al., 2015), in exosomes that are released from and taken up by dendritic cells (Alexander et al., 2015). The Authors demonstrated that injection of miR-146a-containig exosomes inhibited, while that of miR-155 promoted, the endotoxin-induced inflammation in mice. Moreover, they showed that one dendritic cell produces near 500 exosomes after 24 h of culture and that each one contains about one copy of miR-146a.

It is presently considered that there are four main processes for sorting miRNAs into exosomes: (i) The neural sphingomyelinase 2-dependent pathway; (ii) The miRNA motif and sumoylated heterogeneous nuclear ribonucleoproteins-dependent pathway; (iii) The 3′-end of the miRNA sequence-

dependent pathway; and (iv) The miRNA induced silencing complex-related pathway (Zhang et al., 2015). It should be considered that besides the involvement of specific sequences in certain miRNAs in guiding their incorporation into exosomes, sorting of exosomal miRNAs may be processed under the control of enzymes or other proteins in a miRNA sequence-independent manner (Khalyfa and Gozal, 2014).

Exosomal miRNAs have been indicated as a promising platform for compartment biomarker and early detection of diseases, including brain disorders (Cheng et al., 2014; Khalyfa and Gozal, 2014; Hornick et al., 2015; Lin et al., 2015; Schwarzenbach, 2015). Recent advances in the research of exosomal biomarkers and their potential application in clinical diagnostics will be addressed in the last section of this review.

MICROGLIA ACTIVATION AND MICROVESICLE RELEASE

Emerging data indicate that activation of microglia is associated to both secretion of soluble molecules and release of EVs into the pericellular space. In the healthy CNS, microglia have highly ramified morphology with thin processes, which dynamically monitor the neural cell microenvironment for surveillance in efforts to maintain homeostasis (Nimmerjahn et al., 2005; Kettenmann et al., 2011; see **Figure 1**). Reactive or activated microglia acquire several altered morphologies, including a hypertrophic cell with enlarged processes, or an amoeboid shape (reviewed in Gemma and Bachstetter, 2013). Depending on the stimuli and on the extent of activation, microglia acquire several phenotypes, being the M1 (inflammatory cell) and M2 subtypes (pro-regenerative cells) the most commonly indicated (Brites and Vaz, 2014).

The proinflammatory phenotype intends to protect and repair the CNS from being damaged (Czeh et al., 2011; Chhor et al., 2013; Brites and Vaz, 2014). However, excessive and prolonged neuroinflammation can be cytotoxic and harmful (for review, see Cherry et al., 2014). The M2 phenotype, or the alternatively activated state, inhibits inflammation and works to restore homeostasis. This phenotype may include different subtypes: (i) M2a associated with the production of anti-inflammatory cytokines and trophic factors (Colton, 2009); (ii) M2b considered to be a combined M1/M2a subtypes (Brites et al., 2015); and (iii) M2c associated with phagocytosis and suppression of the innate immune system (Brites and Vaz, 2014). However, one should be cautious when considering that M2 microglia is always advantageous, once it was suggested to correspond to a deactivated, irresponsive population (Chakrabarty et al., 2012), and M2a together with M2c cells were shown to be implicated in AD progression in the APP/PS1 transgenic mice (Weekman et al., 2014). Actually, the notion of microglia as either "good" or "bad" is believed to be too simplistic because microglia switch between these phenotypes and may exist in many intermediate states. Today it is also believed that surveillant microglia is able to phagocytose spines and apoptotic cells (Sierra et al., 2013). Further studies are required to decipher the participation of microglia subtypes in cell homeostasis and neuroinflammation under normal and pathological circumstances.

Non-vesicular mechanisms of microglial secretion mediated by connexin (Cx)43, Cx36, and P2X7 ATP channels are increased in M1 microglia (Eugenín et al., 2003; Dobrenis et al., 2005; Choi et al., 2007). Recent data have shown that reactive microglia release EVs, which are implicated in communication with the brain microenvironment (for review, see Prada et al., 2013). MVs, but not exosomes, seem to contain the cytokine IL-1β and its proform, together with pro-caspase-1, the inflammasome-associated enzyme responsible for IL-1β maturation, when microglia is stimulated by LPS (Bianco et al., 2005). Most interesting, MVs derived from either M1 or surveillant microglia were shown to modulate synaptic activity (Prada et al., 2013). Recent studies evidenced that active synapses promote the pruning of inactive ones by stimulating microglial phagocytosis with exosomes (Bahrini et al., 2015).

Through these processes, microglia influence brain cell functions, either by propagating inflammation and causing neurodegeneration or by playing a neuroprotective role. Indeed, it was demonstrated that MVs from reactive microglia induce an inflammatory reaction in target cells (Verderio et al., 2012a) and that α-synuclein treated microglia release activated exosomes, triggering increased apoptosis and with a suggestive role in the progression of PD (Chang et al., 2013). Microglia-derived MVs were also shown to participate in AD neurodegeneration by promoting the formation of soluble Aβ species and the propagation of such toxic forms (Joshi et al., 2014). On the other way, it was demonstrated that interferon (IFN)-γ stimulated microglia release nutritive exosomes that conferred protection to the neighboring cells (Pusic and Kraig, 2015). The release of MVs, at least from macrophages, was shown to be enhanced when cells are treated with ATP and when polarized either in M1 or in M2 phenotypes. The content of such MVS seems to be determined by the polarization state in that nucleic acid content was shown to be specific (Garzetti et al., 2013). Moreover, different stimuli may trigger the release of MVs with distinct properties, as observed with monocytes (Bernimoulin et al., 2009). Some studies also evidence that induced and proliferative senescence is associated with increased release of MVs, mainly exosomes (Lehmann et al., 2008). Actually, exosome production and secretion are altered during *in vivo* aging and in cancer, and their cargo in miRNAs was suggested to contribute to aging (Xu and Tahara, 2013). One may then assume that activation lead to an increased release of MVs that may differ on their cargo and be associated with several physiological and pathological processes in which neuroinflammation play a pivotal role. Moreover, microglial decline and senescence was suggested to also be implicated in depression-associated impairments such as neuroplasticity and neurogenesis (Yirmiya et al., 2015). Recently, it was demonstrated two principal mechanisms of transmembrane protein release from senescent cells, one related with tumour necrosis factor receptor 1 (TNFR1) by ectodomain shedding and the other of the full-length intercellular adhesion molecule 1 (ICAM1) through MVs, probably exosomes (Effenberger et al., 2014). Interestingly

while autophagy was shown to decrease with age, the release of exosomes seems to be enhanced in senescent cells (Brites, 2015).

EXTRACELLULAR VESICLES IN ADVANCED MEDICINE

EVs have been investigated in neurodegenerative diseases as potential biomarkers, improving diagnosis and disease monitoring, as well as vehicles for targeted delivery of pharmacological compounds and gene therapies. Most attractive is that each EV relies on its source cell. They share expression level, presence, absence, mutation, copy number variation, truncation, duplication, insertion, modification, sequence variation, or molecular association of a given molecule being defined as a bio-signature. According with this concept of EVs bio-signature, analysis of EVs in circulating fluids may allow the identification of their cellular source and constitute important disease biomarkers, either in diagnosis or prognosis.

It has been proposed that serotonin dysfunctions are implicated in the pathophysiology of MDD and decreased levels were found in MDD patients (Paul-Savoie et al., 2011). Thus, serotonin remains at the core of recent advances to elucidate the underlying mechanisms of depression (Albert et al., 2012). Most interesting serotonin was demonstrated to stimulate the secretion of exosomes from microglia cells (Glebov et al., 2015). We may speculate that low levels of serotonin in MDD patients may influence the microglia release of exosomes and be related with the pathology. Indeed, decreased exosome formation was demonstrated to trigger Aβ aggregation for instance, reason why exosome administration or enhancement of exosome generation was suggested as a novel therapeutic approach to AD (Yuyama et al., 2014). In the future, development of engineered nanovesicles might be a valuable tool for the therapy of pathologies associated with inflammation and oxidative stress, including MDD. Future work on the role of exosomes in MDD is required.

As previously noted exosomes may be used as delivery platforms, encapsulating agents or siRNAs, but also as a diagnostic tool due to their content in miRNAs and misfolded proteins. The potential of miRNAs to serve as biomarkers in a noninvasive manner for psychiatric and neurodegenerative diseases and to monitor antidepressant response has been progressing (Dwivedi, 2011, 2014; Serafini et al., 2012; Chana et al., 2013; Dorval et al., 2013). Based on studies demonstrating the role of miRNAs in neural plasticity, neurogenesis and stress response, it is believed that they may contribute to the pathogenesis and progression of MDD. For example, acute stress and chronic stress induce an increase in the expression of selected miRNAs, including miR-134, miR-183, miR-132, Let-7a-l, miR-9–1, and miR-124a-l in the brain (for review, see Dwivedi, 2014). Dysregulated miRNAs were observed in PBMCs from patients with MDD and consistent overexpression along 8-weeks interval was obtained for miR-941 and miR-589 (Belzeaux et al., 2012). As already mentioned, actively secreted miRNAs are enclosed in exosomes, which are excreted in response to stress signaling (Mendell and Olson, 2012) and thus considered as potential biomarkers in depression and antidepressant response (Dwivedi, 2014).

Exosomal miRNAs may constitute biomarkers of a specific disease, as their serum levels are altered in a variety of pathological conditions (Kosaka et al., 2010a; Etheridge et al., 2011; Mo et al., 2012). Indeed, serum enrichment of exosomal miR-21 was positively correlated with tumour progression and aggressiveness in patients with oesophageal squamous cell carcinoma (Tanaka et al., 2013), miR-19a with the recurrence in human colorectal cancer (Matsumura et al., 2015) and miR-141 with metastatic prostate cancer (Kim and Kim, 2013). Increased levels of serum exosomal miR-150, -155, miR-342 and -1246 may differentiate patients with acute myeloid leukemia (Fayyad-Kazan et al., 2013; Hornick et al., 2015) and miR-122 can be an indicator of liver injury and portal hypertension (Jansen et al., 2015).

Brain exosomal miRNAs were shown to distinguish patients with schizophrenia (increased expression of miR-497) from those with bipolar disorder (increased expression of miR-29c; Banigan et al., 2013). Brain-derived EVs can travel and be detected in CSF (Verderio et al., 2012b) and based on the fact that they cross the blood-brain barrier (BBB) from the periphery into the brain (Gupta and Pulliam, 2014) we may hypothesize that the opposite is also true. Therefore, a significant body of literature indicate exosomes as novel biomarkers in clinical diagnosis with high specificity and sensitivity derived from their excellent stability, although their potential value in clinical diagnostics still needs to be fully explored. Recent studies in PD provided evidence on the existence of a circulating subpopulation of MVs showing exosomal properties and enriched Integrin β1 content (Tomlinson et al., 2015).

Nowadays it is considered that by engineering microglia it will be possible to modulate derived EVs and redirect microglia towards a neuroprotective phenotype able to promote tissue repair and with promising therapeutic effects in neurodegenerative diseases associated to inflammatory processes. In particular, short interfering RNAs (siRNAs) are now recognized as therapeutic tools that despite their poor bioavailability can cross the BBB when administered by systemic injection of target exosomes (Alvarez-Erviti et al., 2011b; El Andaloussi et al., 2013). When purified exosomes were loaded with exogenous siRNA by electroporation, strong mRNA and BACE1 protein knockdown, a therapeutic target in AD, was observed in wild-type mice (Alvarez-Erviti et al., 2011b). This was the first demonstration of an exosome-based drug delivery system. Furthermore, intracerebrally administered exosomes revealed to act as potent scavengers for Aβ by carrying it on the exosome surface, thus improving Aβ clearance what may have potential benefits in AD (Yuyama et al., 2014). It was additionally demonstrated that exosomes are able to transfer siRNA to monocytes and lymphocytes triggering the silencing of the target gene *MAPK* (Wahlgren et al., 2012) or knocking down the target gene *RAD51* in recipient cancer cells (Shtam et al., 2013). It was shown that exosomes can also be loaded with interference RNA (iRNA) and most curious, when injected intravenously in mice they were identified only in the target cells (Alvarez-Erviti et al., 2011b).

There is a growing interest in exploring exosomes and their cargo as a tool to monitor disease and as having therapeutic potential as delivery vehicles for specific miRNAs and/or their inhibitors (Kim et al., 2006; Bhatnagar et al., 2007; Lai et al., 2011; Yang et al., 2011; Hu et al., 2012; Ohno et al., 2013). The modulation of miRNA functioning may be achieved through overexpression or by knocking down in EVs. As an example, transfer of anti-miR-9 from mesenchymal stem cells (MSCs) into GBM cells was able to reverse the chemoresistance by blocking the expression of P-glycoprotein in GMB cells and was mediated by the release of MVs (Munoz et al., 2013). Exosomes are considered ideal for drug delivery due to the low immunogenicity, capacity to transport molecules, ability to interact with target cells and willingness to be manipulated for personalized medicine (Gupta and Pulliam, 2014). Such approach is of particular significance once the utilization of miRNAs as therapeutics using a systemic approach needs to overcome the gastrointestinal system, cross the BBB and produce the desired effect in a specific part of the brain (O'Connor et al., 2012).

There is a large body of information showing that soluble factors and EVs within the secretome provide a major contribution to paracrine activity generating a tissue microenvironment that may be neurotoxic or beneficial to regeneration. Secretome from NSCs and MSCs was shown to contain NGF, glial derived neurotrophic factor (GDNF), and BDNF, among others, and suggested to modulate the neurogenic niche (Salgado et al., 2015). As the neurogenic process in the adult brain constitutes a new dimension of plasticity, ways to repair impairments in neuroplasticity may turn useful to treat depression (Bessa et al., 2009; Mateus-Pinheiro et al., 2013a,b). Other studies provided data showing that MSCs and their secretome are able to rescue the AD cell model from misfolded truncated tau-induced cell death (Zilka et al., 2011). Therefore, another therapeutic option will be to inject the stem cell secretome for repair of the damaged tissue, which may even be improved through the use of gene expression methodologies or culture preconditioning of modified stem cells increasing the ability to secrete pro-regenerative factors (Drago et al., 2013).

EVs may be produced from stem cells and their cargo modified to be enriched in growth factors, cytokines, chemokines and regulatory miRNAs to achieve a faster and better regeneration of the injured tissue (Ratajczak et al., 2012; Drago et al., 2013). Most encouraging, EVs can be generated from patient's cells and used for autologous therapies after modulation of their miRNA cargo. Although requiring a great deal of future studies to understand how miRNAs are transferred into exosomes and delivered to target cells, it is expected that exosomes and/or ectosomes will be soon used in clinics. Considering the link between neuroinflammation and depression, we may assume that circulating exosomes from activated glia may contain increased levels of MDD-related miRNAs, which if modulated can switch exosomal neurotoxic effects to neuroprotective activities. This is a field not yet explored in MDD that deserves to be investigated in the future inasmuch pathophysiology leading to mood disorders may differ among patients.

FUTURE PERSPECTIVES

MDD is a disabling condition with impact on well-being and a leading cause of disease burden in high-income countries. Treatment of MDD is very challenging since some patients do not respond to first-line pharmacotherapy. Current guidelines for the management of MDD recommend selective serotonin receptor inhibitors or serotonin-norepinephrine receptor inhibitors, but due to side effects, including potential increased suicidal ideation, clinicians are considering atypical antipsychotics as alternative therapies. Augmentation of MDD patients that do not respond adequately to antipsychotics has increased over the past decade. Whether exosomes may help in the treatment of MDD deserves additional research.

The need to target exact cell types with a specific treatment, without changing the physiology of other cells or tissues, has been one of the major challenges during the most recent years. Furthermore, for *in vivo* application, there is also the need to develop transport systems that cross major biological barriers including the BBB. Under this concept, exosomes have been postulated as potential new delivery vehicles for specific miRNAs or their inhibitors (Kim et al., 2006; Bhatnagar et al., 2007; Lai et al., 2011; Yang et al., 2011; Hu et al., 2012; Ohno et al., 2013). Recent advances have been made to target the brain using a systemic delivery route and to specifically target immune cells in order to reduce neuroinflammation and treat associated disorders as MDD. A link between immune dysregulation and the pathophysiology of at least some forms of major affective disorder has long been hypothesized and abnormalities in peripheral cytokines in depressed patients have led to propose a primary immunological etiology for MDD (Eyre and Baune, 2012). While increased IL-6 was observed to dysregulate genes involved in miRNA machinery, IL-10 revealed neuroprotective properties in first episode psychotic patients with depression (Noto et al., 2015). A better understanding of the pathophysiology of MDD, mainly the immune mechanisms, may help in selecting more powerful biomarkers and discovering target effective treatments using exosomes as delivering vehicles.

Neurons use glial cell exosomes to improve stress tolerance by their role as multifunctional signal emitters. The low levels of serotonin found in MDD patients (Jacobsen et al., 2012) may compromise cell exosome release (Glebov et al., 2015), establishing a bridge between MDD and the potential role of exosomes in disease progression, either for not releasing adequate growth factors and neurotransmitters or by transporting inflammatory miRNAs. In addition, we should consider that exosomes represent a mechanism to get rid of toxic proteins having a neuroprotective action (Joshi et al., 2015), but can also be mediators of neuroinflammation (Gupta and Pulliam, 2014) by their cargo in miRNAs. Alvarez-Erviti et al. (2011b) provided the proof-of-concept that exosomes are a good delivery system of siRNAs to the mice brain. The Authors showed that intravenously administered exosomes derived from autologous murine dendritic cells are able to knockdown specific proteins in neurons, microglia, and oligodendrocytes in the brain of treated animals, proving the

therapeutic potential of autologous exosome-based delivery system. Most attractive, Bryniarski et al. (2013) recently showed that exosomes can deliver their cargo into precise cell types in antigen-specific manner by expressing on their surface specific antibodies.

One preferential route of exosome administration into brain tissue is intranasal delivery. An initial study showed that exosomes derived from activated dendritic cells are efficiently delivered by intranasal administration. They showed to be preferentially taken up by oligodendrocytes and to improve baseline myelination (Pusic et al., 2014), thus suggesting to be a promising strategy for myelin-impaired disorders as MDD (Rajkowska et al., 2015). Interestingly, modified exosomes containing the signal transducer and activator of transcription 3 (Stat3) inhibitor JSI124, have previously been shown to be specifically delivered to microglia cells via the intranasal route, showing a significant protection from LPS-induced brain inflammation (Zhuang et al., 2011).

Most attractively, recent studies showed that intraperitoneal injection of the microglia inhibitor minocycline in adult mice partially prevents LPS (Henry et al., 2008) or Interferon-α-induced (Zheng et al., 2015) depressive-like symptoms. However, studies on the use of non-steroid anti-inflammatory drugs (NSAIDs; Jiang and Chang, 1999) or COX-1 inhibitor (Choi et al., 2009), being COX-1 mainly activated in microglial cells, reported an increase of depressive episodes in psychiatrically healthy individuals. Moreover, a single administration of LPS to severely depressed patients led to a short-term elevation of first-line cytokines improving the depressed state in the first 24 h (Bauer et al., 1995). This finding suggests that the loss of microglia proper function, which may be induced by a strong proinflammatory stimulus as LPS, may also be in the basis of depression-like symptoms. So, it may be hypothesized that deliver of microglia inhibitors or microglia inducers (depending on the immune activation state of the depressed patient) within vesicles targeting microglia may result in promising therapeutic strategies for MDD.

Overall, the production of EVs from patients' own cells that may be engineered to load specific miRNAs cargos and express at cell surface known proteins to be recognized by specific cell types, may in the future be an ideal strategy for personalized autologous therapies. In addition, the induction of exosome release and cell-to-cell communication for transmission of cytoprotective signals may have additional benefits in some specific circumstances. One could imagine combined therapy content in EVs targeting many different sorts of natural MDD resistances at once. However, we must not forget that a great deal of work must be performed before such therapeutic approaches may become feasible in the clinic for neurodegenerative and psychiatric disorders, including MDD.

AUTHOR CONTRIBUTIONS

DB contributed to the conception, design, data collection and critical analysis of the literature, as well as to the writing of the review. AF have given a substantial contribution to data collection, critical analysis of the literature and writing of the review.

FUNDING

Supported by the projects PTDC/SAU-FAR/118787/2010 (to DB), EXPL/NEU-NMC/1003/2013 (to AF) and in part, by iMed.ULisboa (UID/DTP/04138/2013), from Fundação para a Ciência e a Tecnologia (FCT). The funding organization had no role in study design, data collection and analysis, decision to publish, or preparation of the present manuscript.

REFERENCES

Absalon, S., Kochanek, D. M., Raghavan, V., and Krichevsky, A. M. (2013). MiR-26b, upregulated in Alzheimer's disease, activates cell cycle entry, tau-phosphorylation and apoptosis in postmitotic neurons. *J. Neurosci.* 33, 14645–14659. doi: 10.1523/JNEUROSCI.1327-13.2013

Albert, P. R., Benkelfat, C., and Descarries, L. (2012). The neurobiology of depression—revisiting the serotonin hypothesis. I. Cellular and molecular mechanisms. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 2378–2381. doi: 10.1098/rstb.2012.0190

Alexander, M., Hu, R., Runtsch, M. C., Kagele, D. A., Mosbruger, T. L., Tolmachova, T., et al. (2015). Exosome-delivered microRNAs modulate the inflammatory response to endotoxin. *Nat. Commun.* 6:7321. doi: 10.1038/ncomms8321

Altshuler, L. L., Abulseoud, O. A., Foland-Ross, L., Bartzokis, G., Chang, S., Mintz, J., et al. (2010). Amygdala astrocyte reduction in subjects with major depressive disorder but not bipolar disorder. *Bipolar Dis.* 12, 541–549. doi: 10.1111/j.1399-5618.2010.00838.x

Alvarez-Erviti, L., Seow, Y., Schapira, A. H., Gardiner, C., Sargent, I. L., Wood, M. J., et al. (2011a). Lysosomal dysfunction increases exosome-mediated alpha-synuclein release and transmission. *Neurobiol. Dis.* 42, 360–367. doi: 10.1016/j.nbd.2011.01.029

Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S., and Wood, M. J. (2011b). Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat. Biotechnol.* 29, 341–345. doi: 10.1038/nbt.1807

Aston, C., Jiang, L., and Sokolov, B. P. (2005). Transcriptional profiling reveals evidence for signaling and oligodendroglial abnormalities in the temporal cortex from patients with major depressive disorder. *Mol. Psychiatry* 10, 309–322. doi: 10.1038/sj.mp.4001565

Bahrini, I., Song, J. H., Diez, D., and Hanayama, R. (2015). Neuronal exosomes facilitate synaptic pruning by up-regulating complement factors in microglia. *Sci. Rep.* 5:7989. doi: 10.1038/srep07989

Baixauli, F., López-Otin, C., and Mittelbrunn, M. (2014). Exosomes and autophagy: coordinated mechanisms for the maintenance of cellular fitness. *Front. Immunol.* 5:403. doi: 10.3389/fimmu.2014.00403

Bakhti, M., Winter, C., and Simons, M. (2011). Inhibition of myelin membrane sheath formation by oligodendrocyte-derived exosome-like vesicles. *J. Biol. Chem.* 286, 787–796. doi: 10.1074/jbc.m110.190009

Banigan, M. G., Kao, P. F., Kozubek, J. A., Winslow, A. R., Medina, J., Costa, J., et al. (2013). Differential expression of exosomal microRNAs in prefrontal cortices of schizophrenia and bipolar disorder patients. *PLoS One* 8:e48814. doi: 10.1371/journal.pone.0048814

Bargaje, R., Gupta, S., Sarkeshik, A., Park, R., Xu, T., Sarkar, M., et al. (2012). Identification of novel targets for miR-29a using miRNA proteomics. *PLoS One* 7:e43243. doi: 10.1371/journal.pone.0043243

Basso, M., Pozzi, S., Tortarolo, M., Fiordaliso, F., Bisighini, C., Pasetto, L., et al. (2013). Mutant copper-zinc superoxide dismutase (SOD1) induces protein secretion pathway alterations and exosome release in astrocytes: implications for disease spreading and motor neuron pathology in amyotrophic lateral sclerosis. *J. Biol. Chem.* 288, 15699–15711. doi: 10.1074/jbc.M112.425066

Bauer, J., Hohagen, F., Gimmel, E., Bruns, F., Lis, S., Krieger, S., et al. (1995). Induction of cytokine synthesis and fever suppresses REM sleep and improves mood in patients with major depression. *Biol. Psychiatry* 38, 611–621. doi: 10.1016/0006-3223(95)00374-x

Bellingham, S. A., Coleman, B. M., and Hill, A. F. (2012a). Small RNA deep sequencing reveals a distinct miRNA signature released in exosomes from prion-infected neuronal cells. *Nucleic Acids Res.* 40, 10937–10949. doi: 10.1093/nar/gks832

Bellingham, S. A., Guo, B. B., Coleman, B. M., and Hill, A. F. (2012b). Exosomes: vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? *Front. Physiol.* 3:124. doi: 10.3389/fphys.2012.00124

Belzeaux, R., Bergon, A., Jeanjean, V., Loriod, B., Formisano-Tréziny, C., Verrier, L., et al. (2012). Responder and nonresponder patients exhibit different peripheral transcriptional signatures during major depressive episode. *Transl. Psychiatry* 2:e185. doi: 10.1038/tp.2012.112

Benton, T., Staab, J., and Evans, D. L. (2007). Medical co-morbidity in depressive disorders. *Ann. Clin. Psychiatry* 19, 289–303. doi: 10.1080/10401230701653542

Bernimoulin, M., Waters, E. K., Foy, M., Steele, B. M., Sullivan, M., Falet, H., et al. (2009). Differential stimulation of monocytic cells results in distinct populations of microparticles. *J. Thromb. Haemost.* 7, 1019–1028. doi: 10.1111/j.1538-7836.2009.03434.x

Bessa, J. M., Ferreira, D., Melo, I., Marques, F., Cerqueira, J. J., Palha, J. A., et al. (2009). The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Mol. Psychiatry* 14, 764–773, 739. doi: 10.1038/mp.2008.119

Bhatnagar, S., Shinagawa, K., Castellino, F. J., and Schorey, J. S. (2007). Exosomes released from macrophages infected with intracellular pathogens stimulate a proinflammatory response *in vitro* and *in vivo*. *Blood* 110, 3234–3244. doi: 10.1182/blood-2007-03-079152

Bianco, F., Perrotta, C., Novellino, L., Francolini, M., Riganti, L., Menna, E., et al. (2009). Acid sphingomyelinase activity triggers microparticle release from glial cells. *EMBO J.* 28, 1043–1054. doi: 10.1038/emboj.2009.45

Bianco, F., Pravettoni, E., Colombo, A., Schenk, U., Moller, T., Matteoli, M., et al. (2005). Astrocyte-derived ATP induces vesicle shedding and IL-1 beta release from microglia. *J Immunol* 174, 7268–7277. doi: 10.4049/jimmunol.174.11.7268

Biggar, K. K., and Storey, K. B. (2011). The emerging roles of microRNAs in the molecular responses of metabolic rate depression. *J. Mol. Cell Biol.* 3, 167–175. doi: 10.1093/jmcb/mjq045

Brites, D. (2015). Cell ageing: a flourishing field for neurodegenerative diseases. *AIMS Mol. Sci.* 2, 225–258. doi: 10.3934/molsci.2015.3.225

Brites, D., Gomes, C., Pinheiro, R., and Falcão, A. S. (2015). "New Insights in the pro- and anti-neurogenic interventions of microglia and their association to gliomas," in *Microglia: Physiology, Regulation and Health Implications*, ed. E. R. Giffard (New York: Nova Biomedical), 67–111.

Brites, D., and Vaz, A. R. (2014). Microglia centered pathogenesis in ALS: insights in cell interconnectivity. *Front. Cell. Neurosci.* 8:117. doi: 10.3389/fncel.2014.00117

Bronisz, A., Wang, Y., Nowicki, M. O., Peruzzi, P., Ansari, K. I., Ogawa, D., et al. (2014). Extracellular vesicles modulate the glioblastoma microenvironment via a tumor suppression signaling network directed by miR-1. *Cancer Res.* 74, 738–750. doi: 10.1158/0008-5472.CAN-13-2650

Bryniarski, K., Ptak, W., Jayakumar, A., Püllmann, K., Caplan, M. J., Chairoungdua, A., et al. (2013). Antigen-specific, antibody-coated, exosome-like nanovesicles deliver suppressor T-cell microRNA-150 to effector T cells to inhibit contact sensitivity. *J. Allergy Clin. Immunol.* 132, 170–181. doi: 10.1016/j.jaci.2013.04.048

Buzas, E. I., György, B., Nagy, G., Falus, A., and Gay, S. (2014). Emerging role of extracellular vesicles in inflammatory diseases. *Nat. Rev. Rheumatol.* 10, 356–364. doi: 10.1038/nrrheum.2014.19

Cardoso, A. L., Guedes, J. R., and De Lima, M. C. (2015). Role of microRNAs in the regulation of innate immune cells under neuroinflammatory conditions. *Curr. Opin. Pharmacol.* 26, 1–9. doi: 10.1016/j.coph.2015.09.001

Carvalho, A. F., Kohler, C. A., Mcintyre, R. S., Knöchel, C., Brunoni, A. R., Thase, M. E., et al. (2015). Peripheral vascular endothelial growth factor as a novel depression biomarker: a meta-analysis. *Psychoneuroendocrinology* 62, 18–26. doi: 10.1016/j.psyneuen.2015.07.002

Castrén, E., and Rantamäki, T. (2010). The role of BDNF and its receptors in depression and antidepressant drug action: reactivation of developmental plasticity. *Dev. Neurobiol.* 70, 289–297. doi: 10.1002/dneu.20758

Chakrabarty, P., Tianbai, L., Herring, A., Ceballos-Diaz, C., Das, P., and Golde, T. E. (2012). Hippocampal expression of murine IL-4 results in exacerbation of amyloid deposition. *Mol. Neurodegener.* 7:36. doi: 10.1186/1750-1326-7-36

Chana, G., Bousman, C. A., Money, T. T., Gibbons, A., Gillett, P., Dean, B., et al. (2013). Biomarker investigations related to pathophysiological pathways in schizophrenia and psychosis. *Front. Cell. Neurosci.* 7:95. doi: 10.3389/fncel.2013.00095

Chang, C., Lang, H., Geng, N., Wang, J., Li, N., and Wang, X. (2013). Exosomes of BV-2 cells induced by alpha-synuclein: important mediator of neurodegeneration in PD. *Neurosci. Lett.* 548, 190–195. doi: 10.1016/j.neulet.2013.06.009

Cheng, L. C., Pastrana, E., Tavazoie, M., and Doetsch, F. (2009). miR-124 regulates adult neurogenesis in the subventricular zone stem cell niche. *Nat. Neurosci.* 12, 399–408. doi: 10.1038/nn.2294

Cheng, L., Sharples, R. A., Scicluna, B. J., and Hill, A. F. (2014). Exosomes provide a protective and enriched source of miRNA for biomarker profiling compared to intracellular and cell-free blood. *J. Extracell. Vesicles* 3:23743 doi: 10.3402/jev.v3.23743

Cherry, J. D., Olschowka, J. A., and O'Banion, M. K. (2014). Neuroinflammation and M2 microglia: the good, the bad and the inflamed. *J. Neuroinflammation* 11:98. doi: 10.1186/1742-2094-11-98

Chhor, V., Le Charpentier, T., Lebon, S., Oré, M. V., Celador, I. L., Josserand, J., et al. (2013). Characterization of phenotype markers and neuronotoxic potential of polarised primary microglia *in vitro*. *Brain Behav. Immun.* 32, 70–85. doi: 10.1016/j.bbi.2013.02.005

Chivet, M., Javalet, C., Laulagnier, K., Blot, B., Hemming, F. J., and Sadoul, R. (2014). Exosomes secreted by cortical neurons upon glutamatergic synapse activation specifically interact with neurons. *J. Extracell. Vesicles* 3:24722. doi: 10.3402/jev.v3.24722

Choi, S. H., Aid, S., and Bosetti, F. (2009). The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. *Trends Pharmacol. Sci.* 30, 174–181. doi: 10.1016/j.tips.2009.01.002

Choi, H. B., Ryu, J. K., Kim, S. U., and McLarnon, J. G. (2007). Modulation of the purinergic P2X7 receptor attenuates lipopolysaccharide-mediated microglial activation and neuronal damage in inflamed brain. *J. Neurosci.* 27, 4957–4968. doi: 10.1523/jneurosci.5417-06.2007

Cocucci, E., and Meldolesi, J. (2011). Ectosomes. *Curr. Biol.* 21, R940–R941. doi: 10.1016/j.cub.2011.10.011

Cocucci, E., and Meldolesi, J. (2015). Ectosomes and exosomes: shedding the confusion between extracellular vesicles. *Trends Cell Biol.* 25, 364–372. doi: 10.1016/j.tcb.2015.01.004

Colton, C. A. (2009). Heterogeneity of microglial activation in the innate immune response in the brain. *J. Neuroimmune. Pharmacol.* 4, 399–418. doi: 10.1007/s11481-009-9164-4

Covington, H. E. III, Lobo, M. K., Maze, I., Vialou, V., Hyman, J. M., Zaman, S., et al. (2010). Antidepressant effect of optogenetic stimulation of the medial prefrontal cortex. *J. Neurosci.* 30, 16082–16090. doi: 10.1523/JNEUROSCI.1731-10.2010

Croce, N., Gelfo, F., Ciotti, M. T., Federici, G., Caltagirone, C., Bernardini, S., et al. (2013). NPY modulates miR-30a-5p and BDNF in opposite direction in an *in vitro* model of Alzheimer disease: a possible role in neuroprotection? *Mol. Cell Biochem.* 376, 189–195. doi: 10.1007/s11010-013-1567-0

Czeh, M., Gressens, P., and Kaindl, A. M. (2011). The yin and yang of microglia. *Dev. Neurosci.* 33, 199–209. doi: 10.1159/000328989

Dahl, J., Ormstad, H., Aass, H. C., Malt, U. F., Bendz, L. T., Sandvik, L., et al. (2014). The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology* 45, 77–86. doi: 10.1016/j.psyneuen.2014.03.019

Dantzer, R., O'Connor, J. C., Freund, G. G., Johnson, R. W., and Kelley, K. W. (2008). From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56. doi: 10.1038/nrn2297

Dantzer, R., O'Connor, J. C., Lawson, M. A., and Kelley, K. W. (2011). Inflammation-associated depression: from serotonin to kynurenine. *Psychoneuroendocrinology* 36, 426–436. doi: 10.1016/j.psyneuen.2010.09.012

Dantzer, R., and Walker, A. K. (2014). Is there a role for glutamate-mediated excitotoxicity in inflammation-induced depression? *J. Neural. Transm. (Vienna)* 121, 925–932. doi: 10.1007/s00702-014-1187-1

Davis, M. J., Haley, T., Duvoisin, R. M., and Raber, J. (2012). Measures of anxiety, sensorimotor function and memory in male and female mGluR4(−)/(−) mice. *Behav. Brain Res.* 229, 21–28. doi: 10.1016/j.bbr.2011.12.037

Dobrenis, K., Chang, H. Y., Pina-Benabou, M. H., Woodroffe, A., Lee, S. C., Rozental, R., et al. (2005). Human and mouse microglia express connexin36 and functional gap junctions are formed between rodent microglia and neurons. *J. Neurosci. Res.* 82, 306–315. doi: 10.1002/jnr.20650

Dorval, V., Nelson, P. T., and Hébert, S. S. (2013). Circulating microRNAs in Alzheimer's disease: the search for novel biomarkers. *Front. Mol. Neurosci.* 6:24. doi: 10.3389/fnmol.2013.00024

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., et al. (2010). A meta-analysis of cytokines in major depression. *Biol. Psychiatry* 67, 446–457. doi: 10.1016/j.biopsych.2009.09.033

Drago, D., Cossetti, C., Iraci, N., Gaude, E., Musco, G., Bachi, A., et al. (2013). The stem cell secretome and its role in brain repair. *Biochimie* 95, 2271–2285. doi: 10.1016/j.biochi.2013.06.020

Dubovický, M., Császár, E., Melicherčíková, K., Kuniaková, M., and Račková, L. (2014). Modulation of microglial function by the antidepressant drug venlafaxine. *Interdiscip. Toxicol.* 7, 201–207. doi: 10.2478/intox-2014-0029

Dwivedi, Y. (2009). Brain-derived neurotrophic factor: role in depression and suicide. *Neuropsychiatr. Dis. Treat.* 5, 433–449. doi: 10.2147/ndt.s5700

Dwivedi, Y. (2011). Evidence demonstrating role of microRNAs in the etiopathology of major depression. *J. Chem. Neuroanat.* 42, 142–156. doi: 10.1016/j.jchemneu.2011.04.002

Dwivedi, Y. (2014). Emerging role of microRNAs in major depressive disorder: diagnosis and therapeutic implications. *Dialogues Clin. Neurosci.* 16, 43–61.

Effenberger, T., Von Der Heyde, J., Bartsch, K., Garbers, C., Schulze-Osthoff, K., Chalaris, A., et al. (2014). Senescence-associated release of transmembrane proteins involves proteolytic processing by ADAM17 and microvesicle shedding. *FASEB J.* 28, 4847–4856. doi: 10.1096/fj.14-254565

El Andaloussi, S., Lakhal, S., Mäger, I., and Wood, M. J. (2013). Exosomes for targeted siRNA delivery across biological barriers. *Adv. Drug Deliv. Rev.* 65, 391–397. doi: 10.1016/j.addr.2012.08.008

El-Etr, M., Rame, M., Boucher, C., Ghoumari, A. M., Kumar, N., Liere, P., et al. (2015). Progesterone and nestorone promote myelin regeneration in chronic demyelinating lesions of corpus callosum and cerebral cortex. *Glia* 63, 104–117. doi: 10.1002/glia.22736

Erny, D., Hrabě De Angelis, A. L., Jaitin, D., Wieghofer, P., Staszewski, O., David, E., et al. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* 18, 965–977. doi: 10.1038/nn.4030

Etheridge, A., Lee, I., Hood, L., Galas, D., and Wang, K. (2011). Extracellular microRNA: a new source of biomarkers. *Mutat. Res.* 717, 85–90. doi: 10.1016/j.mrfmmm.2011.03.004

Eugenín, E. A., Brañes, M. C., Berman, J. W., and Sáaez, J. C. (2003). TNF-alpha plus IFN-gamma induce connexin43 expression and formation of gap junctions between human monocytes/macrophages that enhance physiological responses. *J. Immunol.* 170, 1320–1328. doi: 10.4049/jimmunol.170.3.1320

Eyre, H., and Baune, B. T. (2012). Neuroplastic changes in depression: a role for the immune system. *Psychoneuroendocrinology* 37, 1397–1416. doi: 10.1016/j.psyneuen.2012.03.019

Falchi, A. M., Sogos, V., Saba, F., Piras, M., Congiu, T., and Piludu, M. (2013). Astrocytes shed large membrane vesicles that contain mitochondria, lipid droplets and ATP. *Histochem. Cell Biol.* 139, 221–231. doi: 10.1007/s00418-012-1045-x

Fan, H. M., Sun, X. Y., Guo, W., Zhong, A. F., Niu, W., Zhao, L., et al. (2014). Differential expression of microRNA in peripheral blood mononuclear cells as specific biomarker for major depressive disorder patients. *J. Psychiatr. Res.* 59, 45–52. doi: 10.1016/j.jpsychires.2014.08.007

Fayyad-Kazan, H., Bitar, N., Najar, M., Lewalle, P., Fayyad-Kazan, M., Badran, R., et al. (2013). Circulating miR-150 and miR-342 in plasma are novel potential biomarkers for acute myeloid leukemia. *J. Transl. Med.* 11:31. doi: 10.1186/1479-5876-11-31

Fertig, E. T., Gherghiceanu, M., and Popescu, L. M. (2014). Extracellular vesicles release by cardiac telocytes: electron microscopy and electron tomography. *J. Cell. Mol. Med.* 18, 1938–1943. doi: 10.1111/jcmm.12436

Fiandaca, M. S., Kapogiannis, D., Mapstone, M., Boxer, A., Eitan, E., Schwartz, J. B., et al. (2015). Identification of preclinical Alzheimer's disease by a profile of pathogenic proteins in neurally derived blood exosomes: a case-control study. *Alzheimers Dement.* 11, 600.e1–607.e1. doi: 10.1016/j.jalz.2014.06.008

Fitzner, D., Schnaars, M., Van Rossum, D., Krishnamoorthy, G., Dibaj, P., Bakhti, M., et al. (2011). Selective transfer of exosomes from oligodendrocytes to microglia by macropinocytosis. *J. Cell Sci.* 124, 447–458. doi: 10.1242/jcs.074088

Frenois, F., Moreau, M., O'Connor, J., Lawson, M., Micon, C., Lestage, J., et al. (2007). Lipopolysaccharide induces delayed FosB/DeltaFosB immunostaining within the mouse extended amygdala, hippocampus and hypothalamus, that parallel the expression of depressive-like behavior. *Psychoneuroendocrinology* 32, 516–531. doi: 10.1016/j.psyneuen.2007.03.005

Frohlich, D., Kuo, W. P., Fruhbeis, C., Sun, J. J., Zehendner, C. M., Luhmann, H. J., et al. (2014). Multifaceted effects of oligodendroglial exosomes on neurons: impact on neuronal firing rate, signal transduction and gene regulation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 369:20130510. doi: 10.1098/rstb.2013.0510

Frühbeis, C., Fröhlich, D., and Krämer-Albers, E. M. (2012). Emerging roles of exosomes in neuron-glia communication. *Front. Physiol.* 3:119. doi: 10.3389/fphys.2012.00119

Frühbeis, C., Fröhlich, D., Kuo, W. P., Amphornrat, J., Thilemann, S., Saab, A. S., et al. (2013). Neurotransmitter-triggered transfer of exosomes mediates oligodendrocyte-neuron communication. *PLoS Biol.* 11:e1001604. doi: 10.1371/journal.pbio.1001604

Frühbeis, C., Fröhlich, D., Kuo, W. P., and Krämer-Albers, E. M. (2013). Extracellular vesicles as mediators of neuron-glia communication. *Front. Cell. Neurosci.* 7:182. doi: 10.3389/fncel.2013.00182

Garzetti, L., Menon, R., Finardi, A., Bergami, A., Sica, A., Martino, G., et al. (2013). Activated macrophages release microvesicles containing polarized M1 or M2 mRNAs. *J. Leukoc. Biol.* 95, 817–825. doi: 10.1189/jlb.0913485

Gemma, C., and Bachstetter, A. D. (2013). The role of microglia in adult hippocampal neurogenesis. *Front. Cell. Neurosci.* 7:229. doi: 10.3389/fncel.2013.00229

Giusti, S. A., Vogl, A. M., Brockmann, M. M., Vercelli, C. A., Rein, M. L., Trumbach, D., et al. (2014). microRNA-9 controls dendritic development by targeting REST. *Elife* 3:e02755. doi: 10.7554/elife.02755

Glebov, K., Löchner, M., Jabs, R., Lau, T., Merkel, O., Schloss, P., et al. (2015). Serotonin stimulates secretion of exosomes from microglia cells. *Glia* 63, 626–634. doi: 10.1002/glia.22772

Goldberg, D. (2010). The detection and treatment of depression in the physically ill. *World Psychiatry* 9, 16–20. doi: 10.1002/j.2051-5545.2010.tb00256.x

Gomes, C., Keller, S., Altevogt, P., and Costa, J. (2007). Evidence for secretion of Cu,Zn superoxide dismutase via exosomes from a cell model of amyotrophic lateral sclerosis. *Neurosci. Lett.* 428, 43–46. doi: 10.1016/j.neulet.2007.09.024

Gosselin, R. D., Meylan, P., and Decosterd, I. (2013). Extracellular microvesicles from astrocytes contain functional glutamate transporters: regulation by protein kinase C and cell activation. *Front. Cell. Neurosci.* 7:251. doi: 10.3389/fncel.2013.00251

Grad, L. I., Yerbury, J. J., Turner, B. J., Guest, W. C., Pokrishevsky, E., O'Neill, M. A., et al. (2014). Intercellular propagated misfolding of wild-type Cu/Zn superoxide dismutase occurs via exosome-dependent and -independent mechanisms. *Proc. Natl. Acad. Sci. U S A* 111, 3620–3625. doi: 10.1073/pnas.1312245111

Gregory, R. I., and Shiekhattar, R. (2005). microRNA biogenesis and cancer. *Cancer Res.* 65, 3509–3512. doi: 10.1158/0008-5472.can-05-0298

Gu, B. J., Saunders, B. M., Petrou, S., and Wiley, J. S. (2011). P2X(7) is a scavenger receptor for apoptotic cells in the absence of its ligand, extracellular ATP. *J. Immunol.* 187, 2365–2375. doi: 10.4049/jimmunol.1101178

Gupta, A., and Pulliam, L. (2014). Exosomes as mediators of neuroinflammation. *J. Neuroinflammation* 11:68. doi: 10.1186/1742-2094-11-68

Hamidi, M., Drevets, W. C., and Price, J. L. (2004). Glial reduction in amygdala in major depressive disorder is due to oligodendrocytes. *Biol. Psychiatry* 55, 563–569. doi: 10.1016/j.biopsych.2003.11.006

Hannestad, J., Dellagioia, N., Gallezot, J. D., Lim, K., Nabulsi, N., Esterlis, I., et al. (2013). The neuroinflammation marker translocator protein is not elevated in individuals with mild-to-moderate depression: a [(1)(1)C]PBR28 PET study. *Brain Behav. Immun.* 33, 131–138. doi: 10.1016/j.bbi.2013.06.010

He, Y., Zhou, Y., Xi, Q., Cui, H., Luo, T., Song, H., et al. (2012). Genetic variations in microRNA processing genes are associated with susceptibility in depression. *DNA Cell Biol.* 31, 1499–1506. doi: 10.1089/dna.2012.1660

Henry, C. J., Huang, Y., Wynne, A., Hanke, M., Himler, J., Bailey, M. T., et al. (2008). Minocycline attenuates lipopolysaccharide (LPS)-induced neuroinflammation, sickness behavior, and anhedonia. *J. Neuroinflammation* 5:15. doi: 10.1186/1742-2094-5-15

Hornick, N. I., Huan, J., Doron, B., Goloviznina, N. A., Lapidus, J., Chang, B. H., et al. (2015). Serum exosome microRNA as a minimally-invasive early biomarker of AML. *Sci. Rep.* 5:11295. doi: 10.1038/srep11295

Hu, G., Drescher, K. M., and Chen, X. M. (2012). Exosomal miRNAs: biological properties and therapeutic potential. *Front. Genet.* 3:56. doi: 10.3389/fgene.2012.00056

Jacobsen, J. P., Medvedev, I. O., and Caron, M. G. (2012). The 5-HT deficiency theory of depression: perspectives from a naturalistic 5-HT deficiency model, the tryptophan hydroxylase 2Arg439His knockin mouse. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 367, 2444–2459. doi: 10.1098/rstb.2012.0109

Jansen, C., Reiberger, T., Huang, J., Eischeid, H., Schierwagen, R., Mandorfer, M., et al. (2015). Circulating miRNA-122 levels are associated with hepatic necroinflammation and portal hypertension in HIV/HCV coinfection. *PLoS One* 10:e0116768. doi: 10.1371/journal.pone.0116768

Jiang, H. K., and Chang, D. M. (1999). Non-steroidal anti-inflammatory drugs with adverse psychiatric reactions: five case reports. *Clin. Rheumatol.* 18, 339–345. doi: 10.1007/s100670050114

Jo, W. K., Zhang, Y., Emrich, H. M., and Dietrich, D. E. (2015). Glia in the cytokine-mediated onset of depression: fine tuning the immune response. *Front. Cell. Neurosci.* 9:268. doi: 10.3389/fncel.2015.00268

Joshi, P., Benussi, L., Furlan, R., Ghidoni, R., and Verderio, C. (2015). Extracellular vesicles in Alzheimer's disease: friends or foes? Focus on aβ-vesicle interaction. *Int. J. Mol. Sci.* 16, 4800–4813. doi: 10.3390/ijms16034800

Joshi, P., Turola, E., Ruiz, A., Bergami, A., Libera, D. D., Benussi, L., et al. (2014). Microglia convert aggregated amyloid-β into neurotoxic forms through the shedding of microvesicles. *Cell Death Differ.* 21, 582–593. doi: 10.1038/cdd.2013.180

Kahlert, C., and Kalluri, R. (2013). Exosomes in tumor microenvironment influence cancer progression and metastasis. *J. Mol. Med. (Berl).* 91, 431–437. doi: 10.1007/s00109-013-1020-6

Kaminska, B., Gozdz, A., Zawadzka, M., Ellert-Miklaszewska, A., and Lipko, M. (2009). MAPK signal transduction underlying brain inflammation and gliosis as therapeutic target. *Anat. Rec. Hoboken* 292, 1902–1913. doi: 10.1002/ar.21047

Katsuura, S., Kuwano, Y., Yamagishi, N., Kurokawa, K., Kajita, K., Akaike, Y., et al. (2012). microRNAs miR-144/144* and miR-16 in peripheral blood are potential biomarkers for naturalistic stress in healthy Japanese medical students. *Neurosci. Lett.* 516, 79–84. doi: 10.1016/j.neulet.2012.03.062

Kettenmann, H., Hanisch, U. K., Noda, M., and Verkhratsky, A. (2011). Physiology of microglia. *Physiol. Rev.* 91, 461–553. doi: 10.1152/physrev.00011.2010

Khalyfa, A., and Gozal, D. (2014). Exosomal miRNAs as potential biomarkers of cardiovascular risk in children. *J. Transl. Med.* 12:162. doi: 10.1186/1479-5876-12-162

Kim, S. H., Bianco, N., Menon, R., Lechman, E. R., Shufesky, W. J., Morelli, A. E., et al. (2006). Exosomes derived from genetically modified DC expressing FasL are anti-inflammatory and immunosuppressive. *Mol. Ther.* 13, 289–300. doi: 10.1016/j.ymthe.2005.09.015

Kim, W. T., and Kim, W. J. (2013). microRNAs in prostate cancer. *Prostate. Int.* 1, 3–9. doi: 10.12954/pi.12011

Kogure, T., Lin, W. L., Yan, I. K., Braconi, C., and Patel, T. (2011). Intercellular nanovesicle-mediated microRNA transfer: a mechanism of environmental modulation of hepatocellular cancer cell growth. *Hepatology* 54, 1237–1248. doi: 10.1002/hep.24504

Konradi, C., Sillivan, S. E., and Clay, H. B. (2012). Mitochondria, oligodendrocytes and inflammation in bipolar disorder: evidence from transcriptome studies points to intriguing parallels with multiple sclerosis. *Neurobiol. Dis.* 45, 37–47. doi: 10.1016/j.nbd.2011.01.025

Kosaka, N., Iguchi, H., and Ochiya, T. (2010a). Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *Cancer Sci.* 101, 2087–2092. doi: 10.1111/j.1349-7006.2010.01650.x

Kosaka, N., Iguchi, H., Yoshioka, Y., Takeshita, F., Matsuki, Y., and Ochiya, T. (2010b). Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J. Biol. Chem.* 285, 17442–17452. doi: 10.1074/jbc.M110.107821

Krämer-Albers, E. M., Bretz, N., Tenzer, S., Winterstein, C., Mobius, W., Berger, H., et al. (2007). Oligodendrocytes secrete exosomes containing major myelin and stress-protective proteins: trophic support for axons? *Proteomics Clin. Appl.* 1, 1446–1461. doi: 10.1002/prca.200700522

Kreft, M., Potokar, M., Stenovec, M., Pangrsic, T., and Zorec, R. (2009). Regulated exocytosis and vesicle trafficking in astrocytes. *Ann. N Y Acad. Sci.* 1152, 30–42. doi: 10.1111/j.1749-6632.2008.04005.x

Krol, J., Loedige, I., and Filipowicz, W. (2010). The widespread regulation of microRNA biogenesis, function and decay. *Nat. Rev. Genet.* 11, 597–610. doi: 10.1038/nrg2843

Ksiazek-Winiarek, D. J., Kacperska, M. J., and Glabinski, A. (2013). microRNAs as novel regulators of neuroinflammation. *Mediators Inflamm.* 2013:172351. doi: 10.1155/2013/172351

Lai, R. C., Chen, T. S., and Lim, S. K. (2011). Mesenchymal stem cell exosome: a novel stem cell-based therapy for cardiovascular disease. *Regen. Med.* 6, 481–492. doi: 10.2217/rme.11.35

Le, M. T., Xie, H., Zhou, B., Chia, P. H., Rizk, P., Um, M., et al. (2009). microRNA-125b promotes neuronal differentiation in human cells by repressing multiple targets. *Mol. Cell. Biol.* 29, 5290–5305. doi: 10.1128/MCB.01694-08

Lee, Y., Morrison, B. M., Li, Y., Lengacher, S., Farah, M. H., Hoffman, P. N., et al. (2012). Oligodendroglia metabolically support axons and contribute to neurodegeneration. *Nature* 487, 443–448. doi: 10.1038/nature11314

Lehmann, B. D., Paine, M. S., Brooks, A. M., McCubrey, J. A., Renegar, R. H., Wang, R., et al. (2008). Senescence-associated exosome release from human prostate cancer cells. *Cancer Res.* 68, 7864–7871. doi: 10.1158/0008-5472.CAN-07-6538

Li, B., and Sun, H. (2013). MiR-26a promotes neurite outgrowth by repressing PTEN expression. *Mol. Med. Rep.* 8, 676–680. doi: 10.3892/mmr.2013.1534

Li, Y. J., Xu, M., Gao, Z. H., Wang, Y. Q., Yue, Z., Zhang, Y. X., et al. (2013). Alterations of serum levels of BDNF-related miRNAs in patients with depression. *PLoS One* 8:e63648. doi: 10.1371/journal.pone.0063648

Lin, S., and Gregory, R. I. (2015). microRNA biogenesis pathways in cancer. *Nat. Rev. Cancer* 15, 321–333. doi: 10.1038/nrc3932

Lin, J., Li, J., Huang, B., Liu, J., Chen, X., Chen, X. M., et al. (2015). Exosomes: novel biomarkers for clinical diagnosis. *ScientificWorldJournal* 2015:657086. doi: 10.1155/2015/657086

Liu, H., Kaur, J., Dashtipour, K., Kinyamu, R., Ribak, C. E., and Friedman, L. K. (2003). Suppression of hippocampal neurogenesis is associated with developmental stage, number of perinatal seizure episodes and glucocorticosteroid level. *Exp. Neurol.* 184, 196–213. doi: 10.1016/s0014-4886(03)00207-3

Lo Cicero, A., Schiera, G., Proia, P., Saladino, P., Savettieri, G., Di Liegro, C. M., et al. (2011). Oligodendroglioma cells shed microvesicles which contain TRAIL as well as molecular chaperones and induce cell death in astrocytes. *Int. J. Oncol.* 39, 1353–1357. doi: 10.3892/ijo.2011.1160

Lopez, J. P., Lim, R., Cruceanu, C., Crapper, L., Fasano, C., Labonte, B., et al. (2014). miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. *Nat. Med.* 20, 764–768. doi: 10.1038/nm.3582

Lopez-Verrilli, M. A., Picou, F., and Court, F. A. (2013). Schwann cell-derived exosomes enhance axonal regeneration in the peripheral nervous system. *Glia* 61, 1795–1806. doi: 10.1002/glia.22558

Maslanik, T., Mahaffey, L., Tannura, K., Beninson, L., Greenwood, B. N., and Fleshner, M. (2013). The inflammasome and danger associated molecular patterns (DAMPs) are implicated in cytokine and chemokine responses following stressor exposure. *Brain Behav. Immun.* 28, 54–62. doi: 10.1016/j.bbi.2012.10.014

Mateus-Pinheiro, A., Patricio, P., Bessa, J. M., Sousa, N., and Pinto, L. (2013a). Cell genesis and dendritic plasticity: a neuroplastic pas de deux in the onset and remission from depression. *Mol. Psychiatry* 18, 748–750. doi: 10.1038/mp.2013.56

Mateus-Pinheiro, A., Pinto, L., Bessa, J. M., Morais, M., Alves, N. D., Monteiro, S., et al. (2013b). Sustained remission from depressive-like behavior depends on hippocampal neurogenesis. *Transl. Psychiatry* 3:e210. doi: 10.1038/tp.2012.141

Mathivanan, S., Fahner, C. J., Reid, G. E., and Simpson, R. J. (2012). ExoCarta 2012: database of exosomal proteins, RNA and lipids. *Nucleic Acids Res.* 40, D1241–D1244. doi: 10.1093/nar/gkr828

Matsumura, T., Sugimachi, K., Iinuma, H., Takahashi, Y., Kurashige, J., Sawada, G., et al. (2015). Exosomal microRNA in serum is a novel biomarker of recurrence in human colorectal cancer. *Br. J. Cancer* 113, 275–281. doi: 10.1038/bjc.2015.201

Meerson, A., Cacheaux, L., Goosens, K. A., Sapolsky, R. M., Soreq, H., and Kaufer, D. (2010). Changes in brain microRNAs contribute to cholinergic stress reactions. *J. Mol. Neurosci.* 40, 47–55. doi: 10.1007/s12031-009-9252-1

Mendell, J. T., and Olson, E. N. (2012). microRNAs in stress signaling and human disease. *Cell* 148, 1172–1187. doi: 10.1016/j.cell.2012.02.005

Metcalfe, M. J., and Figueiredo-Pereira, M. E. (2010). Relationship between tau pathology and neuroinflammation in Alzheimer's disease. *Mt. Sinai J. Med.* 77, 50–58. doi: 10.1002/msj.20163

Miller, A. H., Maletic, V., and Raison, C. L. (2009). Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol. Psychiatry* 65, 732–741. doi: 10.1016/j.biopsych.2008.11.029

Mishra, P. J. (2014). microRNAs as promising biomarkers in cancer diagnostics. *Biomark. Res.* 2:19. doi: 10.1186/2050-7771-2-19

Mittelbrunn, M., Gutiérrez-Vázquez, C., Villarroya-Beltri, C., González, S., Sánchez-Cabo, F., González, M. A., et al. (2011). Unidirectional transfer of microRNA-loaded exosomes from T cells to antigen-presenting cells. *Nat. Commun.* 2:282. doi: 10.1038/ncomms1285

Mo, M. H., Chen, L., Fu, Y., Wang, W., and Fu, S. W. (2012). Cell-free circulating miRNA biomarkers in cancer. *J. Cancer* 3, 432–448. doi: 10.7150/jca.4919

Momen-Heravi, F., Balaj, L., Alian, S., Tigges, J., Toxavidis, V., Ericsson, M., et al. (2012). Alternative methods for characterization of extracellular vesicles. *Front. Physiol.* 3:354. doi: 10.3389/fphys.2012.00354

Mouillet-Richard, S., Baudry, A., Launay, J. M., and Kellermann, O. (2012). microRNAs and depression. *Neurobiol. Dis.* 46, 272–278. doi: 10.1016/j.nbd.2011.12.035

Müller, N., and Ackenheil, M. (1998). Psychoneuroimmunology and the cytokine action in the CNS: implications for psychiatric disorders. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 22, 1–33. doi: 10.1016/s0278-5846(97)00179-6

Munoz, J. L., Bliss, S. A., Greco, S. J., Ramkissoon, S. H., Ligon, K. L., and Rameshwar, P. (2013). Delivery of functional Anti-miR-9 by mesenchymal stem cell-derived exosomes to glioblastoma multiforme cells conferred chemosensitivity. *Mol. Ther. Nucleic Acids* 2:e126. doi: 10.1038/mtna.2013.60

Nave, K. A. (2010). Myelination and support of axonal integrity by glia. *Nature* 468, 244–252. doi: 10.1038/nature09614

Nimmerjahn, A., Kirchhoff, F., and Helmchen, F. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma *in vivo*. *Science* 308, 1314–1318. doi: 10.1126/science.1110647

Noto, C., Ota, V. K., Santoro, M. L., Gouvea, E. S., Silva, P. N., Spindola, L. M., et al. (2015). Depression, cytokine and cytokine by treatment interactions modulate gene expression in antipsychotic naive first episode psychosis. *Mol. Neurobiol.* doi: 10.1007/s12035-015-9489-3 [Epub ahead of print].

O'Brien, S. M., Scully, P., Fitzgerald, P., Scott, L. V., and Dinan, T. G. (2007). Plasma cytokine profiles in depressed patients who fail to respond to selective serotonin reuptake inhibitor therapy. *J. Psychiatr. Res.* 41, 326–331. doi: 10.1016/j.jpsychires.2006.05.013

O'Connor, R. M., Dinan, T. G., and Cryan, J. F. (2012). Little things on which happiness depends: microRNAs as novel therapeutic targets for the treatment of anxiety and depression. *Mol. Psychiatry* 17, 359–376. doi: 10.1038/mp.2011.162

O'Connor, J. C., Lawson, M. A., André, C., Moreau, M., Lestage, J., Castanon, N., et al. (2009). Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine 2,3-dioxygenase activation in mice. *Mol. Psychiatry* 14, 511–522. doi: 10.1038/sj.mp.4002148

Ohno, S., Takanashi, M., Sudo, K., Ueda, S., Ishikawa, A., Matsuyama, N., et al. (2013). Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. *Mol. Ther.* 21, 185–191. doi: 10.1038/mt.2012.180

Pallavi, P., Sagar, R., Mehta, M., Sharma, S., Subramanium, A., Shamshi, F., et al. (2013). Serum neurotrophic factors in adolescent depression: gender difference and correlation with clinical severity. *J. Affect. Disord.* 150, 415–423. doi: 10.1016/j.jad.2013.04.033

Pan, Y., Chen, X. Y., Zhang, Q. Y., and Kong, L. D. (2014). Microglial NLRP3 inflammasome activation mediates IL-1beta-related inflammation in prefrontal cortex of depressive rats. *Brain Behav. Immun.* 41, 90–100. doi: 10.1016/j.bbi.2014.04.007

Parachikova, A., Vasilevko, V., Cribbs, D. H., Laferla, F. M., and Green, K. N. (2010). Reductions in amyloid-beta-derived neuroinflammation, with minocycline, restore cognition but do not significantly affect tau hyperphosphorylation. *J. Alzheimers Dis.* 21, 527–542. doi: 10.3233/JAD-2010-100204

Paul-Savoie, E., Potvin, S., Daigle, K., Normand, E., Corbin, J. F., Gagnon, R., et al. (2011). A deficit in peripheral serotonin levels in major depressive disorder but not in chronic widespread pain. *Clin. J. Pain* 27, 529–534. doi: 10.1097/AJP.0b013e31820dfede

Peferoen, L., Kipp, M., Van Der Valk, P., Van Noort, J. M., and Amor, S. (2014). Oligodendrocyte-microglia cross-talk in the central nervous system. *Immunology* 141, 302–313. doi: 10.1111/imm.12163

Pegtel, D. M., Peferoen, L., and Amor, S. (2014). Extracellular vesicles as modulators of cell-to-cell communication in the healthy and diseased brain. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 369:20130516. doi: 10.1098/rstb.2013.0516

Phillips, A. C., Carroll, D., and Der, G. (2015). Negative life events and symptoms of depression and anxiety: stress causation and/or stress generation. *Anxiety Stress Coping* 28, 357–371. doi: 10.1080/10615806.2015.1005078

Pilc, A., Chaki, S., Nowak, G., and Witkin, J. M. (2008). Mood disorders: regulation by metabotropic glutamate receptors. *Biochem. Pharmacol.* 75, 997–1006. doi: 10.1016/j.bcp.2007.09.021

Potolicchio, I., Carven, G. J., Xu, X., Stipp, C., Riese, R. J., Stern, L. J., et al. (2005). Proteomic analysis of microglia-derived exosomes: metabolic role of the aminopeptidase CD13 in neuropeptide catabolism. *J. Immunol.* 175, 2237–2243. doi: 10.4049/jimmunol.175.4.2237

Prada, I., Furlan, R., Matteoli, M., and Verderio, C. (2013). Classical and unconventional pathways of vesicular release in microglia. *Glia* 61, 1003–1017. doi: 10.1002/glia.22497

Proia, P., Schiera, G., Mineo, M., Ingrassia, A. M., Santoro, G., Savettieri, G., et al. (2008). Astrocytes shed extracellular vesicles that contain fibroblast growth factor-2 and vascular endothelial growth factor. *Int. J. Mol. Med.* 21, 63–67. doi: 10.3892/ijmm.21.1.63

Pusic, A. D., and Kraig, R. P. (2015). Phasic treatment with interferon gamma stimulates release of exosomes that protect against spreading depression. *J. Interferon Cytokine Res.* 35, 795–807. doi: 10.1089/jir.2015.0010

Pusic, A. D., Pusic, K. M., Clayton, B. L., and Kraig, R. P. (2014). IFNgamma-stimulated dendritic cell exosomes as a potential therapeutic for remyelination. *J. Neuroimmunol.* 266, 12–23. doi: 10.1016/j.jneuroim.2013.10.014

Rahman, O. A., Sasvari-Szekely, M., Szekely, A., Faludi, G., Guttman, A., and Nemoda, Z. (2010). Analysis of a polymorphic microRNA target site in the purinergic receptor P2RX7 gene. *Electrophoresis* 31, 1790–1795. doi: 10.1002/elps.200900664

Rajasethupathy, P., Fiumara, F., Sheridan, R., Betel, D., Puthanveettil, S. V., Russo, J. J., et al. (2009). Characterization of small RNAs in Aplysia reveals a role for miR-124 in constraining synaptic plasticity through CREB. *Neuron* 63, 803–817. doi: 10.1016/j.neuron.2009.05.029

Rajendran, L., Bali, J., Barr, M. M., Court, F. A., Krämer-Albers, E. M., Picou, F., et al. (2014). Emerging roles of extracellular vesicles in the nervous system. *J. Neurosci.* 34, 15482–15489. doi: 10.1523/JNEUROSCI.3258-14.2014

Rajkowska, G., and Miguel-Hidalgo, J. J. (2007). Gliogenesis and glial pathology in depression. *CNS Neurol. Disord. Drug Targets* 6, 219–233. doi: 10.2174/187152707780619326

Rajkowska, G., Mahajan, G., Maciag, D., Sathyanesan, M., Iyo, A. H., Moulana, M., et al. (2015). Oligodendrocyte morphometry and expression of myelin - Related mRNA in ventral prefrontal white matter in major depressive disorder. *J. Psychiatr. Res.* 65, 53–62. doi: 10.1016/j.jpsychires.2015.04.010

Raposo, G., and Stoorvogel, W. (2013). Extracellular vesicles: exosomes, microvesicles and friends. *J. Cell Biol.* 200, 373–383. doi: 10.1083/jcb.201211138

Ratajczak, M. Z., Kucia, M., Jadczyk, T., Greco, N. J., Wojakowski, W., Tendera, M., et al. (2012). Pivotal role of paracrine effects in stem cell therapies in

regenerative medicine: can we translate stem cell-secreted paracrine factors and microvesicles into better therapeutic strategies? *Leukemia* 26, 1166–1173. doi: 10.1038/leu.2011.389

Réus, G. Z., Fries, G. R., Stertz, L., Badawy, M., Passos, I. C., Barichello, T., et al. (2015). The role of inflammation and microglial activation in the pathophysiology of psychiatric disorders. *Neuroscience* 300, 141–154. doi: 10.1016/j.neuroscience.2015.05.018

Rinaldi, A., Vincenti, S., De Vito, F., Bozzoni, I., Oliverio, A., Presutti, C., et al. (2010). Stress induces region specific alterations in microRNAs expression in mice. *Behav. Brain Res.* 208, 265–269. doi: 10.1016/j.bbr.2009.11.012

Roman, A., Kreiner, G., and Nalepa, I. (2013). Macrophages and depression - a misalliance or well-arranged marriage? *Pharmacol. Rep.* 65, 1663–1672. doi: 10.1016/s1734-1140(13)71528-7

Rota, R., Ciarapica, R., Giordano, A., Miele, L., and Locatelli, F. (2011). microRNAs in rhabdomyosarcoma: pathogenetic implications and translational potentiality. *Mol. Cancer* 10:120. doi: 10.1186/1476-4598-10-120

Rotheneichner, P., Lange, S., O'sullivan, A., Marschallinger, J., Zaunmair, P., Geretsegger, C., et al. (2014). Hippocampal neurogenesis and antidepressive therapy: shocking relations. *Neural Plast.* 2014:723915. doi: 10.1155/2014/723915

Rucker, J. J., and McGuffin, P. (2014). Chipping away at major depressive disorder. *Genome Biol.* 15:421. doi: 10.1186/s13059-014-0421-3

Russo, I., Bubacco, L., and Greggio, E. (2012). Exosomes-associated neurodegeneration and progression of Parkinson's disease. *Am. J. Neurodegener. Dis.* 1, 217–225.

Sadallah, S., Eken, C., and Schifferli, J. A. (2011). Ectosomes as modulators of inflammation and immunity. *Clin. Exp. Immunol.* 163, 26–32. doi: 10.1111/j.1365-2249.2010.04271.x

Salgado, A. J., Sousa, J. C., Costa, B. M., Pires, A. O., Mateus-Pinheiro, A., Teixeira, F. G., et al. (2015). Mesenchymal stem cells secretome as a modulator of the neurogenic niche: basic insights and therapeutic opportunities. *Front. Cell. Neurosci.* 9:249. doi: 10.3389/fncel.2015.00249

Saman, S., Lee, N. C., Inoyo, I., Jin, J., Li, Z., Doyle, T., et al. (2014). Proteins recruited to exosomes by tau overexpression implicate novel cellular mechanisms linking tau secretion with Alzheimer's disease. *J. Alzheimers Dis.* 40(Suppl. 1), S47–S70. doi: 10.3233/JAD-132135

Saus, E., Soria, V., Escaramis, G., Vivarelli, F., Crespo, J. M., Kagerbauer, B., et al. (2010). Genetic variants and abnormal processing of pre-miR-182, a circadian clock modulator, in major depression patients with late insomnia. *Hum. Mol. Genet.* 19, 4017–4025. doi: 10.1093/hmg/ddq316

Sbai, O., Ould-Yahoui, A., Ferhat, L., Gueye, Y., Bernard, A., Charrat, E., et al. (2010). Differential vesicular distribution and trafficking of MMP-2, MMP-9 and their inhibitors in astrocytes. *Glia* 58, 344–366. doi: 10.1002/glia.20927

Schiepers, O. J., Wichers, M. C., and Maes, M. (2005). Cytokines and major depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 29, 201–217. doi: 10.1016/j.pnpbp.2004.11.003

Schneider, A., and Simons, M. (2013). Exosomes: vesicular carriers for intercellular communication in neurodegenerative disorders. *Cell Tissue Res.* 352, 33–47. doi: 10.1007/s00441-012-1428-2

Schnieder, T. P., Trencevska, I., Rosoklija, G., Stankov, A., Mann, J. J., Smiley, J., et al. (2014). Microglia of prefrontal white matter in suicide. *J. Neuropathol. Exp. Neurol.* 73, 880–890. doi: 10.1097/NEN.0000000000000107

Schratt, G. M., Tuebing, F., Nigh, E. A., Kane, C. G., Sabatini, M. E., Kiebler, M., et al. (2006). A brain-specific microRNA regulates dendritic spine development. *Nature* 439, 283–289. doi: 10.1038/nature04367

Schwamborn, J. C., Berezikov, E., and Knoblich, J. A. (2009). The TRIM-NHL protein TRIM32 activates microRNAs and prevents self-renewal in mouse neural progenitors. *Cell* 136, 913–925. doi: 10.1016/j.cell.2008.12.024

Schwarzenbach, H. (2015). The clinical relevance of circulating, exosomal miRNAs as biomarkers for cancer. *Expert Rev. Mol. Diagn.* 15, 1159–1169. doi: 10.1586/14737159.2015.1069183

Serafini, G., Pompili, M., Hansen, K. F., Obrietan, K., Dwivedi, Y., Shomron, N., et al. (2014). The involvement of microRNAs in major depression, suicidal behavior and related disorders: a focus on miR-185 and miR-491–3p. *Cell. Mol. Neurobiol.* 34, 17–30. doi: 10.1007/s10571-013-9997-5

Serafini, G., Pompili, M., Innamorati, M., Giordano, G., Montebovi, F., Sher, L., et al. (2012). The role of microRNAs in synaptic plasticity, major affective disorders and suicidal behavior. *Neurosci. Res.* 73, 179–190. doi: 10.1016/j.neures.2012.04.001

Serafini, G., Rihmer, Z., and Amore, M. (2015). The role of glutamate excitotoxicity and neuroinflammation in depression and suicidal behavior: focus on microglia cells. *Neuroimmunol Neuroinflamm.* 2, 127–130. doi: 10.4103/2347-8659.157955

Shelton, R. C., Claiborne, J., Sidoryk-Wegrzynowicz, M., Reddy, R., Aschner, M., Lewis, D. A., et al. (2011). Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Mol. Psychiatry* 16, 751–762. doi: 10.1038/mp.2010.52

Shifrin, D. A. Jr., Demory Beckler, M., Coffey, R. J., and Tyska, M. J. (2013). Extracellular vesicles: communication, coercion and conditioning. *Mol. Biol. Cell* 24, 1253–1259. doi: 10.1091/mbc.E12-08-0572

Shtam, T. A., Kovalev, R. A., Varfolomeeva, E. Y., Makarov, E. M., Kil, Y. V., and Filatov, M. V. (2013). Exosomes are natural carriers of exogenous siRNA to human cells *in vitro*. *Cell Commun. Signal.* 11:88. doi: 10.1186/1478-811X-11-88

Sierra, A., Abiega, O., Shahraz, A., and Neumann, H. (2013). Janus-faced microglia: beneficial and detrimental consequences of microglial phagocytosis. *Front. Cell. Neurosci.* 7:6. doi: 10.3389/fncel.2013.00006

Smalheiser, N. R., Lugli, G., Rizavi, H. S., Torvik, V. I., Turecki, G., and Dwivedi, Y. (2012). microRNA expression is down-regulated and reorganized in prefrontal cortex of depressed suicide subjects. *PLoS One* 7:e33201. doi: 10.1371/journal.pone.0033201

Smirnova, L., Gräfe, A., Seiler, A., Schumacher, S., Nitsch, R., and Wulczyn, F. G. (2005). Regulation of miRNA expression during neural cell specification. *Eur. J. Neurosci.* 21, 1469–1477. doi: 10.1111/j.1460-9568.2005.03978.x

Song, C., Halbreich, U., Han, C., Leonard, B. E., and Luo, H. (2009). Imbalance between pro- and anti-inflammatory cytokines and between Th1 and Th2 cytokines in depressed patients: the effect of electroacupuncture or fluoxetine treatment. *Pharmacopsychiatry* 42, 182–188. doi: 10.1055/s-0029-1202263

Song, C., and Wang, H. (2011). Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 760–768. doi: 10.1016/j.pnpbp.2010.06.020

Sorrells, S. F., and Sapolsky, R. M. (2007). An inflammatory review of glucocorticoid actions in the CNS. *Brain Behav. Immun.* 21, 259–272. doi: 10.1016/j.bbi.2006.11.006

Sperlágh, B., Vizi, E. S., Wirkner, K., and Illes, P. (2006). P2X7 receptors in the nervous system. *Prog. Neurobiol.* 78, 327–346. doi: 10.1016/j.pneurobio.2006.03.007

Steiner, J., Bielau, H., Brisch, R., Danos, P., Ullrich, O., Mawrin, C., et al. (2008). Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J. Psychiatr. Res.* 42, 151–157. doi: 10.1016/j.jpsychires.2006.10.013

Stockmeier, C. A., and Rajkowska, G. (2004). Cellular abnormalities in depression: evidence from postmortem brain tissue. *Dialogues Clin. Neurosci.* 6, 185–197. doi: 10.1111/j.1582-4934.2002.tb00522.x

Stokes, L., Spencer, S. J., and Jenkins, T. A. (2015). Understanding the role of P2X7 in affective disorders-are glial cells the major players? *Front. Cell. Neurosci.* 9:258. doi: 10.3389/fncel.2015.00258

Strawbridge, R., Arnone, D., Danese, A., Papadopoulos, A., Herane Vives, A., and Cleare, A. J. (2015). Inflammation and clinical response to treatment in depression: a meta-analysis. *Eur. Neuropsychopharmacol.* 25, 1532–1543. doi: 10.1016/j.euroneuro.2015.06.007

Sun, Y., Luo, Z. M., Guo, X. M., Su, D. F., and Liu, X. (2015). An updated role of microRNA-124 in central nervous system disorders: a review. *Front. Cell. Neurosci.* 9:193. doi: 10.3389/fncel.2015.00193

Szulwach, K. E., Li, X., Smrt, R. D., Li, Y., Luo, Y., Lin, L., et al. (2010). Cross talk between microRNA and epigenetic regulation in adult neurogenesis. *J. Cell Biol.* 189, 127–141. doi: 10.1083/jcb.200908151

Takeuchi, T., Suzuki, M., Fujikake, N., Popiel, H. A., Kikuchi, H., Futaki, S., et al. (2015). Intercellular chaperone transmission via exosomes contributes to maintenance of protein homeostasis at the organismal level. *Proc. Natl. Acad. Sci. U S A* 112, E2497–E2506. doi: 10.1073/pnas.1412651112

Tanaka, Y., Kamohara, H., Kinoshita, K., Kurashige, J., Ishimoto, T., Iwatsuki, M., et al. (2013). Clinical impact of serum exosomal microRNA-21 as a clinical biomarker in human esophageal squamous cell carcinoma. *Cancer* 119, 1159–1167. doi: 10.1002/cncr.27895

Taylor, A. R., Robinson, M. B., Gifondorwa, D. J., Tytell, M., and Milligan, C. E. (2007). Regulation of heat shock protein 70 release in astrocytes: role of signaling kinases. *Dev. Neurobiol.* 67, 1815–1829. doi: 10.1002/dneu.20559

Terasawa, K., Ichimura, A., Sato, F., Shimizu, K., and Tsujimoto, G. (2009). Sustained activation of ERK1/2 by NGF induces microRNA-221 and 222 in PC12 cells. *FEBS J.* 276, 3269–3276. doi: 10.1111/j.1742-4658.2009.07041.x

Théry, C., Amigorena, S., Raposo, G., and Clayton, A. (2006). Isolation and characterization of exosomes from cell culture supernatants and biological fluids. *Curr. Protoc. Cell Biol.* 3:3.22. doi: 10.1002/0471143030.cb0322s30

Théry, C., Zitvogel, L., and Amigorena, S. (2002). Exosomes: composition, biogenesis and function. *Nat. Rev. Immunol.* 2, 569–579. doi: 10.1038/nri855

Tomlinson, P. R., Zheng, Y., Fischer, R., Heidasch, R., Gardiner, C., Evetts, S., et al. (2015). Identification of distinct circulating exosomes in Parkinson's disease. *Ann. Clin. Transl. Neurol.* 2, 353–361. doi: 10.1002/acn3.175

Torres-Platas, S. G., Cruceanu, C., Chen, G. G., Turecki, G., and Mechawar, N. (2014). Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. *Brain Behav. Immun.* 42, 50–59. doi: 10.1016/j.bbi.2014.05.007

Trajkovic, K., Hsu, C., Chiantia, S., Rajendran, L., Wenzel, D., Wieland, F., et al. (2008). Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* 319, 1244–1247. doi: 10.1126/science.1153124

Turchinovich, A., Samatov, T. R., Tonevitsky, A. G., and Burwinkel, B. (2013). Circulating miRNAs: cell-cell communication function? *Front. Genet.* 4:119. doi: 10.3389/fgene.2013.00119

Turola, E., Furlan, R., Bianco, F., Matteoli, M., and Verderio, C. (2012). Microglial microvesicle secretion and intercellular signaling. *Front. Physiol.* 3:149. doi: 10.3389/fphys.2012.00149

Tynan, R. J., Beynon, S. B., Hinwood, M., Johnson, S. J., Nilsson, M., Woods, J. J., et al. (2013). Chronic stress-induced disruption of the astrocyte network is driven by structural atrophy and not loss of astrocytes. *Acta Neuropathol.* 126, 75–91. doi: 10.1007/s00401-013-1102-0

Uchida, S., Hara, K., Kobayashi, A., Funato, H., Hobara, T., Otsuki, K., et al. (2010). Early life stress enhances behavioral vulnerability to stress through the activation of REST4-mediated gene transcription in the medial prefrontal cortex of rodents. *J. Neurosci.* 30, 15007–15018. doi: 10.1523/JNEUROSCI.1436-10.2010

Valadi, H., Ekström, K., Bossios, A., Sjöstrand, M., Lee, J. J., and Lötvall, J. O. (2007). Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* 9, 654–659. doi: 10.1038/ncb1596

Van Buel, E. M., Patas, K., Peters, M., Bosker, F. J., Eisel, U. L., and Klein, H. C. (2015). Immune and neurotrophin stimulation by electroconvulsive therapy: is some inflammation needed after all? *Transl. Psychiatry* 5:e609. doi: 10.1038/tp.2015.100

Vaváková, M., Ďuračková, Z., and Trebatická, J. (2015). Markers of oxidative stress and neuroprogression in depression disorder. *Oxid. Med. Cell. Longev.* 2015:898393. doi: 10.1155/2015/898393

Verderio, C., Cagnoli, C., Bergami, M., Francolini, M., Schenk, U., Colombo, A., et al. (2012a). TI-VAMP/VAMP7 is the SNARE of secretory lysosomes contributing to ATP secretion from astrocytes. *Biol. Cell* 104, 213–228. doi: 10.1111/boc.201100070

Verderio, C., Muzio, L., Turola, E., Bergami, A., Novellino, L., Ruffini, F., et al. (2012b). Myeloid microvesicles are a marker and therapeutic target for neuroinflammation. *Ann. Neurol.* 72, 610–624. doi: 10.1002/ana.23627

Vingtdeux, V., Sergeant, N., and Buée, L. (2012). Potential contribution of exosomes to the prion-like propagation of lesions in Alzheimer's disease. *Front. Physiol.* 3:229. doi: 10.3389/fphys.2012.00229

Wahlgren, J., De L Karlson, T., Brisslert, M., Vaziri Sani, F., Telemo, E., Sunnerhagen, P., et al. (2012). Plasma exosomes can deliver exogenous short interfering RNA to monocytes and lymphocytes. *Nucleic Acids Res.* 40:e130. doi: 10.1093/nar/gks463

Walker, F. R., Nilsson, M., and Jones, K. (2013). Acute and chronic stress-induced disturbances of microglial plasticity, phenotype and function. *Curr. Drug Targets* 14, 1262–1276. doi: 10.2174/13894501131499902O8

Wang, S., Cesca, F., Loers, G., Schweizer, M., Buck, F., Benfenati, F., et al. (2011). Synapsin I is an oligomannose-carrying glycoprotein, acts as an oligomannose-binding lectin and promotes neurite outgrowth and neuronal survival when released via glia-derived exosomes. *J. Neurosci.* 31, 7275–7290. doi: 10.1523/JNEUROSCI.6476-10.2011

Wang, G., Dinkins, M., He, Q., Zhu, G., Poirier, C., Campbell, A., et al. (2012). Astrocytes secrete exosomes enriched with proapoptotic ceramide and prostate apoptosis response 4 (PAR-4): potential mechanism of apoptosis induction in Alzheimer disease (AD). *J. Biol. Chem.* 287, 21384–21395. doi: 10.1074/jbc.M112.340513

Wang, X., Sundquist, K., Hedelius, A., Palmér, K., Memon, A. A., and Sundquist, J. (2015). Circulating microRNA-144–5p is associated with depressive disorders. *Clin. Epigenetics* 7:69. doi: 10.1186/s13148-015-0099-8

Wan, Y., Liu, Y., Wang, X., Wu, J., Liu, K., Zhou, J., et al. (2015). Identification of differential microRNAs in cerebrospinal fluid and serum of patients with major depressive disorder. *PLoS One* 10:e0121975. doi: 10.1371/journal.pone.0121975

Warnecke-Eberz, U., Chon, S. H., Hölscher, A. H., Drebber, U., and Bollschweiler, E. (2015). Exosomal onco-miRs from serum of patients with adenocarcinoma of the esophagus: comparison of miRNA profiles of exosomes and matching tumor. *Tumour Biol.* 36, 4643–4653. doi: 10.1007/s13277-015-3112-0

Weber, C. (2013). microRNAs: from basic mechanisms to clinical application in cardiovascular medicine. *Arterioscler. Thromb. Vasc. Biol.* 33, 168–169. doi: 10.1161/ATVBAHA.112.300920

Weber, M. D., Frank, M. G., Tracey, K. J., Watkins, L. R., and Maier, S. F. (2015). Stress induces the danger-associated molecular pattern HMGB-1 in the hippocampus of male Sprague Dawley rats: a priming stimulus of microglia and the NLRP3 inflammasome. *J. Neurosci.* 35, 316–324. doi: 10.1523/JNEUROSCI.3561-14.2015

Weekman, E. M., Sudduth, T. L., Abner, E. L., Popa, G. J., Mendenhall, M. D., Brothers, H. M., et al. (2014). Transition from an M1 to a mixed neuroinflammatory phenotype increases amyloid deposition in APP/PS1 transgenic mice. *J. Neuroinflammation* 11:127. doi: 10.1186/1742-2094-11-127

Wes, P. D., Easton, A., Corradi, J., Barten, D. M., Devidze, N., DeCarr, L. B., et al. (2014). Tau overexpression impacts a neuroinflammation gene expression network perturbed in Alzheimer's disease. *PLoS One* 9:e106050. doi: 10.1371/journal.pone.0106050

World Health and Organization. (2012). *Media Centre Fact Sheet No. 369*. Available online at: http://www.who.int/mediacentre/factsheets/fs369/en/

Wu, T. Y., Liu, L., Zhang, W., Zhang, Y., Liu, Y. Z., Shen, X. L., et al. (2015). High-mobility group box-1 was released actively and involved in LPS induced depressive-like behavior. *J. Psychiatr. Res.* 64, 99–106. doi: 10.1016/j.jpsychires.2015.02.016

Xanthos, D. N., and Sandkuhler, J. (2014). Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity. *Nat. Rev. Neurosci.* 15, 43–53. doi: 10.1038/nrn3617

Xu, D., and Tahara, H. (2013). The role of exosomes and microRNAs in senescence and aging. *Adv. Drug Deliv. Rev.* 65, 368–375. doi: 10.1016/j.addr.2012.07.010

Xu, S., Witmer, P. D., Lumayag, S., Kovacs, B., and Valle, D. (2007). microRNA (miRNA) transcriptome of mouse retina and identification of a sensory organ-specific miRNA cluster. *J. Biol. Chem.* 282, 25053–25066. doi: 10.1074/jbc.m700501200

Yan, H., Fang, M., and Liu, X. Y. (2013). Role of microRNAs in stroke and poststroke depression. *ScientificWorldJournal* 2013:459692. doi: 10.1155/2013/459692

Yang, M., Chen, J., Su, F., Yu, B., Lin, L., Liu, Y., et al. (2011). Microvesicles secreted by macrophages shuttle invasion-potentiating microRNAs into breast cancer cells. *Mol. Cancer* 10:117. doi: 10.1186/1476-4598-10-117

Yang, P., Gao, Z., Zhang, H., Fang, Z., Wu, C., Xu, H., et al. (2015). Changes in proinflammatory cytokines and white matter in chronically stressed rats. *Neuropsychiatr. Dis. Treat.* 11, 597–607. doi: 10.2147/NDT.s78131

Yirmiya, R., Rimmerman, N., and Reshef, R. (2015). Depression as a microglial disease. *Trends Neurosci.* 38, 637–658. doi: 10.1016/j.tins.2015.08.001

Younger, S. T., and Corey, D. R. (2011). Transcriptional gene silencing in mammalian cells by miRNA mimics that target gene promoters. *Nucleic Acids Res.* 39, 5682–5691. doi: 10.1093/nar/gkr155

Yuyama, K., Sun, H., Mitsutake, S., and Igarashi, Y. (2012). Sphingolipid-modulated exosome secretion promotes clearance of amyloid-beta by microglia. *J. Biol. Chem.* 287, 10977–10989. doi: 10.1074/jbc.M111.324616

Yuyama, K., Sun, H., Sakai, S., Mitsutake, S., Okada, M., Tahara, H., et al. (2014). Decreased amyloid-beta pathologies by intracerebral loading of glycosphingolipid-enriched exosomes in Alzheimer model mice. *J. Biol. Chem.* 289, 24488–24498. doi: 10.1074/jbc.M114.577213

Zhan, X., Jickling, G. C., Ander, B. P., Liu, D., Stamova, B., Cox, C., et al. (2014). Myelin injury and degraded myelin vesicles in Alzheimer's disease. *Curr. Alzheimer Res.* 11, 232–238. doi: 10.2174/1567205011666140131120922

Zheng, L. S., Kaneko, N., and Sawamoto, K. (2015). Minocycline treatment ameliorates interferon-alpha- induced neurogenic defects and depression-like behaviors in mice. *Front. Cell. Neurosci.* 9:5. doi: 10.3389/fncel.2015.00005

Zhang, J., Li, S., Li, L., Li, M., Guo, C., Yao, J., et al. (2015). Exosome and exosomal microRNA: trafficking, sorting and function. *Genomics Proteomics Bioinformatics* 13, 17–24. doi: 10.1016/j.gpb.2015.02.001

Zhou, R., Yuan, P., Wang, Y., Hunsberger, J. G., Elkahloun, A., Wei, Y., et al. (2009). Evidence for selective microRNAs and their effectors as common long-term targets for the actions of mood stabilizers. *Neuropsychopharmacology* 34, 1395–1405. doi: 10.1038/npp.2008.131

Zhuang, X., Xiang, X., Grizzle, W., Sun, D., Zhang, S., Axtell, R. C., et al. (2011). Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. *Mol. Ther.* 19, 1769–1779. doi: 10.1038/mt.2011.164

Zilka, N., Zilkova, M., Kazmerova, Z., Sarissky, M., Cigankova, V., and Novak, M. (2011). Mesenchymal stem cells rescue the Alzheimer's disease cell model from cell death induced by misfolded truncated tau. *Neuroscience* 193, 330–337. doi: 10.1016/j.neuroscience.2011.06.088

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Brites and Fernandes. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution and reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Cellular Neuroscience

ORIGINAL RESEARCH
published: 02 February 2016
doi: 10.3389/fncel.2016.00008

Fluoxetine Requires the Endfeet Protein Aquaporin-4 to Enhance Plasticity of Astrocyte Processes

Barbara Di Benedetto[1], Victoria A. Malik[1], Salina Begum[1], Lena Jablonowski[1], Gabriela B. Gómez-González[1], Inga D. Neumann[2] and Rainer Rupprecht[1]*

[1] Laboratory of Neuro-Glia Pharmacology, Department of Psychiatry and Psychotherapy, University of Regensburg, Regensburg, Germany, [2]Department of Neurobiology, University of Regensburg, Regensburg, Germany

Morphological alterations in astrocytes are characteristic for post mortem brains of patients affected by major depressive disorder (MDD). Recently, a significant reduction in the coverage of blood vessels (BVs) by aquaporin-4 (AQP-4)-positive astrocyte endfeet has been shown in the prefrontal cortex (PFC) of MDD patients, suggesting that either alterations in the morphology of endfeet or in AQP-4 distribution might be responsible for the disease phenotype or constitute a consequence of its progress. Antidepressant drugs (ADs) regulate the expression of several proteins, including astrocyte-specific ones. Thus, they may target AQP-4 to induce morphological changes in astrocytes and restore their proper shape or relocate AQP-4 to endfeet. Using an animal model of depression, rats selectively bred for high anxiety-like behavior (HAB), we confirmed a reduced coverage of BVs in the adult PFC by AQP-4-immunoreactive (AQP-4-IR) astrocyte processes with respect to non-selected Wistar rats (NAB), thereby validating it for our study. A further evaluation of the morphology of astrocyte in brain slices (*ex vivo*) and *in vitro* using an antibody against the astrocyte-specific cytoskeletal protein glial fibrillary acidic protein (GFAP) revealed that HAB astrocytes extended less processes than NAB cells. Furthermore, short-term drug treatment *in vitro* with the AD fluoxetine (FLX) was sufficient to increase the plasticity of astrocyte processes, enhancing their number in NAB-derived cells and recovering their basal number in HAB-derived cells. This enhanced FLX-dependent plasticity occurred, however, only in the presence of intact AQP-4, as demonstrated by the lack of effect after the downregulation of AQP-4 with RNAi in both NAB and HAB cells. Nonetheless, a similar short-term treatment did neither modulate the coverage of BVs with AQP-4-positive astrocyte endfeet in NAB nor in HAB rats, although dosage and time of treatment were sufficient to fully recover GFAP expression in HAB brains. Thus, we suggest that longer treatment regimes may be needed to properly restore the coverage of BVs or to relocate AQP-4 to astrocyte endfeet. In conclusion, FLX requires AQP-4 to modulate the plasticity of astrocyte processes and this effect might be essential to re-establish a functional glia-vasculature interface necessary for a physiological communication between bloodstream and brain parenchyma.

Keywords: fluoxetine, astrocytes, glia-vasculature interface, plasticity, aquaporin-4

OPEN ACCESS

Edited by:
João Filipe Oliveira, University of Minho, Portugal

Reviewed by:
Andrew MacLean, Tulane University School of Medicine, USA
Gael Quesseveur, McGill University, Canada

****Correspondence:***
Barbara Di Benedetto
Barbara.Di-Benedetto@ukr.de

Received: *31 October 2015*
Accepted: *11 January 2016*
Published: *02 February 2016*

Citation:
Di Benedetto B, Malik VA, Begum S, Jablonowski L, Gómez-González GB, Neumann ID and Rupprecht R (2016) Fluoxetine Requires the Endfeet Protein Aquaporin-4 to Enhance Plasticity of Astrocyte Processes. Front. Cell. Neurosci. 10:8. doi: 10.3389/fncel.2016.00008

INTRODUCTION

Morphometric examinations of prefrontal cortical (PFC) regions in post mortem brains of patients with major depressive disorder (MDD) revealed alterations in the density of astrocytes, in addition to neurons (Rajkowska et al., 1999; Rajkowska and Stockmeier, 2013). Moreover, studies in animal models of stress-induced depressive-like behaviors showed an additional astrocyte pathology, with morphological differences affecting specifically this cell type (Czeh et al., 2006). Astrocytes extend processes that wrap around synapses and blood vessels (BVs), thereby regulating the functionality of neuronal circuits and of the blood-brain barrier (BBB; Koehler et al., 2009; Parpura et al., 2012). Recently, a cerebrovascular pathology has been demonstrated in older subjects suffering of MDD and patients who suffered a primary cerebrovascular disease additionally showed symptoms of mood disorders (Miguel-Hidalgo et al., 2013), thus suggesting a strong causal link between a vascular pathology and MDD. Indeed, a recent study revealed a reduced coverage of BVs by astrocytic endfeet positive for aquaporin-4 (AQP-4) in the PFC of post mortem brains from MDD patients (Rajkowska et al., 2013). AQP-4 is a plasma membrane water-transporting protein which is specifically localized to the endfeet of astrocyte processes, thereby regulating several functional properties of such processes. Its regulation of water permeability is particularly important for the modulation of astrocyte plasticity, especially for the extension and migration of astrocyte processes during neuronal activity or activity around BVs (Papadopoulos and Verkman, 2013) and to maintain the integrity of the BBB (Zhou et al., 2008). Because of their localization at the BBB, astrocytes may additionally regulate the transport of therapeutic drugs in/out of the brain through their polarized endfeet which interact with BVs (Pardridge, 1999) and mutations in proteins located to the endfeet can predict a positive or negative response to antidepressants (ADs; Uhr et al., 2008). Interestingly, a recent study has shown how ablation of the mouse gene coding for AQP-4 could disrupt responses to fluoxetine (FLX) in a chronic stress model of depressive-like behavior (Kong et al., 2009). Furthermore, aqp-4 knockout mice present cognitive deficits similar to those implicated in mood disorders (Skucas et al., 2011) and exhibit an exacerbated depressive-like behavioral response after corticosterone treatment, accompanied by impaired astrocytic functions (Kong et al., 2014).

An essential mean to understand the neurobiological underpinnings of brain pathologies is the use of animal models which reproduce the main features of human diseases and several animal models exist, which have been validated to study MDD. Among them, rats selectively bred for high anxiety-related behavior (HAB) have shown to be an appropriate tool (with face, construct and predictive validity) to study molecular risk factors which may predispose to develop depressive-like behavior (Wegener et al., 2012). Although they have been extensively characterized at behavioral and pharmacological levels, it is still unknown whether they show an astrocyte pathology such as the one described in MDD patients. Therefore, the first aim of our study was to determine if HAB rats are a proper animal model to analyze astrocyte alterations in MDD and the role of AQP-4 in depressive-like behavior. Then, we followed the hypothesis that AQP-4 might be either reduced or mislocalized in a depressive-like pathology, thus being absent from endfeet and unable to fulfill its functional role(s) around BVs. Moreover, as ADs target astrocytes (Czeh and Di Benedetto, 2013) and this targeting might be relevant to treat neuropsychiatric disorders (Di Benedetto and Rupprecht, 2013), we examined whether FLX may require AQP-4 to mediate its pharmacological modulatory effects on morphological changes in astrocytes.

MATERIALS AND METHODS

Drugs

Fluoxetine was purchased from Sigma (Taufkirchen, Germany) and was dissolved in water for *in vitro* experiments. For *ex vivo* experiments, FLX was dissolved in phosphate buffer saline (PBS, saline).

Animals and Drug Treatment

Adult male rats (10–12 weeks old, 280–350 g) selectively bred for HAB on Wistar background and weight-matched non-selectively bred Wistar rats (NAB) were used in this study. Breeding of HAB and NAB animals occured at the animal facilities of the University of Regensburg. Animals were housed under standard laboratory conditions in groups of four [12 hours (hrs) light: dark cycle, 22–24°C, lights on at 06:00 am, food and water *ad libitum*]. For drug treatment, FLX was dissolved on the day of the injections. Rats received an i.p. injection of either 0 (saline) or 10 mg/kg FLX twice a day for 2 days. On day 3, the animals were anesthetized with CO2 and perfused transcardially with PBS, followed by 4% paraformaldehyde (PFA, Sigma) in PBS. Brains were removed and post-fixed O/N at 4°C, cryoprotected in 25% sucrose in PBS and cut coronally at 40 μm on a cryostat. Sections were preserved in a solution with 25% ethylene glycol, 25% glycerol in PBS at −20°C until further processed for IF–IHC. Animal experiments were approved by the government of the Oberpfalz, Germany, and performed in accordance with the Guide for the Care and Use of Laboratory Animals by the National Institutes of Health, Bethesda, MD, USA.

Cell Culture

Primary astrocytes (NAB and HAB) were isolated from cerebral cortices of postnatal day 1 (P1) rat brains. In brief, brains were isolated and cortices were dissected, cut in small pieces and digested with 0.25% trypsin containing 1 mM EDTA for 20 min (min) at 37°C with gentle shaking. The remaining tissue pieces were triturated with a fire-polished Pasteur-pipette to yield dissociated cells. Cells were centrifuged at 90 × g for 5 min, resuspended in DMEM supplemented with 10% FCS, 2 mM GlutaMAXI, penicillin (100 units/ml), streptomycin (100 μg/ml) and 0.1 mM MEM Non Essential Amino Acids (Invitrogen, Darmstadt, Germany) and seeded on poly-d-lysine-coated 75 cm^2 flasks (Bressler et al., 1980; Allen et al., 2001). When astrocyte cultures reached 80–90% confluency, flasks were shaked to detach non-astrocytic cells, trypsinized with 0.25%

trypsin containing 1 mM EDTA and seeded on 24-well plates with glass coverslips at a density of about 1×10^5 cells/well.

RNA Interference (RNAi), Cell Transfection and Drug Treatment

Short interfering RNAs (siRNAs) complementary to the rat mRNA sequence coding for AQP4 were synthesized (Dharmacon Research, Lafayette, CO). Two different siRNAs (si2, 5′-CGGACUGAUGUUACUGGUUUU-3′ and si3, 5′-UCAAUUAUACCGGAGCCAGUU-3′) were selected, which were previously published to be effective in primary rat astrocytes (Nicchia et al., 2005), together with a scrambled siRNA (scr, 5′- CCUAAGGUUAAGUCGCCCUUU-3′), which was used as negative control. All sequences were submitted to a BLAST search to verify their specific targeting of Aqp-4 mRNA (si2 and si3) and the lack of targeting of any sequence for scrRNA. Transfections were performed using Lipofectamine™ 2000 (Invitrogen), following manufacturer's instructions. In brief, 24 h before transfection, medium was changed to medium without antibiotics. Afterwards, the appropriate amount of each siRNA to reach a final working concentration of 50 nM was diluted in Opti-MEM I Reduced Serum Medium (Invitrogen), incubated at RT and then mixed with Opti-MEM I containing the appropriate amount of Lipofectamine. After a 20 min incubation time, this mixture was distributed on cells. Cells were then growing for three additional days, before changing the medium to medium without FCS 24 h before drug treatment. FLX was administrated at a final concentration of 10 μM for 48 h. Afterwards, cells were washed with PBS, fixed in PFA 4% for 20 min at room temperature (RT) and then washed again with PBS and maintained at 4°C until further processed for immunofluorescent-immunocytochemistry (IF-ICC).

Immunofluorescent-Immunohistochemistry and Immunocytochemistry (IF-IHC and IF-ICC)

For IF-IHC in rat brains: sections from the prefrontal cortex (PFC) were selected and washed thoroughly, before permeabilization and blocking for 2 h in 0.5% Triton-X 100 + 2% Normal Goat Serum (NGS, Vector Labs, Biozol, Eching, Germany) in PBS. Afterwards, they were incubated with rabbit anti-AQP4 (1:200, Abcam, Cambridge, UK) together with mouse anti-collagen IV (1:500, Sigma) antibodies or with mouse anti-GFAP antibody (1:400, Sigma) in 0.5% TX 100 + 2% NGS in PBS O/N at 4°C. Sections were then incubated with the respective secondary antibodies, the biotinylated anti-rabbit IgG antibody (1:300, Jackson ImmunoResearch, Hamburg, Germany) with anti-mouse-Cy3 (1:250, Jackson ImmunoResearch) in 1% NGS in PBS for 2 h at RT. Afterwards, sections were washed and further incubated with avidin anti-biotin Alexa Fluor 488 (1:1000, Invitrogen) together with DAPI (1:1000, Sigma) in 1% NGS in PBS for additional 2 h at RT. Finally, all sections were then washed and mounted on slides for confocal analysis.

For IF- ICC on primary astrocytes: cells were permeabilized and blocked in 0.2% Triton-X 100 + 2% NGS in PBS for 1 h at RT. Afterwards, they were incubated O/N at 4°C with rabbit anti-AQP4 antibody (1:400, Abcam) together with mouse anti-GFAP + mouse anti-S100ß (1:400 and 1:1000, respectively, Sigma) in 1% NGS in PBS. Cells were then washed and incubated 1 h with biotinylated anti-rabbit IgG antibody (Jackson Immunoresearch, 1:300) and anti-mouse-Cy3 (1:200) in 1% NGS in PBS and afterwards 1 h with avidin anti-biotin Alexa Fluor 488 (1:1000) and DAPI (1:1000) in 1% NGS in PBS. After washing, coverslips were mounted on slides with anti-fading mounting medium (Aquapolymount, Polysciences Europe GmbH, Eppelheim, Germany) and analyzed with confocal microscopy.

Confocal Microscopy

Confocal microscopy was performed with an Olympus confocal microscope (inverted type IX81, Olympus Europe Holding GmbH, Hamburg, Germany). For experiments in adult brains (IF-IHC), mean values for coverage were obtained from an average of 10 images (20 optical sections, 1 μm Z-step size) from at least two slices per each brain. For IF-ICC, an average of 10–25 fields from each treatment condition were acquired (1 μm Z-step size) for morphological examination. The FluoView FV1000 program (Version 2.1c; Olympus FluoView Resource Center) was used to convert the images from the proprietary file format (.oib) into tagged image file format (.tiff) files for further analysis.

Coverage Analysis

One (.tiff) file was generated for each image which contained two color channels: a red for BVs labeled with collagen IV and a green for endfeet immunoreactive (IR) for AQP-4. The area of co-localized immunolabeling in the region of interest (ROI) was measured using a Demo version of AutoQuantX3 Program. Afterwards, to calculate the coverage index (area of co-localization/total area of vessels), the area occupied by the colocalizing pixels was divided by the total area occupied by the red pixels (representing the BVs labeled with collagen IV). This ratio was defined as the "Coverage."

Analysis of Processes

For morphological analysis: in a first experiment (**Figure 2C**), astrocytes were stained with GFAP and S100β to label astrocyte processes and the program NeuronJ, a plugin of ImageJ (http://www.imagescience.org/meijering/software/neuronj/) was used to count the number of processes per cell, after converting images from the confocal microscope into 8-bit color pictures to be compatible with the NeuronJ Program. In a second experiment, AQP-4 staining was used to evaluate whether observed changes in morphology correlated with changes in AQP-4 cellular content. For the analysis, we marked cell boundaries using the "lasso" tool of Photoshop CS3 and then evaluated AQP-4 signal intensity using the Histogramm function. Also for the RNAi experiment we used IF-ICC to identify and choose for the analysis cells which showed a reduction in AQP-4 signal, indicative of an efficient downregulation. Only those cells with reduced signal were further examined for the number of astrocyte processes per cell.

Statistical Analysis

To choose the appropriate statistical test, a normality test was performed for each sample distribution. Data are plotted as means ± SEM and N refers to the number of independent experiments. A one-way ANOVA was used, followed by Tukey HSD *post hoc* or Dunnett's *post hoc* tests for multiple comparisons. For comparisons between two groups, the unpaired Student's t-test was used. Analyses were performed with Prism GraphPad program (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS

HAB Rats are a Valid Animal Model to Study Changes in Coverage of Blood Vessels by the Endfeet Protein Aquaporin-4

First, we evaluated whether the HAB rats as the animal model used for this study, showed a reduced coverage of BVs by AQP-4-IR endfeet in the PFC, as it was shown in human post mortem brains of MDD patients (Rajkowska et al., 2013). Using immunohistochemistry to label brain slices (*ex vivo*) with collagen IV, a specific marker of BVs, together with a specific antibody against AQP-4, which is localized to the endfeet of astrocytes surrounding BVs (Papadopoulos and Verkman, 2013), we revealed that in the PFC of HAB rats the BVs showed a 60% reduction in coverage by AQP-4-IR endfeet with respect to NAB rats, similar to the finding in MDD patients [**Figure 1**; NAB, 0.16 ± 0.04, $N = 8$; HAB, 0.06 ± 0.02, $N = 7$; Student's t-test, $t_{(1,13)} = 2.198$, $^{*}p < 0.05$]. Thus, HAB rats were validated as appropriate animal model for our study.

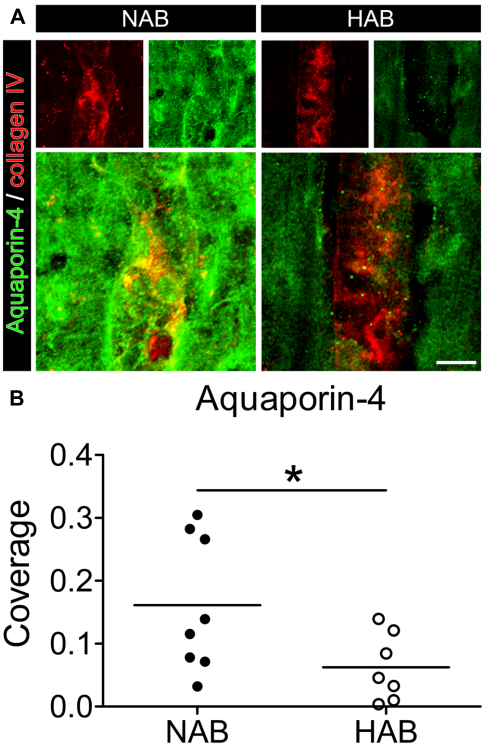


FIGURE 1 | The coverage of blood vessels (BVs) by aquaporin-4 (AQP-4)-immunoreactive astrocytic endfeet is reduced in the PFC of HAB rats with respect to NAB rats. (A) Representative photomicrographs show immunofluorescent stainings for AQP-4 (in green) and collagen IV (to label BVs, in red) in NAB (left) and HAB (right) rats. **(B)** The scattered plot shows a reduction of about 60% in the coverage of BVs by AQP-4-positive endfeet in HAB rats in comparison with NAB rats [NAB ($N = 8$), HAB ($N = 7$); $t_{(1,13)} = 2.198$, $^{*}p < 0.05$]. Scale bar = 10 μm.

The Number of Astrocyte Processes is Reduced in HAB with Respect to NAB Astrocytes and Fluoxetine Treatment can Rescue this Deficit

To investigate whether the observed lack of coverage was dependent on a primary morphological change in astrocyte processes, causing only secondarily a loss of AQP-4-IR endfeet, or on a mislocalization of AQP-4 protein from still intact processes, we first compared *ex vivo* how GFAP, an astrocyte-specific cytoskeletal marker, was expressed in HAB and NAB brains. As previously shown (Rajkowska and Miguel-Hidalgo, 2007), we observed a dramatic reduction in the complexity of astrocytic arborization (**Figure 2A**), thereby suggesting that a main change in the morphology of astrocyte processes may underlie the reduced coverage of BVs in HAB rats. We therefore took advantage of our primary cell culture model using cortical astrocytes derived from both NAB and HAB rats to deeper examine the observed morphological differences. We thereby showed *in vitro* that HAB-derived astrocytes developed a reduced number of processes with respect to NAB-derived cells [**Figures 2B(1,3),C**, NAB C, 9.59 ± 0.75 (22 cells), HAB C, 3.75 ± 0.75 (20 cells), ANOVA, $F_{(1,3)} = 23.91$, with Tukey's *post hoc* test, $^{***}p < 0.001$]. As far as AQP-4 modulates extension of astrocyte processes (Saadoun et al., 2005) and a lack of AQP-4 impairs the recovery from a depressive-like phenotype upon FLX treatment in aqp-4 knockout mice (Kong et al., 2009), we then examined whether the lack of astrocyte processes in HAB cells might be linked to an AQP-4 deficiency which might thereafter disrupt responses to FLX. A short-term (48 h) FLX administration significantly increased the number of processes in NAB astrocytes, indicating such a morphological change as a FLX target. More interestingly, however, we observed that this short drug treatment was also sufficient to significantly enhance the number of astrocyte processes in HAB cells, thereby restoring the basal total number of processes per cell [**Figures 2B(2,4),C**, NAB + FLX, 13.57 ± 0.95 (21 cells), HAB + FLX, 8.94 ± 0.88 (16 cells), ANOVA with Tukey's *post hoc* test, $^{**}p < 0.01$, $^{***}p < 0.001$]. Thus, suggesting that AQP-4 may not be functionally deficient in HAB cells to mediate FLX effect, but maybe only transcriptionally or translationally repressed. To clarify this aspect, since we in fact observed an apparent reduced AQP-4 expression in HAB brains (**Figure 1A**), we used a more quantitative method to estimate whether AQP-4 could be less expressed in HAB astrocytes with

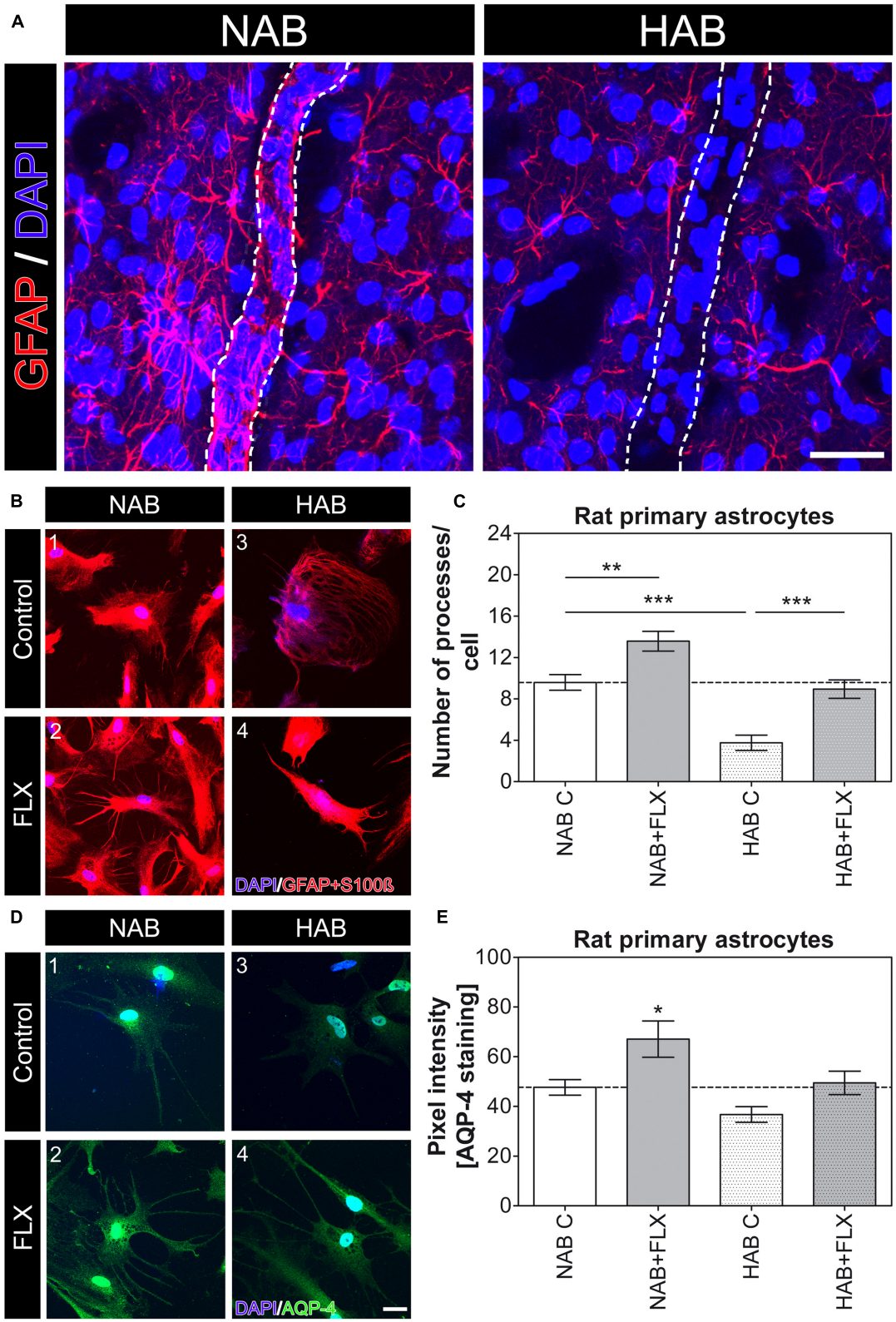


FIGURE 2 | Continued

FIGURE 2 | Continued

Morphological differences in astrocytes are evident in HAB brains and HAB cells with respect to NAB and FLX can modulate astrocyte morphology *in vitro*. **(A)** Representative photomicrographs for GFAP (red) in brain slices (*ex vivo*) show a strong atrophy of astrocytes in the PFC of HAB rats. DAPI (in blue) labels nuclei of single cells to help identifying cells and BVs. Scale bar = 30 μm. **(B)** Representative photomicrographs show NAB (1 and 2) and HAB-derived (3 and 4) astrocytes before and after a 48 h FLX treatment. Cells were labelled with DAPI (blue) and the astrocyte markers GFAP and S100ß (red) to examine morphological changes. **(C)** The graph shows a reduction of about 60% in the number of astrocyte processes in HAB astrocytes in comparison with NAB cells and the effect of FLX in increasing the number of processes in both cell types [ANOVA, $F_{(1,3)} = 23.91$, $^{**}p < 0.01$, $^{***}p < 0.001$]. **(D)** Representative photomicrographs of immunofluorescent stainings show NAB (1 and 2) and HAB-derived (3 and 4) astrocytes before and after a 48 h FLX treatment. Cells were labelled with DAPI (blue) and AQP-4 (green) to measure changes in AQP-4 content per cell. **(E)** The graph shows a reduction of about 23% in the amount of AQP-4 in HAB astrocytes in comparison with NAB cells. FLX reversed AQP-4 amount to basal levels in HAB cells and significantly enhanced AQP-4 expression in NAB cells. ANOVA, $F_{(1,3)} = 6.61$, $^{*}p < 0.05$. Scale bar = 10 μm.

respect to NAB ones. Because commercially available antibodies against AQP-4 are better working in immunochemistry than for western blot, we performed an immunochemical staining to compare AQP-4 expression between NAB and HAB astrocytes and to evaluate how such expression was modulated upon drug administration. We thereby showed that indeed the expression of AQP-4 in HAB astrocytes was 23% less intense in average than in NAB cells, although the difference did not reach statistical significance (**Figure 2E**). More importantly, however, AQP-4 expression was significantly increased by FLX treatment in NAB astrocytes [**Figures 2D(1,2),E**, NAB C, 47.65 ± 3.17 (14 cells), NAB + FLX, 67.06 ± 7.29 (14 cells), ANOVA, $F_{(1,3)} = 6.61$, with Tukey's *post hoc* test, $^{*}p < 0.05$], paralleling the increased amount of astrocyte processes (**Figure 2C**). In addition, AQP-4 expression was normalized in HAB cells after FLX treatment [**Figures 2D(3,4),E**, HAB C, 36.71 ± 3.17 (14 cells), HAB + FLX, 49.46 ± 4.71 (14 cells)], corresponding to the rescued effect on numbers of processes.

Downregulation of AQP-4 Induces a Reduction in Number of Astrocyte Processes in NAB Cells and Hampers both the Effect of FLX in NAB Cells and its Rescue Effect in HAB Cells

To then evaluate whether AQP-4 might be responsible for the altered morphology observed in HAB cells and whether it is required for the rescue effect upon FLX treatment in these cells, we used RNAi to downregulate AQP-4 in primary astrocyte from both NAB and HAB rats. For these experiments we used siRNA sequences which already showed their AQP-4 knockdown efficacy in rat astrocytes (Nicchia et al., 2005). We thereby revealed that AQP-4 was indeed necessary for NAB astrocytes to form their processes, since its knockdown caused a reduction in the total number of processes per cell. Moreover, AQP-4 knockdown caused a loss of response to FLX treatment [**Figure 3**, NAB C, 9.33 ± 0.62 (15 cells), NAB scr, 12.6 ± 0.71 (15 cells), NAB si2, 4.13 ± 0.72 (15 cells), NAB si3, 4.00 ± 0.46 (15 cells), NAB + FLX, 21.86 ± 1.35 (14 cells), NAB scr + FLX, 19.36 ± 0.94 (14 cells), NAB si2 + FLX, 4.38 ± 0.75 (13 cells), NAB si3 + FLX, 4.5 ± 0.43 (12 cells), ANOVA, $F_{(1,7)} = 81.21$, with Tukey's *post hoc* test, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to NAB C and ### $p < 0.001$ with respect to NAB scr]. In this experiment we could observe a higher number of astrocyte processes in response to FLX treatment than those measured in the first experiment (**Figure 2C**). However, baseline number of processes per cell were very consistent, thereby suggesting that drug effects may vary among experiments. Therefore, we focused our analysis on relative changes after FLX treatment and did not make specific claims about absolute numbers.

Furthermore, because we measured a reduced amount of AQP-4 in HAB cells, which correlated to the lowered number of processes (**Figure 2E**), we additionally performed a knockdown experiment in HAB astrocytes to verify whether such variations in AQP-4 content might affect the functional role of AQP-4 in HAB cells too. We confirmed that a partially reduced amount of AQP-4 could indeed be quantified in control HAB cells (HAB C) with respect to NAB C cells (**Supplementary Figure S1**), which might have caused the reduced amount of processes per cell in HAB astrocytes. However, a dysfunctional AQP-4 in HAB astrocytes could be ruled out because FLX was still able to restore the basal number of processes in these cells, whereas the effect of FLX was blunted in AQP-4 knockdown HAB astrocytes in which FLX could not reactivate AQP-4 expression (**Figure 2E**). Thus, further supporting the essential role of AQP-4 to mediate the growth of processes upon FLX administration [**Figure 4**, HAB C, 2.20 ± 0.47 (15 cells), HAB scr, 2.67 ± 0.61 (15 cells), HAB si2, 2.27 ± 0.36 (15 cells), HAB si3, 3.71 ± 0.64 (14 cells), HAB + FLX, 13.79 ± 0.86 (14 cells), HAB scr + FLX, 15.36 ± 0.95 (14 cells), HAB si2 + FLX, 3.80 ± 0.66 (10 cells), HAB si3 + FLX, 2.87 ± 0.38 (15 cells), ANOVA, $F_{(1,7)} = 73.00$, with Tukey's *post hoc* test, $^{***}p < 0.001$ with respect to HAB C and ###$p < 0.001$ with respect to HAB scr]. In this experiment we found that the siRNA si3 showed lower efficiency than si2 in downregulating AQP-4, as it was already evident in NAB cells (**Supplementary Figure S1A**). This lower efficacy of si3 gave the impression of an apparent lack of effect on AQP-4 in HAB cells (**Supplementary Figure S1B**), because in these cells the basal AQP-4 content was already quite low. However, we could still observe its functional efficacy to block the effect of FLX on astrocyte processes. This finding further supported that only in case of a genetic blockade of the drug-dependent increase of AQP-4 expression in HAB cells, which hinders the normalization of its expression (**Figure 2E**), the effect of FLX on the extension of processes was inhibited.

Short Term FLX Treatment Modulates GFAP Expression but it is not Sufficient to Affect Coverage of BVs by Astrocytic Endfeet Neither in NAB Nor in HAB Rats

To verify *in vivo* whether the short-term treatment with FLX was sufficient to modulate the coverage of BVs by AQP-4-IR

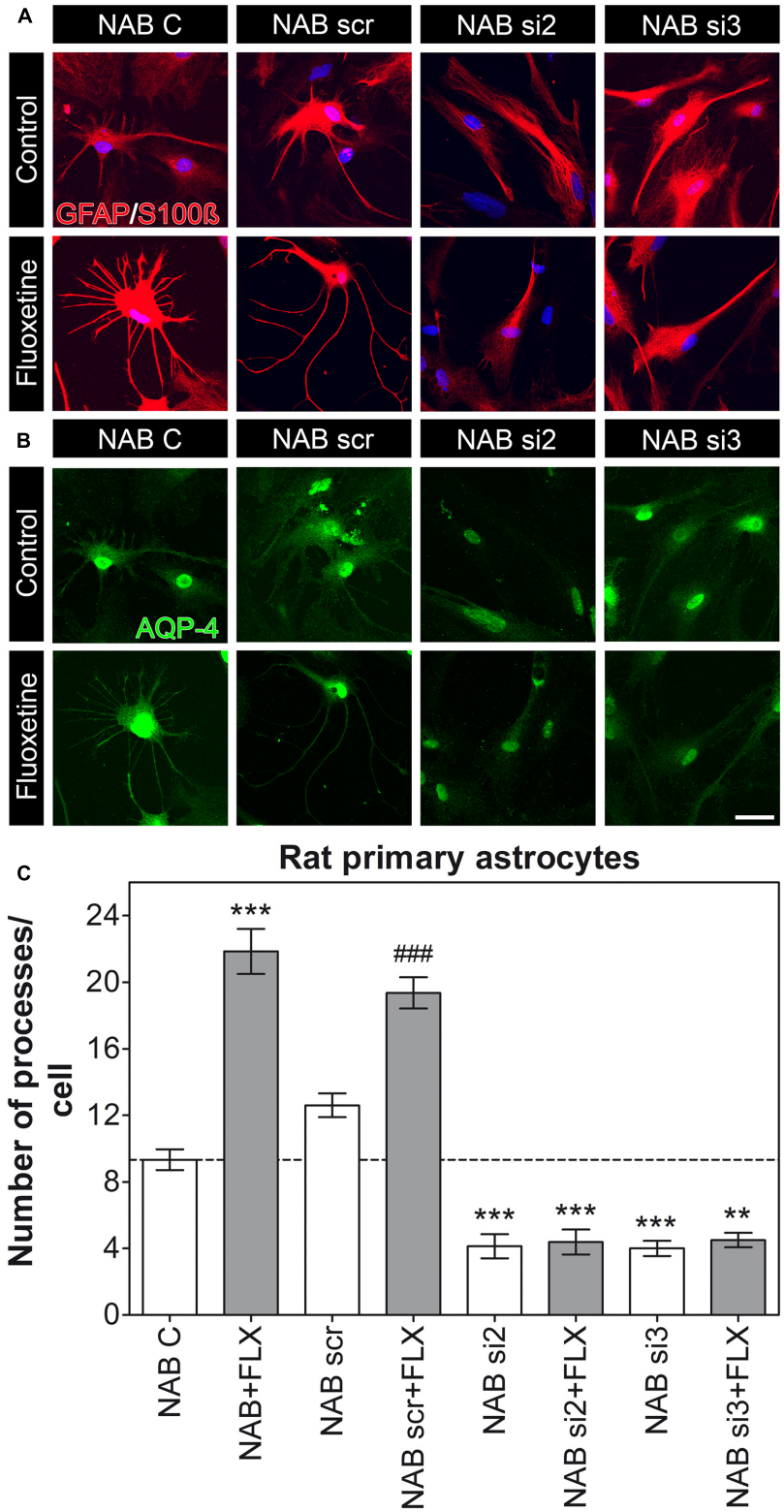


FIGURE 3 | Knockdown of AQP-4 in NAB astrocytes induces a lack of processes per cell. (A,B) Photomicrographs show representative immunofluorescent stainings for GFAP/S100ß (to label astrocytes, in red) and AQP-4 (in green). **(C)** The graph shows that FLX can induce an increased amount of astrocyte processes in NAB C (control) and NAB scr (transfected with a scrambled siRNA) astrocytes, but not in cells transfected with siRNAs against the mRNA coding for AQP-4 (si2 and si3; ANOVA with Tukey HSD *post hoc* test, $^{**}p < 0.01$, $^{***}p < 0.001$ with respect to NAB C and ### $p < 0.001$ with respect to NAB scr). Scale bar = 20 μm.

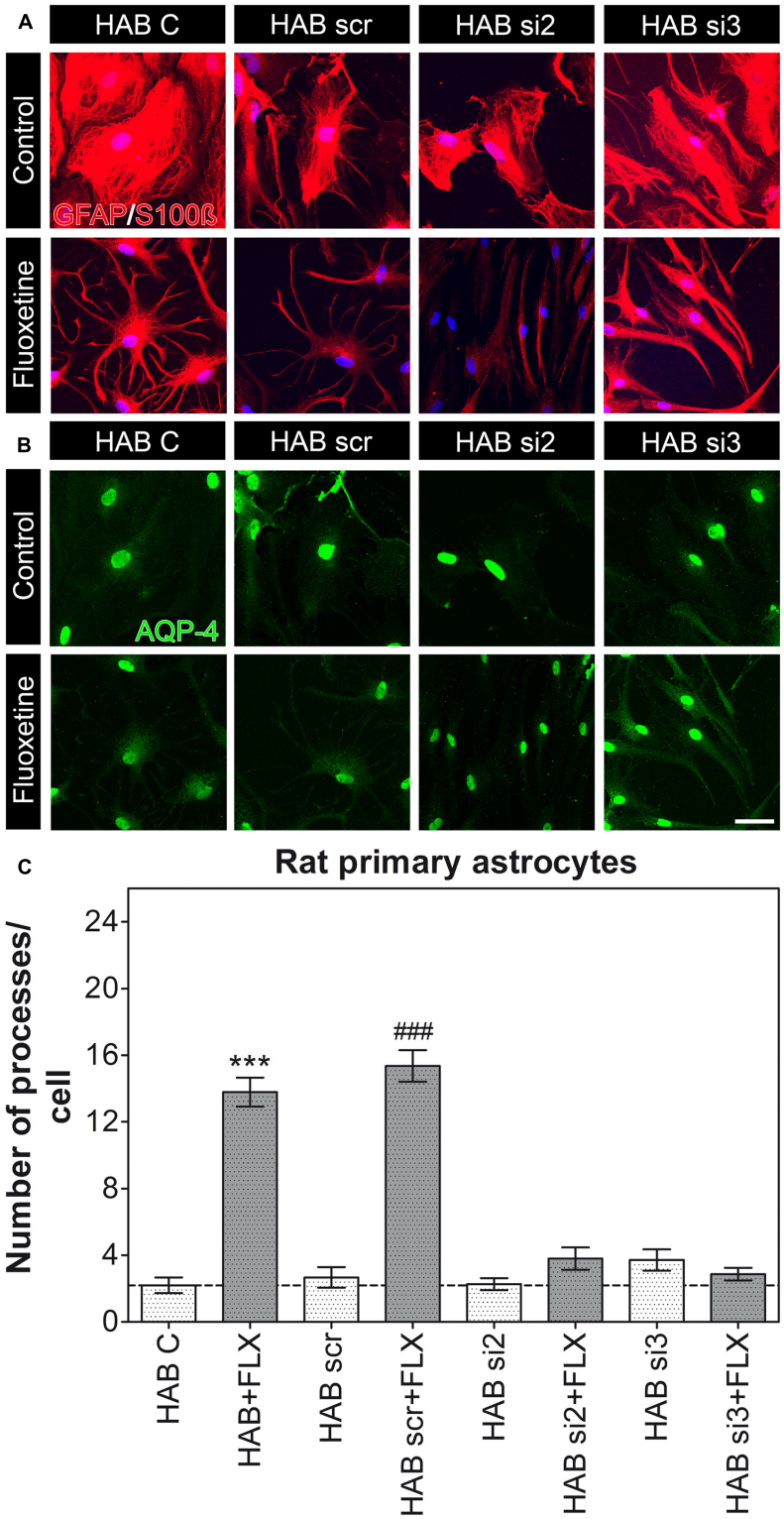


FIGURE 4 | Knockdown of AQP-4 in HAB astrocytes. (A,B) Photomicrographs show representative immunofluorescent stainings for GFAP/S100ß (to label astrocytes, in red) and AQP-4 (in green). **(C)** The graph shows that FLX could still induce an increased amount of astrocyte processes in HAB C (control) and HAB scr astrocytes, thereby suggesting the presence of a still functional AQP-4 to mediate such an effect. However, cells transfected with si2 and si3 did not show the rescue effect after FLX treatment, as they remained lower than NAB (ANOVA with Tukey HSD *post hoc* test, ***$p < 0.001$ with respect to HAB C and ### $p < 0.001$ with respect to HAB scr). Scale bar = 20 μm.

endfeet, we initially injected a small group of NAB and HAB rats with FLX for 48 h. Using IF-IHC on brain slices from the PFC we could show that indeed 48 h were sufficient to modulate the cytoskeletal protein GFAP, with a consequent full recover of its reduced expression in HAB brains [**Figure 5A(3',4')**, yet suggesting that a drug effect indeed occurred after the short-term treatment. However, we observed that FLX did not induce any significant change in the coverage of BVs by AQP4-IR endfeet in neither group of rats, although in NAB rats we found a higher degree of variability in response to FLX with respect to HAB rats [**Figures 5B(1–4),C**; NAB, 0.294 ± 0.017, $N = 4$; NAB + FLX, 0.379 ± 0.08, $N = 4$; HAB, 0.012 ± 0.004, $N = 3$; HAB + FLX, 0.008 ± 0.001, $N = 3$; ANOVA, $F_{(1,3)} = 15.44$, with Tukey's *post hoc* test, $^*p < 0.05$].

DISCUSSION

Several reports in the last years have suggested AQP-4 as an essential brain protein for a variety of astrocytic functions, among which water balance and K+ buffering are the mostly known (Papadopoulos and Verkman, 2013). In addition to that, more recent studies have evidenced a role of AQP-4 as mediator of AD efficacy (Kong et al., 2009) and as a "protective" factor against corticosterone-induced depressive-like phenotype accompanied by an astrocytic pathology (Kong et al., 2014), proposing AQP-4 as a novel putative molecule affected in the etiopathogenesis of MDD. In accordance with these studies, the examination of post mortem brains of MDD patients revealed a lack of coverage of BVs with AQP-4-IR endfeet of astrocytes, further supporting a role for AQP-4 in MDD (Rajkowska et al., 2013). Morphological changes in astrocytes strongly correlate with their functionality and it has been already shown that a reduction in the soma size of hippocampal astrocytes characterize the hippocampus of tree shrews after long-term psychosocial stress, which induces depressive-like behavior (Czeh et al., 2006). However, to identify risk factors which might predispose an individual to develop a depressive disorder, we would need an animal model which displays a depressive-like phenotype with a strong genetic component. We therefore decided to examine the morphology of astrocyte in HAB rats (Neumann et al., 2011; Wegener et al., 2012). Using IF-IHC we could indeed show that HAB rats show a similar reduction in the coverage of BVs by AQP-4-IR endfeet in the PFC as described in the human study by Rajkowska et al. (2013). Our results demonstrate the validity of this animal model to study the importance of an astrocyte pathology for MDD, in addition to the Wistar-Kyoto rat strain (Gosselin et al., 2009), thereby offering an alternative animal model to examine the role of astrocytes in MDD with a particular focus on glia-vasculature dysfunctions.

Furthermore, the lack of AQP-4 positive endfeet observed in the PFC of post mortem brains from MDD patients prompted us to hypothesize that either the endfeet of astrocytes were primarily morphologically affected, becoming only secondarily devoid of AQP-4 expression, or AQP-4 was dyslocalized, although astrocyte processes remained unaffected. Using a staining for GFAP both in brain slices and in our cell culture model, we showed that in fact HAB cells did not show the same morphological complexity in slices *ex vivo* and did not possess the same amount of processes *in vitro* as NAB cells did, thus suggesting that astrocytes from this animal model were primarily morphologically affected. Astrocyte endfeet are essential to maintain the integrity of the BBB (Zhou et al., 2008), but also to mediate the uptake of several substances from the bloodstream. We might speculate that their impairment may explain the slow onset of AD action, as far as the recovery of a properly functional BBB is first necessary before

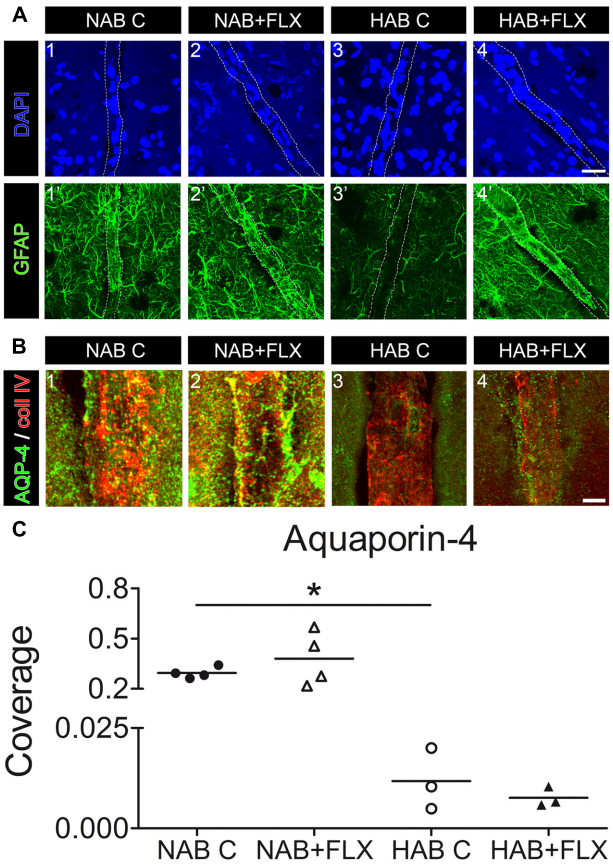


FIGURE 5 | Short-term treatment of NAB and HAB rats with FLX can reverse the reduced GFAP expression in HAB rats, but it is not sufficient to neither modulate coverage of BVs by AQP-4-IR endfeet in NAB brains nor rescue the lack of coverage in HAB brains. **(A)** Representative photomicrographs show immunofluorescent stainings for GFAP (in green) and DAPI (in blue) in NAB (1,1') and HAB (3,3') brains, where it is evident the atrophy of astrocyte processes shown by the reduced GFAP staining in HAB brains (3'). Such a reduced GFAP expression could be rescued by the short-term treatment with FLX (HAB + FLX, 4,4'). Scale bar = 20 μm. **(B)** Representative photomicrographs shows that FLX did not change the coverage of BVs by AQP-4-positive endfeet neither in NAB (1,2) nor in HAB (3,4) treated rats. **(C)** The scattered plot shows that FLX did not change the coverage of BVs by AQP-4-positive endfeet neither in NAB (1,2) nor in HAB (3,4) treated rats after the short-term drug treatment [NAB, 0.294 ± 0.017, $N = 4$; NAB + FLX, 0.379 ± 0.08, $N = 4$; HAB, 0.012 ± 0.004, $N = 3$; HAB + FLX, 0.008 ± 0.001, $N = 3$; ANOVA, $F_{(1,3)} = 15.44$, with Tukey's *post hoc* test, $^*p < 0.05$]. Scale bar = 5 μm.

ADs can be efficiently transported into the brain parenchyma. Therefore, we examined how NAB and HAB astrocytes were reacting to the AD FLX in terms of process extension. To our surprise, however, FLX induced an increased number of astrocyte processes in both cells, suggesting that a lack of AQP-4 on the BVs might not be a primary cause of disease, but may only affect the response to drug treatments. To better understand this aspect, we used RNAi to downregulate AQP-4 and examined how its knockdown was affecting the response to FLX. We thereby showed that, although control HAB astrocytes were still able to protrude an increased number of astrocyte processes in response to drug administration, both NAB and HAB transfected with siRNA targeting the mRNA coding for AQP-4 were not only becoming unable to protrude new astrocyte processes in response to FLX treatment, but they were also losing pre-existing ones (in NAB cells). Thus, suggesting that AQP-4 might exert multiple roles: on the one hand, it is necessary to somehow maintain astrocyte processes in shape, maybe through actin- (Nicchia et al., 2008) or GFAP-mediated mechanisms, as far as both these proteins may be important regulators of structural changes; on the other hand, AQP-4 is necessary to mediate AD efficacy. The latter is actually in line with reports showing that the mouse knockout for aqp-4 cannot recover from the stress-induced depressive-like behavior after FLX treatment (Kong et al., 2009). Our results from the RNAi experiments actually suggest that the observed lack of response to FLX in aqp-4 knockouts may depend on a reduced number of processes in astrocytes due to AQP-4 deficiency, which consequently hinders a FLX-induced process extension necessary to recover a proper astrocytic functionality. Although interesting, our results can only provide limited evidence for either temporary or persistent FLX effects on astrocyte processes after the short-term treatment used here. To clarify this point, washout experiment in cell culture would be needed and, if resulting effects turned out to be stable enough, then cell culture of astrocytes from adult animals treated with FLX might be important to further confirm these *in vitro* findings.

Our *ex vivo* results after short term treatment of NAB and HAB animals with FLX did not show the rescue of coverage of BVs by AQP-4-IR astrocyte processes, probably due to a delay in the uptake of FLX *in vivo* or to an insufficient time to relocate AQP-4 to the endfeet, which might require longer treatment exposures or the supportive role of other cell types, such as endothelial cells, necessary to induce the localization of AQP-4 to endfeet (Camassa et al., 2015). In fact, endothelial cells would be the first cells exposed to FLX, strongly suggesting future studies to analyze their response to FLX and ADs in more details, specifically to dissect the potential contribution of endothelial cells to the polarized distribution of AQP-4 in response to drug administration. Indeed, such studies should help to understand the molecular motors which regulate AQP-4 distribution and function. A better understanding of the mechanism(s) which drive AQP-4 in its appropriate localization may be of help to identify novel target molecules which could fasten the restoration of coverage of BVs by endfeet of astrocytes enriched in AQP-4 to favor the recovery of a functional BBB and substance exchange between bloodstream and brain parenchyma.

Another possibility to explain the lack of rescue effect that we observed in HAB rats treated with FLX might rely on a difference in the type of processes that might form after drug administration. We indeed observed that HAB cells treated with FLX are enriched in processes which appeared to be shorter than those induced in NAB cells, suggesting that longer treatment times may be necessary to fully recover an amount of astrocyte processes with proper functionality, i.e., long enough to reach and completely surround again BVs with their AQP-4-IR endfeet. A similar study has been recently published, which showed that the AD naltrexone could reverse an astrocytic atrophy revealed in macaques with behavioral disorders (Lee et al., 2015).

Although Nicchia et al. (2008) have shown that stellation of astrocytes does not directly depend on the presence of AQP-4, we noticed that AQP-4 knockdown resulted in a lack of stellate morphology and FLX was no longer able to induce such a stellate phenotype in AQP-4 knockdown astrocytes. We cannot rule out that such a phenotype is due to a primary disrupted actin or GFAP cytoskeleton in HAB astrocytes, since we observed that GFAP is strongly impaired in HAB brains. More studies are needed to identify the specific molecular mechanisms behind the lack of stellate morphology in HAB cells.

CONCLUSION

Our results show that AQP-4 might be necessary in order to maintain astrocyte processes, which are the functional unit of astrocytes at the BBB and around synapses (Kimelberg, 2010). Moreover, we could show that the lack of AQP-4 impairs the effect of FLX in restoring basal amounts of processes per cell. We think that a better understanding of the mechanisms which drive AQP-4 to the endfeet and around BVs might help to develop pharmacological compounds which could reverse disease phenotypes with an astrocytic pathology, such as MDD.

AUTHOR CONTRIBUTIONS

BDB conceived and designed the experiments for the work and contributed to acquisition, analysis, interpretation of data and to drafting of the manuscript; VAM, SB, LJ, and GG-G contributed to acquisition, analysis and interpretation of data for the work; IDN contributed to animal experiments (in particular to the generation and maintenance of the HAB rat colony) and to revision of the manuscript for important intellectual content; RR contributed to the conception of the work and to revision of the manuscript for important intellectual content. All authors revised and approved the final version of the manuscript to be published and agreed to be accountable for all aspects of the work.

FUNDING

This work was supported by intramural funding from the University of Regensburg (to BDB) and by the German Federal

Ministry of Education and Research (BMBF Grant Nr 710849 to BDB, IDN, and RR). The sponsors did not have any role in the collection, analysis and interpretation of data, in the writing of the report and in the decision to submit the article for publication.

ACKNOWLEDGMENTS

The authors would like to thank Rodrigue Maloumby (University Regensburg) for excellent technical assistance with animal perfusions for the *ex vivo* experiments and Doris Bayerl for help with HAB animal husbandry.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fncel.2016.00008

FIGURE S1 | The analysis of pixel intensity for AQP-4 staining in NAB (A) and HAB (B) cells revealed that both si2 and si3 showed a high degree of downregulation in both cell types, although with different efficiencies. However, the reduced efficiency of si3 in HAB cells could also be explained through a general reduced amount of AQP-4 signal in HAB C cells with respect to the NAB C (NAB C, 49.01 signal intensity [in arbitrary unit] and HAB C, 36.48). ANOVA with Dunnett's post hoc test, $*p < 0.05$, $**p < 0.01$.

REFERENCES

Allen, J. W., Mutkus, L. A., and Aschner, M. (2001). Isolation of neonatal rat cortical astrocytes for primary cultures. *Curr. Protoc. Toxicol.* Chap. 12:Unit1214. doi: 10.1002/0471140856.tx1204s04

Bressler, J. P., Cole, R., and de Vellis, J. (1980). Cell culture systems to study glial transformation. *Dev. Toxicol. Environ. Sci.* 8, 187–192.

Camassa, L. M., Lunde, L. K., Hoddevik, E. H., Stensland, M., Boldt, H. B., De Souza, G. A., et al. (2015). Mechanisms underlying AQP4 accumulation in astrocyte endfeet. *Glia* doi: 10.1002/glia.22878 [Epub ahead of print].

Czeh, B., and Di Benedetto, B. (2013). Antidepressants act directly on astrocytes: evidences and functional consequences. *Eur. Neuropsychopharmacol.* 23, 171–185. doi: 10.1016/j.euroneuro.2012.04.017

Czeh, B., Simon, M., Schmelting, B., Hiemke, C., and Fuchs, E. (2006). Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology* 31, 1616–1626. doi: 10.1038/sj.npp.1300982

Di Benedetto, B., and Rupprecht, R. (2013). Targeting glia cells: novel perspectives for the treatment of neuropsychiatric diseases. *Curr. Neuropharmacol.* 11, 171–185. doi: 10.2174/1570159X11311020004

Gosselin, R. D., Gibney, S., O'Malley, D., Dinan, T. G., and Cryan, J. F. (2009). Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression. *Neuroscience* 159, 915–925. doi: 10.1016/j.neuroscience.2008.10.018

Kimelberg, H. K. (2010). Functions of mature mammalian astrocytes: a current view. *Neuroscientist* 16, 79–106. doi: 10.1177/1073858409342593

Koehler, R. C., Roman, R. J., and Harder, D. R. (2009). Astrocytes and the regulation of cerebral blood flow. *Trends Neurosci.* 32, 160–169. doi: 10.1016/j.tins.2008.11.005

Kong, H., Sha, L. L., Fan, Y., Xiao, M., Ding, J. H., Wu, J., et al. (2009). Requirement of AQP4 for antidepressive efficiency of fluoxetine: implication in adult hippocampal neurogenesis. *Neuropsychopharmacology* 34, 1263–1276. doi: 10.1038/npp.2008.185

Kong, H., Zeng, X. N., Fan, Y., Yuan, S. T., Ge, S., Xie, W. P., et al. (2014). Aquaporin-4 knockout exacerbates corticosterone-induced depression by inhibiting astrocyte function and hippocampal neurogenesis. *CNS Neurosci. Ther.* 20, 391–402. doi: 10.1111/cns.12222

Lee, K. M., Chiu, K. B., Didier, P. J., Baker, K. C., and MacLean, A. G. (2015). Naltrexone treatment reverses astrocyte atrophy and immune dysfunction in self-harming macaques. *Brain Behav. Immun.* 50, 288–297. doi: 10.1016/j.bbi.2015.07.017

Miguel-Hidalgo, J. J., Jiang, W., Konick, L., Overholser, J. C., Jurjus, G. J., Stockmeier, C. A., et al. (2013). Morphometric analysis of vascular pathology in the orbitofrontal cortex of older subjects with major depression. *Int. J. Geriatr. Psychiatry* 28, 959–970. doi: 10.1002/gps.3911

Neumann, I. D., Wegener, G., Homberg, J. R., Cohen, H., Slattery, D. A., Zohar, J., et al. (2011). Animal models of depression and anxiety: what do they tell us about human condition? *Prog. Neuropsychopharmacol. Biol Psychiatry* 35, 1357–1375. doi: 10.1016/j.pnpbp.2010.11.028

Nicchia, G. P., Rossi, A., Mola, M. G., Procino, G., Frigeri, A., and Svelto, M. (2008). Actin cytoskeleton remodeling governs aquaporin-4 localization in astrocytes. *Glia* 56, 1755–1766. doi: 10.1002/glia.20724

Nicchia, G. P., Srinivas, M., Li, W., Brosnan, C. F., Frigeri, A., and Spray, D. C. (2005). New possible roles for aquaporin-4 in astrocytes: cell cytoskeleton and functional relationship with connexin43. *FASEB J.* 19, 1674–1676. doi: 10.1096/fj.04-3281fje

Papadopoulos, M. C., and Verkman, A. S. (2013). Aquaporin water channels in the nervous system. *Nat. Rev. Neurosci.* 14, 265–277. doi: 10.1038/nrn3468

Pardridge, W. M. (1999). Blood-brain barrier biology and methodology. *J. Neurovirol.* 5, 556–569. doi: 10.3109/13550289909021285

Parpura, V., Heneka, M. T., Montana, V., Oliet, S. H., Schousboe, A., Haydon, P. G., et al. (2012). Glial cells in (patho)physiology. *J. Neurochem.* 121, 4–27. doi: 10.1111/j.1471-4159.2012.07664.x

Rajkowska, G., Hughes, J., Stockmeier, C. A., Javier Miguel-Hidalgo, J., and Maciag, D. (2013). Coverage of blood vessels by astrocytic endfeet is reduced in major depressive disorder. *Biol. Psychiatry* 73, 613–621. doi: 10.1016/j.biopsych.2012.09.024

Rajkowska, G., and Miguel-Hidalgo, J. J. (2007). Gliogenesis and glial pathology in depression. *CNS Neurol. Disord. Drug Targets* 6, 219–233. doi: 10.2174/187152707780619326

Rajkowska, G., Miguel-Hidalgo, J. J., Wei, J., Dilley, G., Pittman, S. D., Meltzer, H. Y., et al. (1999). Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. *Biol. Psychiatry* 45, 1085–1098. doi: 10.1016/S0006-3223(99)00041-4

Rajkowska, G., and Stockmeier, C. A. (2013). Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Curr. Drug Targets* 14, 1225–1236. doi: 10.2174/13894501113149990156

Saadoun, S., Papadopoulos, M. C., Watanabe, H., Yan, D., Manley, G. T., and Verkman, A. S. (2005). Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. *J. Cell Sci.* 118, 5691–5698. doi: 10.1242/jcs.02680

Skucas, V. A., Mathews, I. B., Yang, J., Cheng, Q., Treister, A., Duffy, A. M., et al. (2011). Impairment of select forms of spatial memory and neurotrophin-dependent synaptic plasticity by deletion of glial aquaporin-4. *J. Neurosci.* 31, 6392–6397. doi: 10.1523/JNEUROSCI.6249-10.2011

Uhr, M., Tontsch, A., Namendorf, C., Ripke, S., Lucae, S., Ising, M., et al. (2008). Polymorphisms in the drug transporter gene ABCB1 predict antidepressant treatment response in depression. *Neuron* 57, 203–209. doi: 10.1016/j.neuron.2007.11.017

Wegener, G., Mathe, A. A., and Neumann, I. D. (2012). Selectively bred rodents as models of depression and anxiety. *Curr. Top. Behav. Neurosci.* 12, 139–187. doi: 10.1007/7854_2011_192

Zhou, J., Kong, H., Hua, X., Xiao, M., Ding, J., and Hu, G. (2008). Altered blood-brain barrier integrity in dult aquaporin-4 knockout mice. *Neuroreport* 19, 1–5. doi: 10.1097/WNR.0b013e3282f2b4eb

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Di Benedetto, Malik, Begum, Jablonowski, Gómez-González, Neumann and Rupprecht. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Cellular Neuroscience

ORIGINAL RESEARCH
published: 22 December 2015
doi: 10.3389/fncel.2015.00490

Attenuated Levels of Hippocampal Connexin 43 and its Phosphorylation Correlate with Antidepressant- and Anxiolytic-Like Activities in Mice

*Gaël Quesseveur[1†], Benjamin Portal[2†], Jean-Arnaud Basile[2], Pascal Ezan[3], Alexia Mathou[4], Hélène Halley[2], Corinne Leloup[4], Xavier Fioramonti[4], Nicole Déglon[5], Christian Giaume[3], Claire Rampon[2] and Bruno P. Guiard[1,2]**

[1] Institut National de la Santé et de la Recherche Médicale UMR-S 1178 – Dépression, Plasticité and Résistance Aux Antidépresseurs, Laboratoire de Neuropharmacologie EA 3544, Faculté de Pharmacie, Université Paris-Sud, Châtenay-Malabry, France, [2] Centre National de la Recherche Scientifique, Centre de Recherches sur la Cognition Animale UMR 5169, Centre de Biologie Intégrative, Université Toulouse III– Paul Sabatier, Toulouse, France, [3] Center for Interdisciplinary Research in Biology, Centre National de la Recherche Scientifique UMR 7241, Collège de France, Paris, France, [4] Centre des Sciences du Goût et de l'Alimentation – Centre National de la Recherche Scientifique UMR 6265 – Institut National de la Recherche Agronomique UMR 1324, Université de Bourgogne, Dijon, France, [5] Laboratory of Cellular and Molecular Neurotherapies, Department of Clinical Neurosciences, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

Clinical and preclinical studies have implicated glial anomalies in major depression. Conversely, evidence suggests that the activity of antidepressant drugs is based, at least in part, on their ability to stimulate density and/or activity of astrocytes, a major glial cell population. Despite this recent evidence, little is known about the mechanism(s) by which astrocytes regulate emotionality. Glial cells communicate with each other through gap junction channels (GJCs), while they can also directly interact with neurons by releasing gliotransmitters in the extracellular compartment via an hemichannels (HCs)-dependent process. Both GJCs and HCs are formed by two main protein subunits: connexins (Cx) 30 and 43 (Cx30 and Cx43). Here we investigate the role of hippocampal Cx43 in the regulation of depression-like symptoms using genetic and pharmacological approaches. The first aim of this study was to evaluate the impact of the constitutive knock-down of Cx43 on a set of behaviors known to be affected in depression. Conversely, the expression of Cx43 was assessed in the hippocampus of mice subjected to prolonged corticosterone (CORT) exposure, given either alone or in combination with an antidepressant drug, the selective serotonin reuptake inhibitor fluoxetine. Our results indicate that the constitutive deficiency of Cx43 resulted in the expression of some characteristic hallmarks of antidepressant-/anxiolytic-like behavioral activities along with an improvement of cognitive performances. Moreover, in a new cohort of wild-type mice, we showed that CORT exposure elicited anxiety and depression-like abnormalities that were reversed by chronic administration of fluoxetine. Remarkably, CORT also increased hippocampal amounts of phosphorylated form of Cx43 whereas fluoxetine treatment normalized this parameter. From these results, we envision that antidepressant drugs may exert their therapeutic activity by decreasing the expression and/or activity of Cx43 resulting from a lower level of phosphorylation in the hippocampus.

Keywords: astrocytes, connexin 43, antidepressants, depression, anxiety, hippocampus, stress, behavior

OPEN ACCESS

Edited by:
Luisa Pinto,
University of Minho, Portugal

Reviewed by:
Bernhard J. Mitterauer, Volitronics-Institute for Basic Research, Psychopathology and Brain Philosophy, Austria
Maciej Figiel, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poland

****Correspondence:***
Bruno P. Guiard
bruno.guiard@univ-tlse3.fr

[†]These authors have contributed equally to this work.

Received: *23 October 2015*
Accepted: *03 December 2015*
Published: *22 December 2015*

Citation:
Quesseveur G, Portal B, Basile J-A, Ezan P, Mathou A, Halley H, Leloup C, Fioramonti X, Déglon N, Giaume C, Rampon C and Guiard BP (2015) Attenuated Levels of Hippocampal Connexin 43 and its Phosphorylation Correlate with Antidepressant- and Anxiolytic-Like Activities in Mice. Front. Cell. Neurosci. 9:490. doi: 10.3389/fncel.2015.00490

INTRODUCTION

Major depression (MD) is one of the most common mental disorders, affecting 350 million people worldwide and is a leading cause of disability (Milanovic et al., 2015). MD imposes substantial economic costs and its impact on disability, productivity, and quality of life further accentuates these costs (Lecrubier, 2001). Evidence indicates that stressful life events increase the risk for MD (Binder and Nemeroff, 2010). As a consequence, classically used animal models of depression rely on the activation of the hypothalamus-pituitary-adrenal (HPA) axis in order to reproduce part of the physiological, neurochemical and morphological maladaptive changes detected in depressed patients (Dalvi and Lucki, 1999). Selective serotonin reuptake inhibitors (SSRIs) represent the most widely prescribed class of antidepressant drugs, but approximately 50% of depressed patients do not respond adequately to SSRI. Moreover even in responders, the onset of therapeutic action is of 2–4 weeks (Blier, 2001). Considering the prevalence of MD and its consequences on life's quality, a better knowledge of its pathophysiology is required to envision the identification of new therapeutic targets.

Opening the way for a promising area of pharmacological interventions, recent data suggest that glia plays an important role in MD and in antidepressant drugs response (Quesseveur et al., 2013a). Indeed, reduced expression of the glial fibrillary acidic protein (GFAP) has been observed in the brain of depressed patients (Miguel-Hidalgo et al., 2000; Webster et al., 2001; Si et al., 2004; Nagy et al., 2015). This glial anomaly, specifically identified in limbic regions (Torres-Platas et al., 2015), is not restricted to GFAP since a decrease in cortical mRNA expression of astrocyte-enriched genes including ALDH1L1, SOX9, GLUL, SCL1A3 has been reported in a large cohort of depressed patients (Nagy et al., 2015). In agreement with the latter findings, subjecting animals to unpredictable chronic mild stress (UCMS) or prolonged social defeat, two animal models of depression, also elicits an attenuation of GFAP (mRNA or protein) in the cortex (Banasr et al., 2010) or the hippocampus of rats (Czeh et al., 2006; Araya-Callis et al., 2012). An elevation in stress-induced glucocorticoids might have contributed to this effect as studies indicated that corticosterone (CORT) can lower GFAP levels in the rat brain (Nichols et al., 1990). Despite this evidence, little is known about the mechanism(s) by which astrocytic activity may regulate emotionality and its related brain circuits.

Astrocytes have the ability to communicate with each other through GJCs, while they can also communicate directly with neurons by releasing gliotransmitters in the extracellular compartment via hemichannels (HCs). In astrocytes, both GJCs and HCs are formed by two main subunits: connexins 30 and 43 (Cx30 and Cx43; Rouach and Giaume, 2001). A few months ago, a post-mortem study correlated MD with decreased levels of Cx43 immunoreactivity in the prefrontal cortex (Miguel-Hidalgo et al., 2014). Similarly, rats subjected to UCMS exhibit a significantly decreased expression and function of cortical and hippocampal Cx43 GJCs whereas typical antidepressant drugs such as fluoxetine, duloxetine, or the tricyclic amitriptyline increased astroglial Cx43 protein levels (Li et al., 2010; Sun et al., 2012; Morioka et al., 2014; Mostafavi et al., 2014). Although the latter studies focused their attention on Cx43 without providing information about Cx30, these clinical and preclinical observations raise the possibility that the down-regulation and/or functional inactivation of astroglial Cx GJCs could produce deleterious effects on mood. Conversely, the over-expression and/or activation of astroglial Cxs GJCs might underlie, at least in part, antidepressant drugs response. However, the role of astroglial Cxs in the regulation of emotional states could be more complicated than expected. Indeed, it was recently reported that restraint stress increases the opening of Cx43 HCs in the hippocampus, thereby promoting the release of glutamate in a neurotoxic concentration for neighboring cells (Orellana et al., 2015). Connexin 43 HCs activity may thus constitute a pre-requisite condition for the damaging effects of chronic stress on neuronal activity and related functions. Consequently and given the importance of adult hippocampal neurogenesis in antidepressant drug response (Santarelli et al., 2001; Surget et al., 2008; David et al., 2009), the latter results suggest, that the inactivation of Cx43 HCs might positively influence emotionality.

Here, we questioned the role of hippocampal Cx43 in the regulation of emotion-related behaviors using complementary genetic and pharmacological approaches. In the first part of this study, we evaluated the impact of the constitutive knock-down of Cx43 on a set of well-defined behavioral paradigms known to recapitulate several symptoms of depression. The second part of this work was designed to investigate the putative changes in hippocampal Cx43 expression in mice chronically exposed to the stress hormone CORT, either alone or in combination with the SSRI fluoxetine. The choice of the hippocampus was based on the fact that this brain region is subjected to intense structural changes and remodeling in MD while an important part of the therapeutic activity of antidepressant drugs relies on their ability to block or reverse impairments of plasticity caused by stress or related pathologies (Warner-Schmidt and Duman, 2006).

MATERIALS AND METHODS

Animals

For experiment 1, 7 week-old male transgenic $Cx43^{fl/fl}$ mice were used and wild-type littermates ($Cx43^{wt/wt}$) with a similar mixed 129P2-C57BL/6 background served as controls (Theis et al., 2001). For experiments 2 and 3, 7 week-old wild-type C57BL/6J mice were used. All mice weighed between 25 and 35 g at the time of behavioral assessment. Mice were housed by 4–5 per cage under standard conditions (12:12 h light-dark cycle, light on at 7 am, 22 ± 1°C ambient temperature, 60% relative humidity). The "Centre de Recherches sur la Cognition Animale (CRCA)" has received French legal approval for experiments on living vertebrate animals (Arrêté Préfectoral: 9-02-2011). This work was carried out in accordance with the Policies of the French Committee of Ethics. Animal surgery and experimentations conducted in this study were authorized by the French Direction of Veterinary Services to CR (#31-11555521, 2002) and all efforts were made to improve animals' welfare and minimize animals suffering.

Drugs and Surgery

Corticosterone (from Sigma–Aldrich, Saint-Quentin Fallavier, France) was dissolved in vehicle (beta-cyclodextrin 0.45%) as previously described (David et al., 2009). CORT (35 μg/ml, equivalent 5 mg/kg/day) was delivered alone or in presence of the antidepressant drug fluoxetine in opaque bottles to protect it from light, available *ad libitum* in the drinking water. Fluoxetine hydrochloride (18 mg/kg per day for 1 month) was purchased from Anawa Trading (Wangen, Switzerland) and was used at a dose that reversed CORT-induced behavioral anomalies (David et al., 2009). In this study, we also used the non-selective Cx blocker carbenoxolone (CBX; Rouach et al., 2003) at a concentration of 10 mM based on a previous report showing its ability to modulate memory (Bissiere et al., 2011; Sun et al., 2012). One week before the tests, wild-type mice were anesthetized with chloral hydrate (400 mg/kg; i.p.). They were then placed on a stereotaxic frame and bilaterally implanted with 29 gage guide cannulae (Cooper's needle works LTD) in the ventral hippocampus using the following coordinates: −2.5 posterior from bregma, ±2.8 mm lateral to midline and 2.0 ventral from the dura. The implants were secured to the skull with dental acrylic. Dummy cannulae were inserted from the end of surgery until the infusion day to prevent clogging of the guides. For its bilateral micro-infusion in the ventral hippocampus (0.3 μl per side at a constant rate of 0.1 μl/min), CBX was dissolved in 0.1 M sterile PBS. 33 gage internal injection cannulae, extending 0.5 mm beyond the guide cannulae, were inserted and connected to a microsyringe driven by a microinfusion pump. The cannulae were left in place for an additional 2 min before being withdrawn and the animals were allowed an additional 15 min before the beginning of experimentation. Vehicle animals were also cannulated and received similar volumes of 0.1 M sterile PBS.

Behavioral Tests

All behavioral tests were performed in the morning to avoid differences in locomotor activity and other variables affected by circadian rhythm. Previous studies have indicated that certain test variables are sensitive, whereas others are resistant, to test order (McIlwain et al., 2001). Bearing this in mind, performance was then evaluated from the least to the most stressful test, thereby decreasing the chance that one test might impact the behavior evaluated in the subsequent paradigm. Importantly, since prior handling and testing have been described to reduce exploratory activity and emotionality in mice (Võikar et al., 2004), animals were tested only once in each paradigm. Finally, given that it has been demonstrated that the interval between behavioral tests could be as short as 1 day, with a weak effect on overall performance (Paylor et al., 2006), in the present study, a 2-day recovery period between tests was provided. It is also important to state that by reducing the inter-test interval, we reduced the possible effects of time on drug administration on tests.

The Sucrose preference test (SPT) was performed in singled-house mice habituated for 48 h to drink water from two bottles. On the following 3 days, the mice could choose between a water bottle and a 1% sucrose solution bottle, switched daily. Sucrose solution intake for 24 h was measured during the last day and expressed as a percentage of the total amount of liquid ingested and normalized to the mouse body weight.

The splash test (ST) was performed for a 5-min period as previously described (David et al., 2009). After squirting 200 μl of a 10% sucrose solution on the mouse's snout, grooming time was scored by a single experimenter as an index of self-care.

The tail suspension test (TST) was performed using the BIOSEB's Tail Suspension Test system (Bioseb) during a 6-min session period. Immobility time was scored as an index of resignation. Movements in terms of energy and power in motion were measured to ensure the absence of any locomotor bias.

The Elevated plus maze (EPM) was performed by placing animals into the central area facing one closed arm and were allowed to explore the maze for 5 min. Testing took place in bright dimmed light conditions (800–900 lux). Automated scoring was done using ANY-maze(tm) behavioral video-tracking software from Stoelting Co (Bioseb, Vitrolles, France). Total number of entries and time spent in the open arms were measured.

The Open field (OF) was performed in 40 cm × 40 cm Plexiglas boxes (Mouse Open Field Arena ENV-510; Med Associates Inc.) during a 30-min session period. Activity chambers were computer interfaced for data analysis (SOF-811; Med associates Inc.), and two regions were defined by grid lines that divided each box into center and periphery areas, with each of the four lines located 11 cm away from each wall. The total number of entries, the ambulatory distance and the time spent in each compartment were measured.

The Novelty suppressed feeding (NSF) was performed in a white plastic box (30 cm × 60 cm). Mice were food deprived for 24 h before testing and then placed in the corner of the box with their respective food pellet on a white square filter paper at the center of the arena under a bright light (∼60 W) placed about 60–80 cm above the food pellet. Latency to begin eating was scored by a single experimenter, with a cut-off time of 10 min. Upon return to their home-cage, the total amount of food intake was measured for each animal and for a 5-min period to ensure the absence of differences in hunger/motivation to eat.

The Object location test (OLT) **and** ***the Object recognition test (ORT)*** started with an habituation to the open-field by exposing the mice to the apparatus for 10 min 1 day before starting the trials (day 1). During the training session, two identical objects were placed 15 cm away from the two opposite corners (day 2). Each mouse was placed in the center of the open-field, facing the objects and was allowed to freely explore the objects for 10 min. During the retention session held 24 h later (day 3), one object was displaced to a new position (in the OLT), or replaced by another one (in the ORT). Then, each mouse was placed in the center of the open-field facing the objects and was allowed to freely move for 10 min. Time spent exploring the different objects was recorded using Noldus Ethovision software.

The Fear conditioning (FC) consisted of a single conditioning session. During conditioning, each mouse was introduced into the conditioning chamber for a total of 4 min 30 s. After a 2-min exploration period, a sound (CS) was emitted for 30 s, and a foot-shock (US) was superposed to the tone during the last 2 s. The mice were then maintained in the chamber

for two additional min. After this procedure, the mouse was gently removed from the chamber. 24 h after the conditioning session, freezing to the context was assessed by again placing each mouse in the conditioning chamber. The level of freezing was measured during 4 min, no tone or foot-shock being presented to the animal. Two hours later, mice were tested for freezing to the tone in a modified context. Two minutes after introduction in the modified chamber, freezing was recorded during a 2-min tone presentation. Freezing behavior was both automatically recorded and scored by an experimenter blind to the experimental conditions. Data was calculated for the 4-min context recall test, the 2-min pre-tone, and the 2-min tone tests.

Emotionality *z*-Score

Z-normalization across complementary measures of emotionality-related behaviors assessed from different paradigms was applied after each experimental protocol. Simple mathematical tools were used to normalize data from each individual raw behavioral data to the mean of the control groups within each experimental cohort. Data were then integrated into a single value, named emotionality *z*-scores. Values were obtained by subtracting the average of observations in a population from an individual raw value and then dividing this difference by the population standard deviation as described previously (Guilloux et al., 2011; Petit et al., 2014). This type of normalization allows data on different scales to be compared. The emotionality *z*-score included the parameters measured in the ST (grooming time), the TST (immobility time), the elevated plus maze (open arms entries and open arms times), the open-field (center entries, center time, and center to total distance ratio) and the NSF (latency to feed). It is noteworthy that several parameters were calculated in the OF and EPM to evaluate anxiety. In order to have a same impact of each test on the emotionality *z*-score, we averaged the normalized parameters evaluated in the OF and EPM to obtain a single value per mouse and per behavioral test. More details about the mathematical method for emotionality *z*-score calculation are provided in **Supplementary Table S1**.

Western-Blot

Expression of Cx30 and Cx43 were analyzed by western blot as previously described (Ezan et al., 2012). Briefly, frozen hippocampi or hypothalamus were pulverized on dry ice, resuspended in boiling 2% SDS containing protease inhibitors (Roche), β-glycerophosphate (10 mM) and orthovanadate (1 mM), and sonicated on ice. Lysates were centrifugated at 13,000 rpm at 4°C and supernatants were diluted with 5x Laemmli buffer, and boiled 5 min. After quantification using the BCA protein assay kit (Pierce), protein samples (20 μg/lane) were separated by electrophoresis on 10% polyacrylamide gels (Gel Nupage 4–12%, Bis-Tris) and transferred onto nitrocellulose membranes. Membranes were blocked for 1 h with 5% fat-free dried milk/TBS1x-0.1% Tween and incubated overnight at 4°C either with anti-Cx30 (rabbit polyclonal, Invitrogen 71-2200, 1/500) or anti-Cx43 (mouse monoclonal, BD Biosciences 610062, 1/500). The next day membranes were washed and incubated with peroxydase-conjugated goat anti-rabbit or goat anti-mouse (HRP-IgG, Santa Cruz, respectively, sc-2004 or sc-2005, 1/2500), for 2 h at room temperature. Chemiluminescent signal was obtained using Western Lighting Plus ECL detection kit (Perkin-Elmer NEL104001EA). Blots were reprobed with anti-tubulin or anti-actin (mouse monoclonal, Sigma, 1/10,000 and 1/30,000; respectively, T6199, A3853) to check protein load. Semi-quantitative densitometric analysis was performed using ImageJ software. The analysis of Cx43 activity was assessed through its level of phosphorylation.

Experimental Design

In the experiment 1 (**Supplementary Figure S1A**), control $Cx43^{wt/wt}$ and $Cx43^{fl/fl}$ mice were subjected to a full comprehensive battery of behavioral tests to evaluate their phenotype. Separate cohorts were used to assess putative changes in depressive, anxious and cognitive symptoms.

In the experiment 2 (**Supplementary Figure S1B**), wild-type mice were subjected to CORT or its vehicle for 1 month and then administered with vehicle or fluoxetine for one additional month. The efficacy of CORT or antidepressants on behavioral performances were assessed in different paradigms. At the end of this procedure, mice brains were removed for the analysis of Cx43 activity through its level of phosphorylation.

Statistical Analysis

For all experiments, statistical data analysis (StatView 5.0 Abacus Concepts, Berkeley, CA, USA) used means ± SEM. Student *t*-tests and one-way ANOVA followed on treatment factor by Fisher Protected Least Significance Difference *post hoc* test were used. The linear relationship between emotionality *z*-scores and the level of expression of non-phosphorylated Cx43 was analyzed by the Pearson's correlation coefficient after a Shapiro-Wik normality test. Significant level was set at $p < 0.05$.

RESULTS

Cx43 Controls Behavioral Symptoms Related to Depression and Anxiety

Given the distribution of Cx43 in astroglial and stem cells in the central nervous system (Dermietzel et al., 1989; Kunze et al., 2009), we proposed to generate mice with a specific inactivation of astroglial Cx43 in the hippocampus. To this end, we generated a pseudotyped MOKOLA lentiviral vectors to selectively drive the *in vivo* expression of the Cre-recombinase within hippocampal astrocytes of $Cx43^{fl/fl}$ mice. The envelope confers to the virus a tropism toward these glial cells (Colin et al., 2009; Quesseveur et al., 2013b) and coupling with a detargeting strategy using miRNA9T and miRNA124T eliminates the putative residual expression of this Cre-recombinase in hippocampal stem cells and neurons, respectively, thus providing a selective astrocytic targeting. However, and as previously shown (Theis et al., 2003; Unger et al., 2012), we observed that $Cx43^{fl/fl}$ mice themselves displayed a dramatic decrease in the expression of Cx43 relative to control $Cx43^{wt/wt}$ mice in various brain regions including the hippocampus (∼−95%; $t_{1,11} < 0.001$) and the hypothalamus

(∼−85%; $t_{1,6} < 0.05$; **Figures 1A–D**). On this background, the relevance and the interest of our strategy consisting in over-expressing the Cre-recombinase within hippocampal astrocytes was limited. However, we took advantage of the knock-down of this protein to assess the consequences of constitutive Cx43 deficiency in a set of behavioral aspects known to be affected in depression: hedonic state, behavioral despair, self-care, anxiety, and cognition/memory by comparing $Cx43^{fl/fl}$ mice and $Cx43^{wt/wt}$ mice maintained on the same genetic background.

With respect to hedonic state, no differences in sucrose preference was detected between control $Cx43^{wt/wt}$ and $Cx43^{fl/fl}$ mice ($t_{1,16} = 0.34$; **Figure 2A**) suggesting that the loss of Cx43 did not interfere with mice's ability to experience pleasure. In the ST evaluating self-care, no difference in the time of grooming was noticed between $Cx43^{wt/wt}$ and $Cx43^{fl/fl}$ mice ($t_{1,16} = 0.8$; **Figure 2B**). We also assessed immobility during exposure to inescapable stress using the TST. $Cx43^{fl/fl}$ mice showed a marked decrease in the immobility time compared to $Cx43^{wt/wt}$ mice, indicative of a low level of despair ($t_{1,16} < 0.001$; **Figure 2C**).

Mice were then tested in behavioral paradigms assessing anxiety. In the elevated plus maze, no difference was observed between $Cx43^{wt/wt}$ and $Cx43^{fl/fl}$ mice in the time spent in the open arms ($t_{1,15} = 0.3$; **Figure 2D**) nor in the number of entries in these arms ($t_{1,15} = 0.4$, data not shown). In the OF, which evaluates locomotor activity in a novel environment as well as the level of anxiety, an increase, albeit not statistically significant, in the time spent ($t_{1,12} = 0.07$; **Figure 2E**) and the number of entries in the center area ($t_{1,12} = 0.09$; data not shown) was observed in $Cx43^{fl/fl}$ mice. To eliminate a putative bias, we verified that the loss of Cx43 did not affect the total distance traveled ($Cx43^{wt/wt}$: 8595 ± 575 vs. $Cx43^{fl/fl}$: 7679 ± 598 cm; $t_{1,12} = 0.3$). In the NSF, which evaluates animal's latency to consume food in a novel aversive environment, $Cx43^{fl/fl}$ mice exhibited a decreased latency to feed compared to $Cx43^{wt/wt}$ mice ($t_{1,16} < 0.05$; **Figure 2F** and **Supplementary Figure S2A** for survival curve). Importantly, body weight loss and food consumption in the home-cage were similar in both genotypes (**Supplementary Figures S2B,C**).

Finally, cognitive/memory functions were assessed by the measure of performances in the OLT (spatial memory), in the ORT (non-spatial working memory) and in the fear conditioning (contextual memory) successively. In the OLT, we found that $Cx43^{fl/fl}$ mice did not displayed notable spatial memory deficits compared to control $Cx43^{wt/wt}$ mice. Indeed, during memory testing 24 h after the acquisition phase, exploratory preference for mice of both groups were significantly higher than chance value (50%), indicating that animals from both genotypes remembered the initial position of the objects ($Cx43^{wt/wt}$ mice: $p < 0.01$ and $Cx43^{fl/fl}$ mice: $p < 0.05$; **Figure 2G**). Moreover, $Cx43^{fl/fl}$ and control $Cx43^{wt/wt}$ mice similarly explored the displaced object ($t_{1,15} = 0.1$; **Figure 2G**) indicating that Cx43 know-down had no effect on spatial memory in this task.

During memory testing in the ORT, $Cx43^{fl/fl}$ and control $Cx43^{wt/wt}$ mice also explored to the same extent the novel object ($t_{1,15} = 0.6$) and both values were significantly different from chance value ($Cx43^{wt/wt}$ mice: $p < 0.05$ and $Cx43^{fl/fl}$ mice: $p < 0.01$; **Figure 2H**). It is noteworthy that during the acquisition phase of object location ($p = 0.5$) and ORT ($p = 0.7$), both groups of mice spent the same amount of time exploring the objects (**Supplementary Figures S2D,E**). In the fear conditioning, 24 h after the conditioning session, $Cx43^{fl/fl}$ mice exhibited significantly more freezing than $Cx43^{wt/wt}$ mice ($t_{1,15} < 0.01$; **Figure 2I**). These data are in favor of an improvement of the associative memory in $Cx43^{fl/fl}$ mice. Interestingly, freezing to the tone was also studied in a modified context. As a validation of the experiment, freezing was measured before the shock on day 1 and no differences occurred between $Cx43^{fl/fl}$ and control $Cx43^{wt/wt}$ mice ($t_{1,15} = 0.12$; **Supplementary Figure S2F**).

These results indicate that the genetic loss of Cx43 elicits a behavioral phenotype which is similar to that observed in response to the chronic administration of antidepressant drugs.

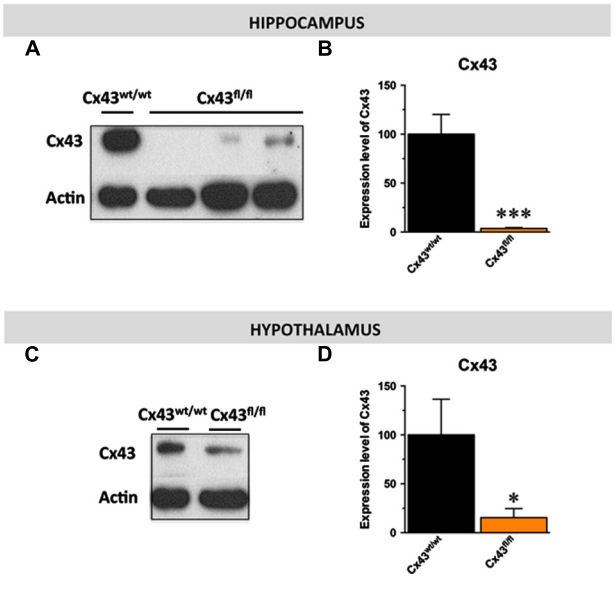


FIGURE 1 | Expression of astroglial Cx43 in $Cx43^{fl/fl}$ mice. Immunoblot analysis **(A–C)** and quantification **(B–D)** of Cx43 levels in the hippocampus **(B)** and the hypothalamus **(D)** of $Cx43^{fl/fl}$ mice relative to control $Cx43^{wt/wt}$ mice. β-actin was used as a loading control. *$p < 0.05$ and ***$p < 0.001$: significantly different from $Cx43^{wt/wt}$ ($n = 6$–7 mice/group for the hippocampus et $n = 4$ for the hypothalamus).

Stress and the Selective Serotonin Reuptake Inhibitor Fluoxetine Display Opposite Effects on the Functional Activity of Hippocampal Cx43

Wild-type mice were first tested in behavioral paradigms assessing mood. In the splash and tail suspension tests, the one-way ANOVA indicated a significant effect of treatment factor [$F_{(2,29)} = 21{,}4$; $p < 0.001$ and $F_{(2,29)} = 3{,}7$; $p < 0.05$; respectively]. In the ST, chronic CORT exposure induced a significant decrease in the time of grooming relative to control mice ($p < 0.001$) whereas this depressive-like phenotype was reversed by the chronic administration of fluoxetine (18 mg/kg/day for 1 month; p.o.; $p < 0.001$; **Figure 3A**). In the TST, although CORT failed

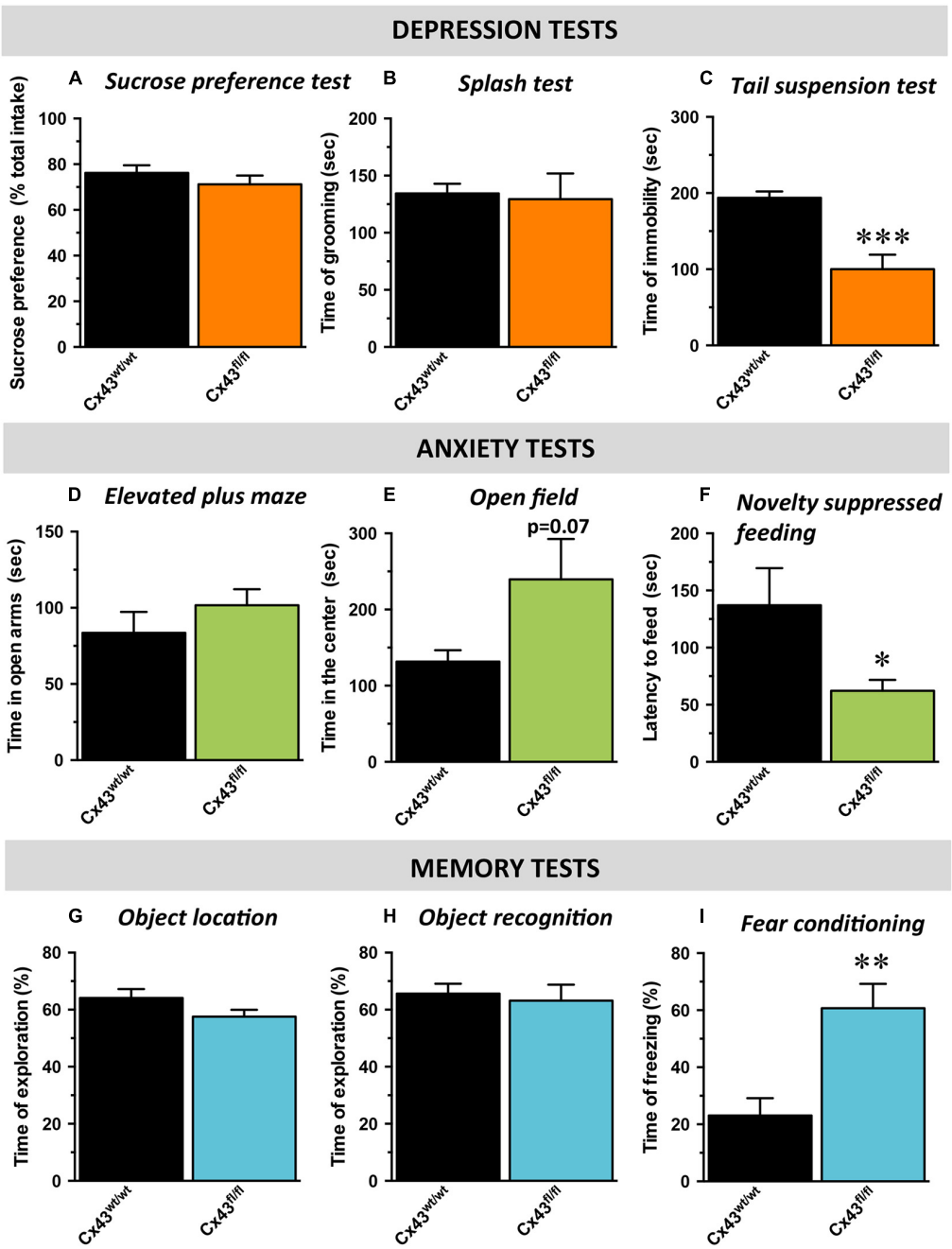


FIGURE 2 | Behavioral phenotype of Cx43$^{fl/fl}$ mice related to mood, anxiety, and memory. Data are expressed as mean ± SEM. **(A–C)** Mood evaluated by the sucrose consumption in the sucrose preference test (SPT; **A)**, the time of grooming in the splash test (ST, **B**) and the time of immobility in the tail suspension test (TST; **C**). **(D,E)** Anxiety evaluated by the time in the open arms of the elevated plus maze (EPM; **D**), the time in the center of the open field (OF; **E**) and the latency to feed in the novelty suppressed feeding (NSF; **F**). **(G–I)** cognitive performances evaluated by the time of exploration of the displaced object in the object location test (OL; **G**), of exploration of the novel object in the object recognition test (OR; **H**) and the time of freezing in the contextual fear conditioning (CFC; **I**). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$: significantly different from Cx43$^{wt/wt}$ ($n = 8$–10 mice/group).

to decrease the time of immobility ($p > 0.05$), the addition of fluoxetine significantly reduced this parameter pointing its antidepressant-like activity ($p < 0.05$; **Figure 3B**).

With respect to anxiety, in the elevated plus maze, the one-way ANOVA on the time spent in the open arms revealed a significant effect of treatment factor [$F_{(2,29)} = 4,3$; $p < 0.05$]. Indeed, CORT decreased this parameter ($p < 0.05$) whereas fluoxetine did not reverse this effect ($p = 0.3$; **Figure 3C**). In the OF, the one-way ANOVA revealed a significant effect of treatment factor on the time spent in the center but not on the number of entries in

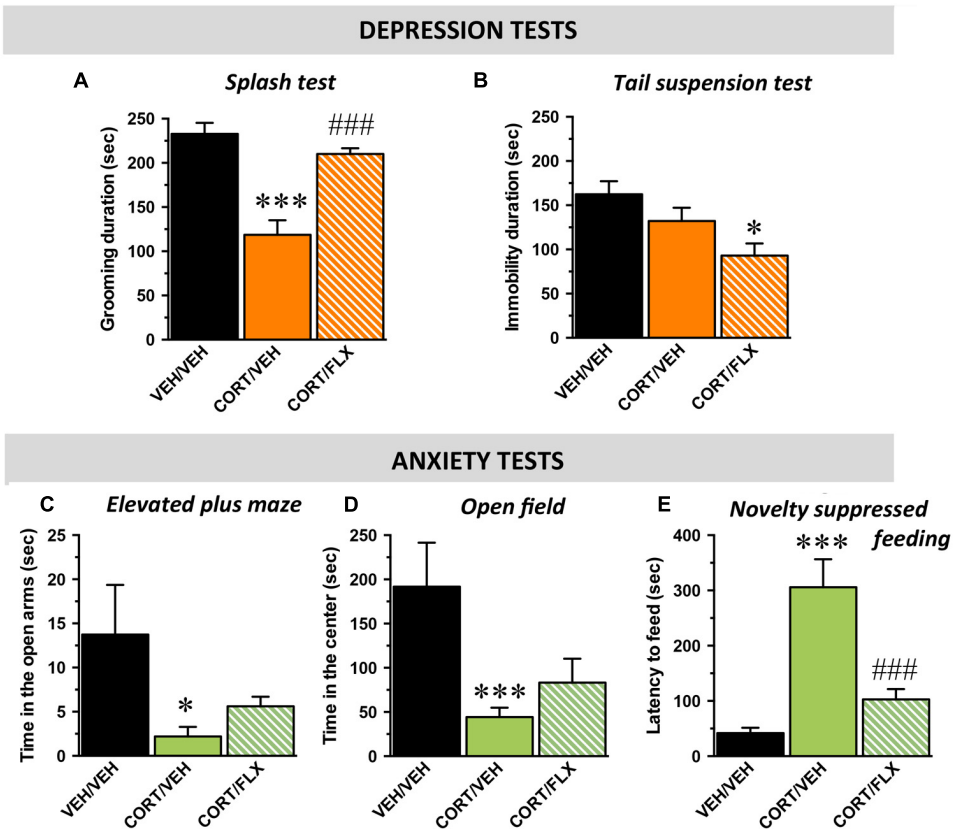


FIGURE 3 | Behavioral phenotype of wild-type mice subjected to corticosterone (CORT) given alone or in combination with fluoxetine. Data are expressed as mean ± SEM in wild-type mice administered with with vehicle (VEH) or CORT(35 μg/ml/day; p.o.) given alone or in combination with fluoxetine (FLX; 18 mg/kg/day; p.o.). **(A,B)** Mood evaluated by the time of grooming in the ST **(A)** and the time of immobility in the TST **(B)**. **(C–E)** Anxiety evaluated by the time in the open arms in the elevated plus maze (EPM; **C**), the time in the center of the arena in the **(D)** and the latency to feed in the **(E)**. $^{*}p < 0.05$, $^{***}p < 0.001$: significantly different from the VEH-treated group· $^{\#\#\#}p < 0.001$: significantly different from the CORT/VEH-treated group. ($n = 10$–11 mice/group).

this compartment [$F_{(2,29)} = 8{,}4$; $p < 0.01$ and $F_{(2,29)} = 2{,}1$; $p = 0.14$; respectively]. Hence, CORT decreased the time in the center ($p < 0.001$) and again, fluoxetine failed to significantly reverse this effect ($p = 0.3$; **Figure 3D**). Finally, in the NSF, the one way-ANOVA showed a significant effect of treatment factor [$F_{(2,29)} = 14{,}6$; $p < 0.001$]. CORT induced a significant increase in the latency to feed ($p < 0.001$) and this effect was reversed by fluoxetine ($p < 0.001$; **Figure 3E**). To eliminate putative bias, body weight loss and food consumption in the home cage were monitored and no differences were observed between the experimental groups [$F_{(2,29)} = 0{,}1$; $p = 0.8$ and $F_{(2,29)} = 0{,}2$; $p = 0.7$; respectively] (**Supplementary Figures S3A–C**).

Because MD is multimodal, quantifiable assessment of depressive-like state is possible when different behavioral parameters can be measured in the same animal. Based on the *z*-score method, we thus normalized each parameter from the average of the corresponding values observed in the control group and integrated these values into a single score (**Supplementary Table S1**). We obtained an emotionality *z*-score for which the one-way ANOVA showed a significant effect of treatment factor [$F_{(2,29)} = 32{,}1$; $p < 0.001$]. As expected, CORT significantly increased the emotionality *z*-score relative to control animals ($p < 0.001$) and this effect was reversed by fluoxetine ($p < 0.001$; **Figure 4A**).

In a subgroup of animal subjected to the behavioral paradigms, we examined their levels of Cx43 expression in the whole hippocampus. Although one way-ANOVA revealed no effect of treatment factor on total Cx43 levels [$F_{(2,14)} = 0{,}6$; $p = 0.5$] (**Supplementary Figure S4A**), a significant effect was reported on its phosphorylated form [$F_{(2,14)} = 5{,}8$; $p < 0.05$]. We showed that CORT increased the levels of phosphorylated Cx43 ($p < 0.05$) and this was reversed by fluoxetine ($p < 0.01$; **Figure 4B**). Interestingly, western blot analysis did not reveal any effect of treatment factor for the expression of total Cx30 in the hippocampus [$F_{(2,14)} = 1{,}2$; $p = 0.3$] (**Supplementary Figure S4B**).

A significant positive correlation between emotionality *z*-scores and the level of expression of phosphorylated Cx43 was unveiled (Pearson $r = 0.64$; $p < 0.01$; **Figure 4C**). The higher the level of phosphorylated Cx43, the higher the emotionality *z*-score.

These results demonstrate that the anxiety and depressive-like symptoms induced by CORT correlate with an increase in the level of Cx43 phosphorylation in the hippocampus

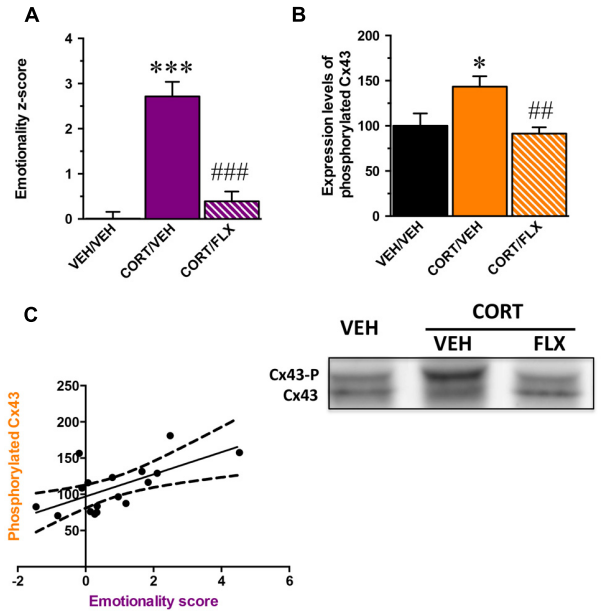


FIGURE 4 | Correlation between the phosphorylation state of Connexin 43 and the emotionality-score. (A) emotionality *z*-score integrating all behavioral parameters assessed in the ST, test suspension test, elevated plus maze, OF, and NSF in mice administered with vehicle (VEH) or CORT(35 μg/ml/day; p.o.) given alone or in combination with fluoxetine (FLX; 18 mg/kg/day; p.o.). Emotionality *z*-scores used in this correlation analysis concern the subpopulation of mice for which the expression of hippocampal Cx43 was examined. **(B)** Quantification of non-phosphorylated Cx43 levels in the hippocampus of mice administered with vehicle VEH or CORT given alone or in combination with FLX. **(C)** Correlation between emotionality *z*-scores and non-phosphorylated Cx43 levels in the hippocampus. Tubulin was used as a loading control. $^{*}p < 0.05$ and $^{***}p < 0.001$: significantly different from the VEH-treated group. $\#\#p < 0.01$ and $\#\#\#p < 0.001$: significantly different from the CORT/VEH-treated group. ($n = 10$–11 mice/group for *z*-scores and $n = 5$–6 for western-blot).

whereas chronic administration with fluoxetine normalizes this parameter.

DISCUSSION

In the present study, we provide evidence that the constitutive knock-down of astroglial Cx43 in mice leads to behavioral changes reminiscent of antidepressant-/anxiolytic-like activities specifically in paradigms producing a high level of stress. In parallel of these experiments and in a mouse model of depression, we also report that the antidepressant-/anxiolytic-like activities of the SSRI fluoxetine is associated with a decrease in the phosphorylation level of Cx43 in the hippocampus.

To examine the role of Cx43 on emotion-related behaviors, our initial strategy was to selectively drive the inactivation of Cx43 within hippocampal astrocytes through a local injection of pseudotyped lentivirus containing the Cre-recombinase (Colin et al., 2009). However, the observation that the expression of Cx43 in $Cx43^{fl/fl}$ mice was dramatically decreased led us to abandon this strategy and challenge the relevance of comparing these floxed mice with their counterparts injected with the Cre-recombinase in discrete brain regions. Although the reason of such a constitutive deletion in $Cx43^{fl/fl}$ mice remains unknown, we decided to compare the behavior of $Cx43^{fl/fl}$ mice having a constitutive decrease in Cx43 expression as previously reported (Theis et al., 2003; Unger et al., 2012) with $Cx43^{wt/wt}$ mice. Our results demonstrate that $Cx43^{fl/fl}$ mice displayed an antidepressant- and anxiolytic-like phenotype in the TST and the NSF but not in the ST, the elevated plus maze and the OF. Hence, we unveiled that the constitutive Cx43 deletion improves mood and decreases anxiety specifically in paradigms producing a high level of stress. Although the role of Cx43 on these behaviors has been poorly investigated, our results are in line with earlier experiments showing increased exploratory and anxiolytic-like behaviors in mice with astrocyte-directed deletion of Cx43 in the whole brain (i.e., $Cx43^{fl/fl}$:GFAP-Cre mice; Frisch et al., 2003). We also examined the mnesic performances of $Cx43^{fl/fl}$ mice because learning and memory impairments represent other symptoms usually affected in MD as shown in clinical (Trivedi and Greer, 2014) and preclinical studies (Darcet et al., 2014). Interestingly, we observed that the constitutive Cx43 deletion improves the long-term memory in the contextual fear conditioning as antidepressant drugs do (for review see Keefe et al., 2014). Again, Cx43 deletion produced beneficial effects in a paradigm for which the intensity of the experienced stress is high whereas it failed to improve memory in the object location and ORTs. Together these data shed new light on the fact that deficiency in Cx43 would favor stress response whereas part of antidepressant activity might rely on the blockade of this protein. This statement is in line with a recent work showing that Cx43 in astrocytes may participate in the pathogenesis of stress-associated psychiatric disorders (Orellana et al., 2015).

To further explore the putative link between stress response and Cx43, we subjected wild-type mice to CORT and evaluated the levels of Cx expression. The chronic administration of CORT in the drinking water is a well-recognized neuroendocrine-based model of depressive-like behavior (David et al., 2009). As expected and previously reported in mice (David et al., 2009; Hache et al., 2012; Quesseveur et al., 2013b; Le Dantec et al., 2014), we showed that CORT elicited behavioral anomalies typically observed in depressed patients such as carelessness, despair, and anxiety. Because full quantifiable assessment of mood-related behavior is possible when the same animal is exposed to multiple tests covering a wide range of representative symptoms of depression, we established an emotionality *z*-score integrating all these behavioral parameters into a single value. We provided evidence that CORT significantly increased emotionality *z*-score compared to control animals as a valid index of pathological state. Indeed, the higher the emotionality *z*-score, the higher the behavioral impairments (Guilloux et al., 2011; Petit et al., 2014). On the contrary, the sustained administration of the SSRI decreased this emotionality *z*-score notably due to its ability to produce antidepressant-/anxiolytic-like activities in the ST, TST, and the NSF. Interestingly, neither CORT nor fluoxetine changed the expression of total Cx43 in the hippocampus but both treatments affected its levels of phosphorylation. One of the most remarkable result obtained herein is the fact that

emotionality *z*-score is positively correlated with the levels of Cx43 phosphorylation in the hippocampus. If we consider that phosphorylation is a prerequisite for acute function of Cx (Solan and Lampe, 2009), then our results suggest that the therapeutic effects of antidepressant drugs might implicate the functional inactivation of Cx43. There is now evidence that SSRIs inhibit 5-HT uptake through the blockade of neuronal and astrocytic SERT. The subsequent enhancement of extracellular 5-HT levels likely contributes to activate astroglial 5-HT receptors (Quesseveur et al., 2013a) which, in turn, might directly regulates Cx function and/or expression. In keeping with this hypothesis, it has been shown that the application of 5-HT on hippocampal primary culture of astrocytes decreases intracellular Ca^{2+} wave propagation or dye transfer between neighboring cells (Blomstrand et al., 1999). The latter findings strengthen the hypothesis that the therapeutic activity of SSRIs would result, at least in part, from the reduction of glial cells coupling. However, these considerations are not necessarily consistent with data showing that chronic treatments with antidepressant drugs such as fluoxetine or duloxetine increase, on the contrary, astrocytic Cx43 GJC coupling (Sun et al., 2012) raising the possibility that the enhancement of astrocyte–astrocyte communication, notably in the cortex, plays an important role in antidepressant drugs response. Several explanations might be advanced to explain the discrepancies between the latter results and the findings described in the present study. The brain region (i.e., the PFC vs. the hippocampus) is an important concern that should be taken into consideration. Functional imaging studies of depressed patients indicate that the clinical response of fluoxetine is associated with a decreased functional activity of limbic regions including the hippocampus and an increased activity of the cortex (Mayberg et al., 2000). It is therefore possible that cortical and hippocampal astrocytes, according to their cellular and molecular environment, differentially impact neuronal activity. If the activation of Cx43 GJCs in the cortex seems to exert beneficial antidepressant-like effects, it is possible that its inactivation in the hippocampus is also a necessary condition to obtain similar therapeutic responses. To test for this hypothesis, we directly injected the Cx inhibitor CBX in the ventral hippocampus. However, our results indicate that the intra-hippocampal CBX elicited depressive-like state in the TST (**Supplementary Figure S5**) similarly to the observation made after its intra-cortical infusion (Sun et al., 2012). The latter results strongly suggest that Cx43 GJC and HC exert different effects on stress and antidepressant drugs response.

Opposite Effects of Gap-Junctions and Hemi-Channels in Response to Stress and Antidepressant Drugs

On this background, the question may then arise as to whether the apparent beneficial effects of Cx43 down-regulation and/or inactivation on antidepressant-/anxiolytic-like activities and cognitive performances reported herein result from change in GJC and/or HC activity? The behavioral phenotyping of Cx43-deficient mice along with pharmacological studies using Cx blockers in relevant animal models of depression emphasize the importance of these proteins in the regulation of mood-related behaviors, while the respective contribution of each function is at present unknown, in particular for the HC function of Cx43.

Our results unveil a deleterious effect of Cx43 on antidepressant response. Besides their role in GJC, Cx43 also form HC to allow a direct communication with neurons by releasing gliotransmitters or other neuroactive substances in the extracellular compartment (Saez et al., 2005; Ransom and Giaume, 2013). Interestingly, acute restraint stress stimulates the opening of astrocytic Cx43 HC and a corollary of such functional change is an enhancement of glutamate release from astrocytes HCs thereby leading to neurotoxic effects (Orellana et al., 2015). These findings along with the observation that CRF stimulate the expression of Cx43 (Hanstein et al., 2009), suggest that enhanced HC activity in astrocytes could contribute to dysfunction in emotional brain circuits. In our experimental conditions, one would assume that chronic CORT exposure stimulated Cx43 HC activity to promote a prolonged and exaggerated release of glutamate in the hippocampus thereby producing neuronal damage and/or alteration in mood-related neurotransmission as previously reported in this model (David et al., 2009; Rainer et al., 2012). In this case, fluoxetine would have exerted its beneficial effects by attenuating Cx43 HC activity reflected herein by its ability to decreased Cx43 phosphorylation in response to stress. In this case, it is, however, unclear why the majority of gliotransmitters (ATP, adenosine, lactate or D-Serine) released by Cx43 are known to elicit antidepressant-like effects (for review Etievant et al., 2013) or to improve fear learning and memory in rodents (Suzuki et al., 2011; Stehberg et al., 2012; Yang et al., 2014). One explanation would be that stress specifically triggers the release of deleterious gliotransmitters in a Cx43 HC-dependent manner. Different ionic selectivities for Cx have already been observed (Wang and Veenstra, 1997) but data on gliotransmitters are still lacking. It is, however, noteworthy that the degree of Cx43 phosphorylation would be an important process to regulate such selectivity (Bao et al., 2007). Finally, we should take into consideration that Cx43 might also allow the secretion of pro-inflammatory factors such as interleukines, cytokines or chemokines. This has been already observed in response to different pathological conditions including neuropathic pain (Chen et al., 2012, 2014) and this would reconcile our results with the inflammatory hypothesis of depression.

The second hypothesis regarding Cx43 and emotionality implies that a normal activity of Cx43-mediated GJC function is required for antidepressant response. Astroglial GJC are permeable for several endogenous molecules, i.e., second messengers [cyclic AMP, inositol-1,4,5-trisphos-phate (InsP3), and Ca^{2+}], amino acids (glutamate, aspartate, and taurine), nucleotides (ADP, ATP, CTP, and NAD) but also energy metabolites and their transfer facilitate information processing and integration from a large number of neurons, in providing metabolites during high neuronal demand (Giaume and Theis, 2010). Considering that antidepressant-like effects of SSRIs are associated with an enhancement of brain plasticity (cell proliferation, neuronal growth and sprouting, dendritic remodeling; Duman et al., 1999), one would expect that the

facilitation of astrocyte–astrocyte communication would be in favor of a therapeutic response. As an example of the physiological importance of communication among these glial cells, we recently demonstrated that astrocytes are able to release Brain-Derived Neurotrophic Factor (BDNF) in response to fluoxetine (Quesseveur et al., 2013b). Given that such a release can be regulated by the intracellular astrocytic concentration of Ca^{2+} (Zafra et al., 1992) or glutamate (Jean et al., 2008) through Cx43 GJC, we can fairly anticipate their beneficial effects on antidepressant drug response notably owing to their proximity with neuronal synapses (Araque et al., 1999; Perea and Araque, 2010). Indeed, this widespread transfer of signaling molecules through Cx43 GJC should stimulate brain plasticity as it represents a neurotrophic supply to neurons. Interestingly, in a recent study exploring the role of astroglial Cx on various stages of hippocampal adult neurogenesis, it was reported that ablation of astroglial Cx43 significantly attenuated proliferation and reduced the survival of adult-born cells (Kunze et al., 2009; Li et al., 2010; Liebmann et al., 2013) confirming that these proteins are required to promote hippocampal adult neurogenesis and related antidepressant-/anxiolytic-like activities.

CONCLUSION

Although the influence of antidepressant drugs on Cx43 expression is well described, little is known about the effects of these treatments on the functional status of Cx43 GJC and Cx43 HC. Our discussion illustrates the fact that both GJC and HC may have different effects on antidepressant drugs response leading to a complex regulation of emotionality by astrocytes. In the present study, we focused our attention on the hippocampus suggesting that the inactivation of Cx43 HC might induce beneficial effects through an attenuation of the stress response. The recent identification of anatomical and functional interactions between the hippocampus and the hypothalamus (Radley and Sawchenko, 2011) reinforces the interest to further explore the influence of hippocampal astroglial Cx43 in the regulation of the neuroendocrine system.

AUTHOR CONTRIBUTIONS

GQ in charge of the behavioral experiments in mice subjected to corticosterone. BP western-blot analysis in $Cx43^{fl/fl}$ mice. J-AB in charge of the behavioral experiments in $Cx43^{fl/fl}$ mice. PE western-blot analysis in mice subjected to corticosterone. AM in charge of behavioral experiments. HH mice genotyping. CL article writing. Scientific expertise. XF in charge of the behavioral experiments in $Cx43^{fl/fl}$ mice. ND article writing. Scientific expertise. CG provide de $Cx43^{fl/fl}$ mice. Article writing. Scientific expertise. CR article writing. Scientific expertise. BG project designer – article writing.

FUNDING

Fondation Recherche Médicale (FRM): DPP20151033959: BG; Région Midi-Pyrénées: BG.

ACKNOWLEDGMENT

We thank Stéphane Pech for his technical support and the ABC facility for housing mice.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fncel.2015.00490

FIGURE S1 | Schematic representation of the experimental designs. **(A)** $Cx43^{wt/wt}$ and $Cx43^{fl/fl}$ mice were subjected to behavioral tests over a 1-week period. **(B)** Wild-type mice were subjected to a 8-week exposure to corticosterone (CORT) followed by a 4-week exposure to fluoxetine or its vehicle. Behavioral tests were then conducted for 1 week with a 2-day period between each test.

FIGURE S2 | (A) Survival curve showing the fraction of animal not eating during the novelty suppressed feeding (NSF). **(B)** Similar body weight loss (in gr) between $Cx43^{wt/wt}$ and $Cx43^{fl/fl}$ mice after a 24-h period of fasting. **(C)** Similar home cage consumption (in gr/gr of mouse body weight) during 5 min between $Cx43^{wt/wt}$ and $Cx43^{fl/fl}$ mice after the NSF test. **(D,E)** Time of exploration of the objects in the OL **(D)** and OR **(E)** during the acquisition phas at D1. **(F)** Time of freezing before the shock in the FCF.

FIGURE S3 | (A) Survival curve showing the fraction of animal not eating during the NSF. **(B)** Similar body weight loss (in gr) between VEH/VEH-, CORT/VEH-, and CORT/FLX-treated mice after a 24-h period of fasting. **(C)** Similar home cage consumption (in gr/gr of mouse body weight) during 5 min between VEH/VEH-, CORT/VEH-, and CORT/FLX-treated mice after the NSF test.

FIGURE S4 | Expression of total astroglial Cxs. (A) Levels of Cx43 in VEH/VEH-, CORT/VEH-, and CORT/FLX-treated mice. **(B)** Levels of Cx30 in VEH/VEH-, CORT/VEH-, and CORT/FLX-treated mice.

FIGURE S5 | Depressive-like effect of intra-hippocampal infusion of an astroglial Cx43 blocker in wild-type mice in the tail suspension test. **(A)** Experimental protocol. Wild-type mice were bilaterally implanted with cannula within the ventral hippocampus and after a 1-week period of recovery, they were tested in the tail suspension test 15 min after the intra-hippocampal infusion of carbenoxolone (CBX 10 mM) or its vehicle. The same animals were tested 1 week later with a higher dose of CBX (100 mM). **(A,B)** Data are expressed as mean ± SEM of the time of immobility after CBX (10 mM) **(B)** or (100 mM) **(C)**. $^*p < 0.05$: significantly different from the VEH-infused group ($n = 7$ mice/group).

TABLE S1 | Mathematical method for Emotionality score calculation. The *Z*-score is a dimensionless mathematical value obtained by subtracting each individual data (X) from the mean of the control group (μ) and then dividing the difference by the standard deviation of the control group (σ). *Z*-score of the Splash Test (ST), the Elevated Plus Maze (EPM) and the Open Field (OF) were converted into opposite values (i.e., by putting the negative sign in front of the formula) in order to standardize the directionality of these scores and to ensure that increased values reflected a pathological state. An individual *z*-score was therefore determined for each parameter. In the case of behavioral tests integrating multiple parameters, such as the EPM and the OF, an averaged *Z*-score was calculated across parameters. Locomotor activity in the EPM and OF was integrated in the averaged *Z*-score of these tests in order to avoid potential biases induced by locomotion on anxiety-like behaviors. Finally, an individual Emotionality score is obtained by averaging *Z*-score of all tests.

REFERENCES

Araque, A., Sanzgiri, R. P., Parpura, V., and Haydon, P. G. (1999). Astrocyte-induced modulation of synaptic transmission. *Can. J. Physiol. Pharmacol.* 77, 699–706. doi: 10.1139/y99-076

Araya-Callis, C., Hiemke, C., Abumaria, N., and Flugge, G. (2012). Chronic psychosocial stress and citalopram modulate the expression of the glial proteins GFAP and NDRG2 in the hippocampus. *Psychopharmacology (Berl.)* 224, 209–222. doi: 10.1007/s00213-012-2741-x

Banasr, M., Chowdhury, G. M., Terwilliger, R., Newton, S. S., Duman, R. S., Behar, K. L., et al. (2010). Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Mol. Psychiatry* 15, 501–511. doi: 10.1038/mp.2008.106

Bao, X., Lee, S. C., Reuss, L., and Altenberg, G. A. (2007). Change in permeant size selectivity by phosphorylation of connexin 43 gap-junctional hemichannels by PKC. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4919–4924. doi: 10.1073/pnas.0603154104

Binder, E. B., and Nemeroff, C. B. (2010). The CRF system, stress, depression and anxiety-insights from human genetic studies. *Mol. Psychiatry* 15, 574–588. doi: 10.1038/mp.2009.141

Bissiere, S., Zelikowsky, M., Ponnusamy, R., Jacobs, N. S., Blair, H. T., and Fanselow, M. S. (2011). Electrical synapses control hippocampal contributions to fear learning and memory. *Science* 331, 87–91. doi: 10.1126/science.1193785

Blier, P. (2001). Pharmacology of rapid-onset antidepressant treatment strategies. *J. Clin. Psychiatry* 62(Suppl. 15), 12–17.

Blomstrand, F., Aberg, N. D., Eriksson, P. S., Hansson, E., and Ronnback, L. (1999). Extent of intercellular calcium wave propagation is related to gap junction permeability and level of connexin-43 expression in astrocytes in primary cultures from four brain regions. *Neuroscience* 92, 255–265. doi: 10.1016/S0306-4522(98)00738-6

Chen, G., Park, C. K., Xie, R. G., Berta, T., Nedergaard, M., and Ji, R. R. (2014). Connexin-43 induces chemokine release from spinal cord astrocytes to maintain late-phase neuropathic pain in mice. *Brain* 137(Pt 8), 2193–2209. doi: 10.1093/brain/awu140

Chen, M. J., Kress, B., Han, X., Moll, K., Peng, W., Ji, R. R., et al. (2012). Astrocytic CX43 hemichannels and gap junctions play a crucial role in development of chronic neuropathic pain following spinal cord injury. *Glia* 60, 1660–1670. doi: 10.1002/glia.22384

Colin, A., Faideau, M., Dufour, N., Auregan, G., Hassig, R., Andrieu, T., et al. (2009). Engineered lentiviral vector targeting astrocytes in vivo. *Glia* 57, 667–679. doi: 10.1002/glia.20795

Czeh, B., Simon, M., Schmelting, B., Hiemke, C., and Fuchs, E. (2006). Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology* 31, 1616–1626. doi: 10.1038/sj.npp.1300982

Dalvi, A., and Lucki, I. (1999). Murine models of depression. *Psychopharmacology (Berl)* 147, 14–16. doi: 10.1007/s002130051131

Darcet, F., Mendez-David, I., Tritschler, L., Gardier, A. M., Guilloux, J. P., and David, D. J. (2014). Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. *Front. Behav. Neurosci.* 8:136. doi: 10.3389/fnbeh.2014.00136

David, D. J., Samuels, B. A., Rainer, Q., Wang, J. W., Marsteller, D., Mendez, I., et al. (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* 62, 479–493. doi: 10.1016/j.neuron.2009.04.017

Dermietzel, R., Traub, O., Hwang, T. K., Beyer, E., Bennett, M. V., Spray, D. C., et al. (1989). Differential expression of three gap junction proteins in developing and mature brain tissues. *Proc. Natl. Acad. Sci. U.S.A.* 86, 10148–10152. doi: 10.1073/pnas.86.24.10148

Duman, R. S., Malberg, J., and Thome, J. (1999). Neural plasticity to stress and antidepressant treatment. *Biol. Psychiatry* 46, 1181–1191. doi: 10.1016/S0006-3223(99)00177-8

Etievant, A., Lambas-Senas, L., Scarna, H., Lucas, G., and Haddjeri, N. (2013). Astrocytes and gliotransmitters: new players in the treatment of major depression? *Curr. Drug. Targets* 14, 1295–1307. doi: 10.2174/13894501131499990197

Ezan, P., Andre, P., Cisternino, S., Saubamea, B., Boulay, A. C., Doutremer, S., et al. (2012). Deletion of astroglial connexins weakens the blood-brain barrier. *J. Cereb. Blood Flow Metab.* 32, 1457–1467. doi: 10.1038/jcbfm.2012.45

Frisch, C., Theis, M., De Souza Silva, M. A., Dere, E., Sohl, G., Teubner, B., et al. (2003). Mice with astrocyte-directed inactivation of connexin43 exhibit increased exploratory behaviour, impaired motor capacities, and changes in brain acetylcholine levels. *Eur. J. Neurosci.* 18, 2313–2318. doi: 10.1046/j.1460-9568.2003.02971.x

Giaume, C., and Theis, M. (2010). Pharmacological and genetic approaches to study connexin-mediated channels in glial cells of the central nervous system. *Brain Res. Rev.* 63, 160–176. doi: 10.1016/j.brainresrev.2009.11.005

Guilloux, J. P., Seney, M., Edgar, N., and Sibille, E. (2011). Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: relevance to emotionality and sex. *J. Neurosci. Methods* 197, 21–31. doi: 10.1016/j.jneumeth.2011.01.019

Hache, G., Guiard, B. P., Le Dantec, Y., Orvoen, S., David, D. J., Gardier, A. M., et al. (2012). Antinociceptive effects of fluoxetine in a mouse model of anxiety/depression. *Neuroreport* 23, 525–529. doi: 10.1097/WNR.0b013e328353d70a

Hanstein, R., Trotter, J., Behl, C., and Clement, A. B. (2009). Increased connexin 43 expression as a potential mediator of the neuroprotective activity of the corticotropin-releasing hormone. *Mol. Endocrinol.* 23, 1479–1493. doi: 10.1210/me.2009-0022

Jean, Y. Y., Lercher, L. D., and Dreyfus, C. F. (2008). Glutamate elicits release of BDNF from basal forebrain astrocytes in a process dependent on metabotropic receptors and the PLC pathway. *Neuron Glia Biol.* 4, 35–42. doi: 10.1017/S1740925X09000052

Keefe, R. S., Fox, K. H., Davis, V. G., Kennel, C., Walker, T. M., Burdick, K. E., et al. (2014). The Brief assessment of cognition in affective disorders (BAC-A): performance of patients with bipolar depression and healthy controls. *J. Affect. Disord.* 166, 86–92. doi: 10.1016/j.jad.2014.05.002

Kunze, A., Congreso, M. R., Hartmann, C., Wallraff-Beck, A., Huttmann, K., Bedner, P., et al. (2009). Connexin expression by radial glia-like cells is required for neurogenesis in the adult dentate gyrus. *Proc. Natl. Acad. Sci. U.S.A.* 106, 11336–11341. doi: 10.1073/pnas.0813160106

Lecrubier, Y. (2001). The burden of depression and anxiety in general medicine. *J. Clin. Psychiatry* 62(Suppl. 8), 4–9.

Le Dantec, Y., Hache, G., Guilloux, J. P., Guiard, B. P., David, D. J., Adrien, J., et al. (2014). NREM sleep hypersomnia and reduced sleep/wake continuity in a neuroendocrine mouse model of anxiety/depression based on chronic corticosterone administration. *Neuroscience* 274, 357–368. doi: 10.1016/j.neuroscience.2014.05.050

Li, D. Q., Li, X. J., Duan, J. F., and Cai, W. (2010). Wuling Capsule promotes hippocampal neurogenesis by improving expression of connexin 43 in rats exposed to chronic unpredictable mild stress. *Zhong Xi Yi Jie He Xue Bao* 8, 662–669. doi: 10.3736/jcim20100710

Liebmann, M., Stahr, A., Guenther, M., Witte, O. W., and Frahm, C. (2013). Astrocytic Cx43 and Cx30 differentially modulate adult neurogenesis in mice. *Neurosci. Lett.* 545, 40–45. doi: 10.1016/j.neulet.2013.04.013

Mayberg, H. S., Brannan, S. K., Tekell, J. L., Silva, J. A., Mahurin, R. K., McGinnis, S., et al. (2000). Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. *Biol. Psychiatry* 48, 830–843. doi: 10.1016/S0006-3223(00)01036-2

McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., and Paylor, R. (2001). The use of behavioral test batteries: effects of training history. *Physiol. Behav.* 73, 705–717. doi: 10.1016/S0031-9384(01)00528-5

Miguel-Hidalgo, J. J., Baucom, C., Dilley, G., Overholser, J. C., Meltzer, H. Y., Stockmeier, C. A., et al. (2000). Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. *Biol. Psychiatry* 48, 861–873. doi: 10.1016/S0006-3223(00)00999-9

Miguel-Hidalgo, J. J., Wilson, B. A., Hussain, S., Meshram, A., Rajkowska, G., and Stockmeier, C. A. (2014). Reduced connexin 43 immunolabeling in the orbitofrontal cortex in alcohol dependence and depression. *J. Psychiatr. Res.* 55, 101–109. doi: 10.1016/j.jpsychires.2014.04.007

Milanovic, S. M., Erjavec, K., Poljicanin, T., Vrabec, B., and Brecic, P. (2015). Prevalence of depression symptoms and associated socio-demographic factors in primary health care patients. *Psychiatr. Danub.* 27, 31–37.

Morioka, N., Suekama, K., Zhang, F. F., Kajitani, N., Hisaoka-Nakashima, K., Takebayashi, M., et al. (2014). Amitriptyline up-regulates connexin43-gap junction in rat cultured cortical astrocytes via activation of the p38 and c-Fos/AP-1 signalling pathway. *Br. J. Pharmacol.* 171, 2854–2867. doi: 10.1111/bph.12614

Mostafavi, H., Khaksarian, M., Joghataei, M. T., Hassanzadeh, G., Soleimani, M., Eftekhari, S., et al. (2014). Fluoxetin upregulates connexin 43 expression in astrocyte. *Basic Clin. Neurosci.* 5, 74–79.

Nagy, C., Suderman, M., Yang, J., Szyf, M., Mechawar, N., Ernst, C., et al. (2015). Astrocytic abnormalities and global DNA methylation patterns in depression and suicide. *Mol. Psychiatry* 20, 320–328. doi: 10.1038/mp.2014.21

Nichols, N. R., Osterburg, H. H., Masters, J. N., Millar, S. L., and Finch, C. E. (1990). Messenger RNA for glial fibrillary acidic protein is decreased in rat brain following acute and chronic corticosterone treatment. *Brain Res. Mol. Brain Res.* 7, 1–7. doi: 10.1016/0169-328X(90)90066-M

Orellana, J. A., Moraga-Amaro, R., Diaz-Galarce, R., Rojas, S., Maturana, C. J., Stehberg, J., et al. (2015). Restraint stress increases hemichannel activity in hippocampal glial cells and neurons. *Front. Cell Neurosci.* 9:102. doi: 10.3389/fncel.2015.00102

Paylor, R., Spencer, C. M., Yuva-Paylor, L. A., and Pieke-Dahl, S. (2006). The use of behavioral test batteries, II: effect of test interval. *Physiol. Behav.* 87, 95–102. doi: 10.1016/j.physbeh.2005.09.002

Perea, G., and Araque, A. (2010). GLIA modulates synaptic transmission. *Brain Res. Rev.* 63, 93–102. doi: 10.1016/j.brainresrev.2009.10.005

Petit, A. C., Quesseveur, G., Gressier, F., Colle, R., David, D. J., Gardier, A. M., et al. (2014). Converging translational evidence for the involvement of the serotonin 2A receptor gene in major depressive disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 54, 76–82. doi: 10.1016/j.pnpbp.2014.04.013

Quesseveur, G., Gardier, A. M., and Guiard, B. P. (2013a). The monoaminergic tripartite synapse: a putative target for currently available antidepressant drugs. *Curr. Drug Targets* 14, 1277–1294. doi: 10.2174/13894501113149990209

Quesseveur, G., David, D. J., Gaillard, M. C., Pla, P., Wu, M. V., Nguyen, H. T., et al. (2013b). BDNF overexpression in mouse hippocampal astrocytes promotes local neurogenesis and elicits anxiolytic-like activities. *Transl. Psychiatry* 3:e253. doi: 10.1038/tp.2013.30

Radley, J. J., and Sawchenko, P. E. (2011). A common substrate for prefrontal and hippocampal inhibition of the neuroendocrine stress response. *J. Neurosci.* 31, 9683–9695. doi: 10.1523/JNEUROSCI.6040-10.2011

Rainer, Q., Nguyen, H. T., Quesseveur, G., Gardier, A. M., David, D. J., and Guiard, B. P. (2012). Functional status of somatodendritic serotonin 1A autoreceptor after long-term treatment with fluoxetine in a mouse model of anxiety/depression based on repeated corticosterone administration. *Mol. Pharmacol.* 81, 106–112. doi: 10.1124/mol.111.075796

Ransom, B. R., and Giaume, C. (2013). "Gap junctions, hemichannels," in *Neuroglia*, 3rd Edn, eds H. Kettenmann and B. R. Ransom (Oxford: Oxford University Press), 292–305.

Rouach, N., and Giaume, C. (2001). Connexins and gap junctional communication in astrocytes are targets for neuroglial interaction. *Prog. Brain Res.* 132, 203–214. doi: 10.1016/S0079-6123(01)32077-0

Rouach, N., Segal, M., Koulakoff, A., Giaume, C., and Avignone, E. (2003). Carbenoxolone blockade of neuronal network activity in culture is not mediated by an action on gap junctions. *J. Physiol.* 553(Pt 3), 729–745. doi: 10.1113/jphysiol.2003.053439

Saez, J. C., Retamal, M. A., Basilio, D., Bukauskas, F. F., and Bennett, M. V. (2005). Connexin-based gap junction hemichannels: gating mechanisms. *Biochim. Biophys. Acta* 1711, 215–224. doi: 10.1016/j.bbamem.2005.01.014

Santarelli, L., Gobbi, G., Debs, P. C., Sibille, E. T., Blier, P., Hen, R., et al. (2001). Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proc. Natl. Acad. Sci. U.S.A.* 98, 1912–1917. doi: 10.1073/pnas.98.4.1912

Si, X., Miguel-Hidalgo, J. J., O'Dwyer, G., Stockmeier, C. A., and Rajkowska, G. (2004). Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression. *Neuropsychopharmacology* 29, 2088–2096. doi: 10.1038/sj.npp.1300525

Solan, J. L., and Lampe, P. D. (2009). Connexin43 phosphorylation: structural changes and biological effects. *Biochem. J.* 419, 261–272. doi: 10.1042/BJ20082319

Stehberg, J., Moraga-Amaro, R., Salazar, C., Becerra, A., Echeverria, C., Orellana, J. A., et al. (2012). Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. *FASEB J.* 26, 3649–3657. doi: 10.1096/fj.11-198416

Sun, J. D., Liu, Y., Yuan, Y. H., Li, J., and Chen, N. H. (2012). Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. *Neuropsychopharmacology* 37, 1305–1320. doi: 10.1038/npp.2011.319

Surget, A., Saxe, M., Leman, S., Ibarguen-Vargas, Y., Chalon, S., Griebel, G., et al. (2008). Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. *Biol. Psychiatry* 64, 293–301. doi: 10.1016/j.biopsych.2008.02.022

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., et al. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810–823. doi: 10.1016/j.cell.2011.02.018

Theis, M., Jauch, R., Zhuo, L., Speidel, D., Wallraff, A., Doring, B., et al. (2003). Accelerated hippocampal spreading depression and enhanced locomotory activity in mice with astrocyte-directed inactivation of connexin43. *J. Neurosci.* 23, 766–776.

Theis, M., Mas, C., Doring, B., Kruger, O., Herrera, P., Meda, P., et al. (2001). General and conditional replacement of connexin43-coding DNA by a lacZ reporter gene for cell-autonomous analysis of expression. *Cell Commun. Adhes* 8, 383–386. doi: 10.3109/15419060109080758

Torres-Platas, S. G., Nagy, C., Wakid, M., Turecki, G., and Mechawar, N. (2015). Glial fibrillary acidic protein is differentially expressed across cortical and subcortical regions in healthy brains and downregulated in the thalamus and caudate nucleus of depressed suicides. *Mol. Psychiatry* doi: 10.1038/mp.2015.65 [Epub ahead of print].

Trivedi, M. H., and Greer, T. L. (2014). Cognitive dysfunction in unipolar depression: implications for treatment. *J. Affect. Disord.* 152–154, 19–27. doi: 10.1016/j.jad.2013.09.012

Unger, T., Bette, S., Zhang, J., Theis, M., and Engele, J. (2012). Connexin-deficiency affects expression levels of glial glutamate transporters within the cerebrum. *Neurosci. Lett.* 506, 12–16. doi: 10.1016/j.neulet.2011.10.032

Võikar, V., Vasar, E., and Rauvala, H. (2004). Behavioral alterations induced by repeated testing in C57BL/6J and 129S2/Sv mice: implications for phenotyping screens. *Genes Brain Behav.* 3, 27–38. doi: 10.1046/j.1601-183X.2003.0044.x

Wang, H. Z., and Veenstra, R. D. (1997). Monovalent ion selectivity sequences of the rat connexin43 gap junction channel. *J. Gen. Physiol.* 109, 491–507. doi: 10.1085/jgp.109.4.491

Warner-Schmidt, J. L., and Duman, R. S. (2006). Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. *Hippocampus* 16, 239–249. doi: 10.1002/hipo.20156

Webster, M. J., Knable, M. B., Johnston-Wilson, N., Nagata, K., Inagaki, M., and Yolken, R. H. (2001). Immunohistochemical localization of phosphorylated glial fibrillary acidic protein in the prefrontal cortex and hippocampus from patients with schizophrenia, bipolar disorder, and depression. *Brain Behav. Immun.* 15, 388–400. doi: 10.1006/brbi.2001.0646

Yang, J., Ruchti, E., Petit, J. M., Jourdain, P., Grenningloh, G., Allaman, I., et al. (2014). Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. *Proc. Natl. Acad. Sci. U.S.A.* 111, 12228–12233. doi: 10.1073/pnas.1322912111

Zafra, F., Lindholm, D., Castren, E., Hartikka, J., and Thoenen, H. (1992). Regulation of brain-derived neurotrophic factor and nerve growth factor mRNA in primary cultures of hippocampal neurons and astrocytes. *J. Neurosci.* 12, 4793–4799.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2015 Quesseveur, Portal, Basile, Ezan, Mathou, Halley, Leloup, Fioramonti, Déglon, Giaume, Rampon and Guiard. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Cellular Neuroscience

ORIGINAL RESEARCH
published: 07 January 2016
doi: 10.3389/fncel.2015.00495

Antidepressants Impact Connexin 43 Channel Functions in Astrocytes

*Tiffany Jeanson[1,2,3,4], Audrey Pondaven[1,2,3], Pascal Ezan[1,2,3], Franck Mouthon[4], Mathieu Charvériat[4] and Christian Giaume[1,2,3]**

[1] Collège de France, Center for Interdisciplinary Research in Biology/Centre National de la Recherche Scientifique, Unité Mixte de Recherche 7241/Institut National de la Santé et de la Recherche Médicale U1050, Paris, France, [2] University Pierre et Marie Curie, Paris, France, [3] MemoLife Laboratory of Excellence and Paris Science Lettre Research University, Paris, France, [4] Theranexus, Lyon, France

Glial cells, and in particular astrocytes, are crucial to maintain neuronal microenvironment by regulating energy metabolism, neurotransmitter uptake, gliotransmission, and synaptic development. Moreover, a typical feature of astrocytes is their high expression level of connexins, a family of membrane proteins that form gap junction channels allowing intercellular exchanges and hemichannels that provide release and uptake pathways for neuroactive molecules. Interestingly, several studies have revealed unexpected changes in astrocytes from depressive patients and rodent models of depressive-like behavior. Moreover, changes in the expression level of the astroglial connexin 43 (Cx43) have been reported in a depressive context. On the other hand, antidepressive drugs have also been shown to impact the expression of this connexin in astrocytes. However, so far there is little information concerning the functional consequence of these changes, i.e., the status of gap junctional communication and hemichannel activity in astrocytes exposed to antidepressants. In the present work we focused our attention on the action of seven antidepressants from four different therapeutic classes and tested their effects on Cx43 expression and on the two connexin-based channels functions studied in cultured astrocytes. We here report that when used at non-toxic and clinically relevant concentrations they have no effects on Cx43 expression but differential effects on Cx43 gap junction channels. Moreover, all tested antidepressants inhibit Cx43 hemichannel with different efficiency depending on their therapeutic classe. By studying the impact of antidepressants on the functional status of astroglial connexin channels, contributing to dynamic neuroglial interactions, our observations should help to better understand the mechanism by which these drugs provide their effect in the brain.

Keywords: gap Junctions, hemichannels, glial cells, depression, inflammation

OPEN ACCESS

Edited by:
Egidio D'Angelo,
University of Pavia, Italy

Reviewed by:
Ping Liu,
University of Connecticut Health Center, USA
Sandra Henriques Vaz,
Universidade de Lisboa, Portugal

****Correspondence:***
Christian Giaume
christian.giaume@college-de-france.fr

Received: *28 September 2015*
Accepted: *09 December 2015*
Published: *07 January 2016*

Citation:
Jeanson T, Pondaven A, Ezan P, Mouthon F, Charvériat M and Giaume C (2016) Antidepressants Impact Connexin 43 Channel Functions in Astrocytes. Front. Cell. Neurosci. 9:495. doi: 10.3389/fncel.2015.00495

INTRODUCTION

During the two last decades, a major step in the understanding of brain functions and dysfunctions has been to consider that not only neurons are at the center of these processes but that also their glial environment is actively involved. This statement is particularly true for astrocytes, a major glial cell population that establishes tight morphological and functional interactions with neurons (Halassa and Haydon, 2010; Verkhratsky et al., 2012) leading to the concept of the "tripartite

synapse" (see Araque et al., 1999; Pérez-Alvarez and Araque, 2013). In brain pathologies and mental illness this partnership is impaired, contributing to severe neuronal defects and even in certain cases leading to neuronal death (Giaume et al., 2007; Bennett et al., 2012; Parpura et al., 2012). Such alterations in neuroglial interaction start to be investigated in order to identify and develop alternative therapeutic approaches that target astrocytes instead of solely neurons (Colangelo et al., 2014; Lundgaard et al., 2014; Vardjan et al., 2015). Following this strategy, the objective is to act on a specific astroglial molecular constituent known to regulate neuronal activity and/or survival. Based on these requirements astroglial connexins (Cxs), a family of membrane proteins, may be considered as a good candidate. Indeed, Cxs are highly expressed in astrocytes compared to all other brain cell populations (see Ransom and Giaume, 2013), including neurons, and they have been reported to interplay with synaptic activity and plasticity (Pannasch et al., 2011), animal behavior (Stehberg et al., 2012), and neuronal survival (Froger et al., 2010; Freitas-Andrade and Naus, 2015). Besides, the expression and function of astroglial Cxs are affected in neurodegenerative diseases (Kawasaki et al., 2009; Koulakoff et al., 2012; Takeuchi and Suzumura, 2014), ischemia and stroke (Orellana et al., 2014), epilepsy (Mylvaganam et al., 2014), demyelinating diseases (Cotrina and Nedergaard, 2012) and cancer (Naus and Laird, 2010). Much less is known about the status, i.e., expression and function, of Cxs in astrocytes in non-neurodegenerative mood disorders such as depression, while those pathologies are associated to a reduction in the number of astrocytes and a decrease in GFAP immunoreactivity (see Rajkowska and Stockmeier, 2013).

Connexins are the molecular constituents of gap junctions that are membrane specializations consisting of dense aggregates of large pore channels formed by two paired hexamers of Cxs. These gap junction channels extend from one cell into an adjacent cell and mediate a unique direct cytoplasm-to-cytoplasm communication. These channels are poorly selective for ions and for small molecular weight signaling molecules, thus they allow extensive ionic and biochemical exchanges between cells (Harris, 2007). In astrocytes, gap junction channels provide the basis for ionic homeostasis, particularly for potassium buffering and intercellular calcium signaling. They are also involved in biochemical and metabolic coupling (see Ransom and Giaume, 2013). Under certain conditions Cxs can also operate as half of a gap junction channel, named "hemichannel," representing another functional state that provides a pathway suitable for autocrine as well as paracrine interactions in the brain. In astrocytes, connexin hemichannels are permeable to ions and are involved in the release of gliotransmitters such as ATP and glutamate (Ye et al., 2003; Kang et al., 2008; Abudara et al., 2015), the uptake of glucose (Retamal et al., 2007) and the efflux of glutathione (Rana and Dringen, 2007; Ye et al., 2015). In astrocytes two major Cxs have been identified, Cx43 and Cx30, which are not expressed in other brain cell types and are characterized by different developmental and regional patterns of expression (Nagy et al., 2004). Both Cxs contribute to gap junctional communication but so far only hemichannels made by Cx43 have been reported to be functional in astrocytes (see Giaume et al., 2013).

The information concerning astroglial Cxs and depression is based on two kinds of observations available from the literature. Firstly, those that report changes in the expression of astroglial Cxs from depressive patients, persons having committed suicide or from animal models of depression. Indeed, a Canadian study of postmortem generated microarray data suicide completers indicated that the expression level of Cx43 and Cx30 is reduced in dorsal lateral prefrontal cortex (Ernst et al., 2011) and in the locus coeruleus (Bernard et al., 2011). In addition, a recent study has reported that the expression of Cx43 is reduced in postmortem brains from patients suffering from major depressive disorder or comorbid depression relative to healthy subjects (Miguel-Hidalgo et al., 2014). Also, Sun et al. (2012) have reported a decrease in diffusion of gap junction channel-permeable dye and expression of Cx43 in the prefrontal cortex in rats subjected to chronic unpredictable stress. Secondly, on the other side treatment with antidepressants also results in changes in the expression level of Cx43 in astrocytes as indicated in **Table 1**. Five antidepressants, from three different therapeutic classes, have been tested in cellular and animal models; results indicate that 24–48 h treatment induces an increase in Cx43 expression at mRNA and/or protein levels. This is particularly the case for fluoxetine that has been tested in several models (Fatemi et al., 2008; Mostafavi et al., 2008; Sun et al., 2012). However, there is little information about the effect of these drugs on the functional status of gap junctional communication and none about hemichannel activity. So far based on this literature it is tempting to deduce and summarize that depressive brains show a down-regulation of Cx43 while antidepressant treatments favor its up-regulation (see Rajkowska and Stockmeier, 2013). Nevertheless, an important clue concerning Cxs is to identify the functional consequences of these treatments on Cx43-based channels since changes in level of expression can have unpredictable consequences on their function. In order to address this question we have carried out a systematic test of seven antidepressants on gap junctional communication and hemichannel activity in primary cultures of astrocytes, known to express only Cx43 (Dermietzel et al., 1991; Giaume et al., 1991; Koulakoff et al., 2008). The present study indicates that when used at a non-toxic and clinically relevant concentration they have differential effects on both channel functions leading to a more complicated global view of their action on intercellular communication mediated by Cx43 in astrocytes.

MATERIALS AND METHODS

All experiments were performed according to the European Community Council Directives of 2010/63/UE and all efforts were made to minimize the number of animals. This study was carried out in accordance with the recommendations of the Ethic Committee 59, Paris, France and received the approval of the

TABLE 1 | Summary table of the effects of antidepressants on Cx43 expression and gap junctional function in astrocytes.

Antidepressant	Class	Effect on Cx43 expression	Effect on Cx43 function	Model	Treatment (dose and time)	Reference
Amitriptyline	TCA	Increase (mRNA, protein)	Increase (gap junction)	Primary cultures rat cortical astrocytes	25 μM, 48 h	Morioka et al., 2014
Clomipramine	TCA	Increase (protein)	NT	Primary culture rat cortical astrocytes	10 μM, 48 h	Morioka et al., 2014
Fluoxetine	SSRI	Increase (protein)	NT	Rat *in vivo* Prefrontal cortex	20 mg/kg i.p. for 21 days	Fatemi et al., 2008
Fluoxetine	SSRI	Increase (mRNA, protein)	NT	Human astrocytoma cell line	10, 20 μg/ml 24 h	Mostafavi et al., 2008
Fluoxetine	SSRI	Increase (protein)	No effect (gap junction)	Rat *in vivo* Prefrontal cortex	10 mg/kg 21 days	Sun et al., 2012
Duloxetine	SNRI	Increase (mRNA, protein)	No effect (gap junction)	Rat *in vivo* Prefrontal cortex	10 mg/kg 21 days	Sun et al., 2012
Fluvoxamine	SSRI	Increase (protein)	NT	Primary cultures rat cortical astrocytes	25 μM 48 h	Morioka et al., 2014

Scientific Committee of the animal facilities of the Collège de France.

Cortical Astrocyte Cultures

Primary astrocyte cultures were prepared from the cortex of newborn (1–2 days) OF1 mice as previously described (Meme et al., 2006). For western blot and scrape-loading dye-transfer experiments, cells were seeded on polyornithine-coated 35-mm-diameter dishes (Nunc, Roskilde, Denmark) at a density of 5×10^5cells/mL. For hemichannel experiments cells were seeded (2×10^5 cells per well) on glass coverslips (Gassalem, Limeil-Brévannes, France) placed inside 24-round-well plate; area 1.9 cm2/well; (NunClon, Thermoscientific, Atlanta, GA, USA). Cellular medium, DMEM (Sigma–Aldrich, St-Louis MO, USA), supplemented with penicillin (5 U/ml), streptomycin (5 μg/ml; Invitrogen, Carlsbad, CA, USA), fungizone amphotericin B (500 ng/mL; Gibco, Life Technologies, Carlsbad, CA, USA), and 10% FCS (Hyclone, Logan, UT, USA), was changed twice a week. When cells reached confluence, around 10 days *in vitro* (DIV), they were harvested with trypsin-EDTA (Invitrogen). The medium was changed twice a week until the experiments were carried out. In order to characterize the proportion of microglia in primary culture of astrocytes, the two cell types were identified by immunostaining with Isolectine B4 and GFAP antibodies, respectively.

Products and Cell Treatments

Astrocyte cultures were treated for 24 h with lipopolysaccharide (LPS, 1 μg/ml) and/or the following antidepressants: fluoxetine, duloxetine, paroxetine, reboxetine, amitriptyline, imipramine, venlafaxine (5, 10, or 20 μM, Sigma–Aldrich, Saint-Louis, MO, USA). Carbenoxolone (50 μM, Sigma–Aldrich) was used as positive inhibitor control for gap junction channels. Drugs were prepared either in H_20 or DMSO. Control cells received no treatment and were previously studied with vehicle (H20 or DMSO) which induced no changes in comparison with untreated cells.

Determination of Gap Junctional Communication

Experiments were performed by using the scrape-loading dye-transfer technique, as previously described (Meme et al., 2006). Briefly, cells were incubated at room temperature for 10 min in HEPES buffered salt solution containing (in mM): NaCl, 140; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1; glucose, 10; HEPES, 5 at pH 7.4. Cells were then washed with a calcium-free HEPES solution for 1 min and the scrape loading and dye transfer assay (see Giaume et al., 2012) was carried out in the same calcium-free solution containing Lucifer yellow CH (427 Da, 1 mg/ml). One minute after scraping procedure, cells were washed with the HEPES solution and then Lucifer yellow loaded in the cells was allowed to diffuse through gap junction channels for 8 min. Photomicrographs were taken and data were quantified using NIS Nikon software. In all experiments, the fluorescence area of the first row of cells initially loaded, as measured in the presence of the gap junction channel inhibitor carbenoxolone (50 μM, 24 h), was subtracted from the total fluorescence area.

Ethidium Bromide Uptake Experiments in Cortical Astrocyte Cultures

Following 10 min exposure to 5 μM ethydium bromide (EtBr), cells were washed with HEPES buffered salt solution containing (in mM): NaCl, 140; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1; glucose, 10; HEPES, 5 at pH 7.4. After 10 min in fixing solution (4% paraformaldehyde in 0.12 M buffer phosphate) and rinsing with phosphate buffered saline (PBS), cells were mounted in Fluoromount-G mounting medium (Orellana et al., 2011). Images of astrocyte cultures were taken with a 40× objective using a confocal laser-scanning microscope (Leica TBCS SP5). Stacks of consecutive confocal images for 10 μm at 0.49 μm intervals were acquired with an argon ion laser at 488 nm. Confocal images of EthBr uptake were analyzed with Image J software. The EtBr fluorescence intensity in the nuclei of astrocytes in each image was measured and the average of six images of different areas in the same culture was calculated the final measurement of dye uptake in that culture.

Western Blot

After 24 h of treatment, cultures were rinsed with PBS 1X and added 75 μL of a solution containing protease and phosphatase inhibitors (orthovanadate 1 mM; α-glycerophosphate 10 mM), and complete miniprotease inhibitor (Roche Diagnostics, Meylan, France). Cells were then harvested by scraping with a rubber policeman and pelleted cells were added 20 μL of 5X Laemmli sample buffer. Samples were boiled for 5 min, placed on ice, and lysed by sonication (Ultrasonic cell disrupter, Microson, Bruxelles, Belgium). Then, samples were stored at −20°C. Proteins were measured with the Bio-Rad protein assay (Bio-Rad laboratories, Richmond, CA, USA). For each cell lysate sample, 20 μg of proteins were separated on Bis-Tris 4–12% NuPAGE gels and electro-transferred to nitrocellulose sheets as previously described (Orellana et al., 2011). Non-specific protein binding was blocked by incubation of nitrocellulose sheets in tris-buffered saline (TBS) – Tween – milk solution (500 mL TBS 1X; 500 μL Tween 20X; non-fat powder milk 25 g) for 1 h. Blots were then incubated overnight with primary antibody mouse Cx43 1:500 (Transduction Laboratories, Le Pont de Claix, France) at 4°C, followed by 4 × 15 min PBS washes. Blots were incubated with goat anti-mouse antibody 1:2500 conjugated to horseradish peroxidase (Tébu, Le Perray-En-Yveline, France). Immunoreactivity was detected by ECL detection using the SuperSignal kit (Pierce, Rockford, IL, USA) according to instructions. Blots were then reprobed with mouse monoclonal anti-glyceraldehyde 3-phosphate dehydrogenase peroxidase (Sigma–Aldrich, 1:10,000) to check the protein load. Chemiluminescence imaging was performed on a LAS4000 (Fujifilm, Stamford, CT, USA). Semiquantitative densitometric analysis was performed with ImageJ software after scanning the bands.

Statistical Analysis

For each data group, results are expressed as mean ± SEM and *n* refers to the number of independent experiments. Kruskal–Wallis test and one-way ANOVA, followed, respectively, by Dunn and Bonferroni post tests, were used as well as unpaired *t*-test. Differences are considered significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control, $+P < 0.05$, $++P < 0.01$, and $+++P < 0.001$ vs. LPS. GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA) was used for calculations.

RESULTS

The doses of the antidepressants were chosen in accordance with literature addressing the neuropharmacokinetics of the tested molecules of interest. Accordingly, cultured cortical astrocytes were treated with concentrations identical to those reported for brains of human or rodent after treatment with clinically relevant doses from *in vivo* studies: for fluoxetine, 20 μM in human brain is achieved at 20 mg/day (see Henry et al., 2005); for venlafaxine, 10 μM in mice brain is reached at 20 mg/kg (Karlsson et al., 2011); for duloxetine, 4.2 mg/kg in rat leads to 10 μM in brain (Kielbasa and Stratford, 2012). For the other molecules (amitriptyline, imipramine, paroxetine, reboxetine) the doses were selected below cell toxicity that was identified by microscopic examination of astrocyte cultures treated for 24 h, related to changed cell morphology and entry of Lucifer yellow into damaged cells. More precisely the lack of toxicity of the selected doses was routinely validated by the absence of Lucifer yellow unspecific uptake in area far from the scrape lines in scrape-loading dye-transfer experiments (see **Figure 1A**). Based on these criterions all molecules were tested at concentrations between 5 and 20 μM (24 h) for toxicity; we observed that fluoxetine at 20 μM, paroxetine 10 μM, and duloxetine 20 μM were toxic at these indicated doses, consequently these molecules were tested at lower doses.

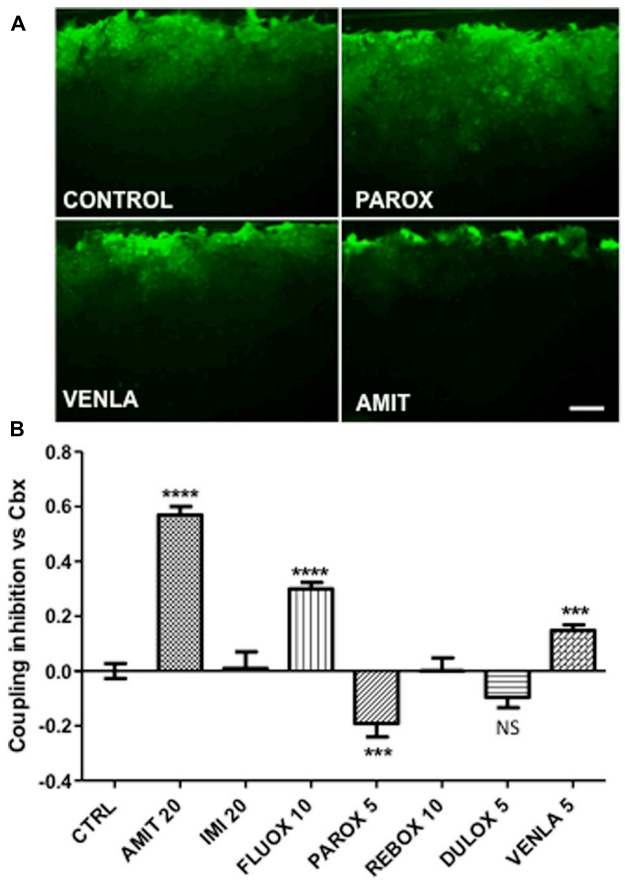


FIGURE 1 | Modulation of astrocyte Cx43 gap junctional communication after 24-h treatment by seven antidepressants in mice cortical astrocyte cultures. Amitriptyline (AMIT), imipramine (IMI), fluoxetine (FLUOX), paroxetine (PAROX), reboxetine (REBOX), duloxetine (DULOX), and venlafaxine (VENLA) were added from 5 to 20 μM in cellular medium during 24 h, gap junctional communication was evaluated by the scrape loading dye transfer method. **(A)** Pictures of control, paroxetine 5 μM, venlafaxine 5 μM, and amitriptyline 20 μM, illustrate the Lucifer yellow spreading through astrocyte gap junctions for these different treatments. **(B)** Summary diagram of junctional communication in vehicle-treated cells (control group) and after antidepressant treatment. Note that the values are normalized to carbenoxolone (50 μM, 24 h). Data are the means ± SEM $n = 3$–7 per group, $^{***}p < 0.001$, $^{****}p < 0.0001$ vs. control, Kruskal–Wallis test and one-way ANOVA followed, respectively, by Dunn and Bonferroni post test. Scale bar 20 μM.

The first question addressed during this screening with antidepressants was their effect on the level of Cx43 expression investigated by western blotting. As indicated in **Table 2**, the seven tested antidepressants did not significantly modify the level of expression of Cx43 studied in cultured cortical mouse astrocytes ($n = 3$–6 per group, $p > 0.05$ One way ANOVA, Dunn post test).

The effect of the selected antidepressants on gap junctional communication was then tested by using the scrape-loading dye transfer technique (see Giaume et al., 2012). In control condition after 10 min the Lucifer yellow diffuses widely perpendicular to the scrape line that indicates a high level of gap junctional communication (**Figure 1A**, control). As illustrated in **Figure 1B**, three different effects were observed. Three antidepressants, amitriptyline (20 μM; **Figure 1A**), fluoxetine (10 μM), and venlafaxine (5 μM; **Figure 1A**) reduced intercellular dye spread by 57% ($n = 6$), 25% ($n = 6$), and 15% ($n = 6$), respectively. In contrast, paroxetine (5 μM; **Figure 1A**) increased dye coupling by 19% ($n = 7$). Finally, imipramine (20 μM, $n = 3$), reboxetine (10 μM, $n = 6$) and duloxetine (5 μM, $n = 9$) had no statistically significant action on the level of intercellular communication between astrocytes, neither at these doses nor at higher non-toxic doses.

In normal condition, astrocytes in culture as well as in acute slices are characterized by a high level of gap junctional communication and low hemichannel activity (Bennett et al., 2003; Retamal et al., 2007; but see Chever et al., 2014). However, in most pathological situations involving brain inflammation, a reactive gliosis is associated with elevated hemichannel activity in astrocytes (see Bennett et al., 2012; Giaume et al., 2013). Such low hemichannel activity was also observed in our culture condition (**Figure 2A**). Therefore, to induce hemichannel activity we treated our cortical primary cultures, in which 11% ($n = 9$) of isolectin B4-positive microglia versus GFAP-positive astrocytes were present, with the endotoxin LPS. As already reported for *in vitro* astrocytes (Retamal et al., 2007), we observed that LPS treatment (1 μg/ml, 24 h) inhibited gap junctional communication by 64% ($n = 6$), in such condition we found that the antidepressants amitriptyline (20 μM, $n = 4$), imipramine (20 μM, $n = 4$), venlafaxine (5 μM, $n = 3$), and duloxetine (5 μM, $n = 4$) did not reverse the inhibition induced by LPS, when co-treated during 24 h in astrocyte cultures. However, paroxetine (5 μM, $n = 4$) and reboxetine (10 μM, $n = 3$) reversed LPS-induced uncoupling, in a low but significant manner, respectively, by 17% ($n = 4$) and 10% ($n = 3$) whereas fluoxetine (10 μM) improved it by 10% ($n = 4$; data not illustrated). Moreover, we found that LPS treatment (1 μg/ml, 24 h) increased by 104% ($n = 7$) the uptake of ethidium bromide (EtBr) in GFAP-positive astrocytes indicating that, as previously reported (Retamal et al., 2007; Abudara et al., 2015) hemichannels in cortical astrocytes were activated (**Figure 2A**). As expected EtBr uptake was inhibited by 93% ($n = 3$) in the presence of carbenoxolone (50 μM; 24 h) indicating that this uptake is mediated through hemichannel activity (**Figure 2B**). All the seven antidepressants tested had a significant inhibitor effect on the LPS-induced EtBr uptake, however, with difference in their efficiency. Indeed, fluoxetine (10 μM), paroxetine (5 μM), and duloxetine (5 μM; **Figure 2A**) had pronounced effect and inhibited EtBr uptake by 97% ($n = 3$), 93% ($n = 3$), and 111% ($n = 4$), respectively (**Figure 2B**). Meanwhile, amitriptyline (20 μM; **Figure 2A**), imipramine (20 μM), reboxetine (10 μM), and venlafaxine (5 μM) had milder effect with 45% ($n = 6$), 28% ($n = 3$), 52% ($n = 3$), and 23% ($n = 4$), respectively (**Figure 2B**).

DISCUSSION

The objective of the present study was to investigate the effects of a panel of antidepressants on the property of the astroglial Cx43, a membrane protein widely expressed in glial cells that contributes actively to neuroglial interaction in normal and disease brain (see Ransom and Giaume, 2013). For this purpose we undertook an *in vitro* screening of seven antidepressants from four different classes (TCA, SSRI, NRI, SNRI), all implied in serotonin and/or noradrenaline reuptake inhibition, to characterize their action on astroglial Cx43 at diverse levels: protein expression, gap junctional communication and hemichannel function. We selected a simple cellular system, primary cultures of mouse cortical and striatal astrocytes in which only Cx43 is detected (Dermietzel et al., 1991; Giaume et al., 1991; Koulakoff et al., 2008). The tested concentrations were determined either based on the literature, when clinically relevant doses were available, or by establishing experimentally their threshold of toxicity for 24 h treatment (see Results section). While we did not observe any significant change in Cx43 protein level, we found that antidepressants had differential effects on astroglial Cx43-based gap junctional communication as summarized in **Table 2**. These

TABLE 2 | Summary table of the effect of seven antidepressants for four different classes: tricyclic (TCA), selective serotonin reuptake inhibitor (SSRI), noradrenaline reuptake inhibitor (NRI), serotonin noradrenaline reuptake inhibitor (SNRI), on Cx43 expression and channel functions in cultured astrocytes.

Antidepressant	Class	Concentration tested (μM)	Cx43 expression	Effect on gap junctional coupling	Effect on hemichannels
Amitriptyline	TCA	20	No significant effect	Inhibition	Mild inhibition
Imipramine	TCA	20	No significant effect	No effect	Mild inhibition
Fluoxetine	SSRI	10	No significant effect	Inhibition	Total inhibition
Paroxetine	SSRI	5	No significant effect	Increase	Total inhibition
Reboxetine	NRI	10	No significant effect	No effect	Mild inhibition
Duloxetine	SNRI	5	No significant effect	No effect	Total inhibition
Venlafaxine	SNRI	5	No significant effect	Inhibition	Mild inhibition

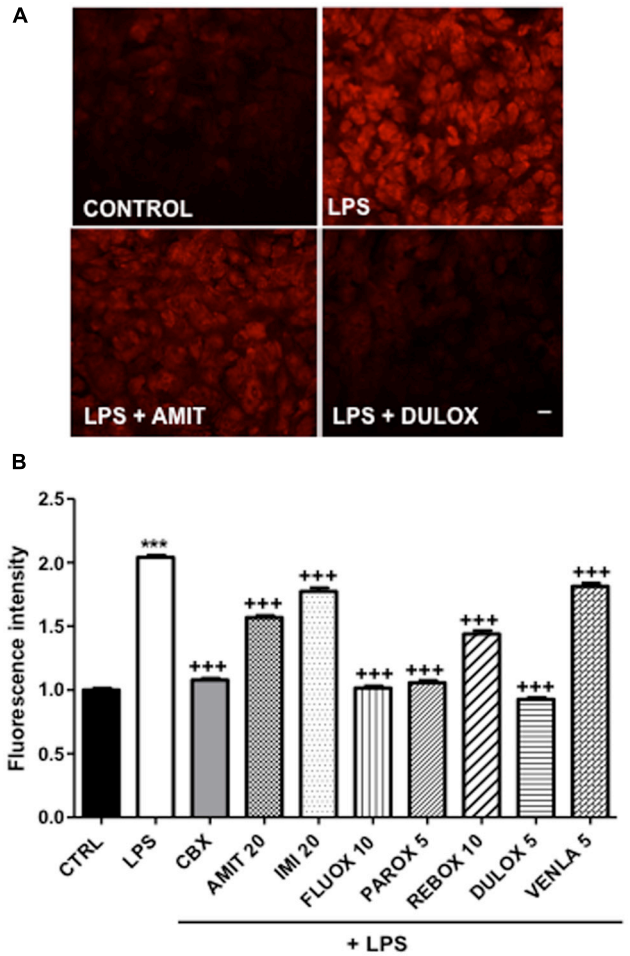


FIGURE 2 | Regulation of hemichannel activity after 24-h antidepressant treatments in mice cortical astrocytic cultures. LPS 1 μg/mL was added 24-h before experiment to induce hemichannel opening and hemichannel activity was evaluated by using the ethidium bromide uptake assay. **(A)** Pictures of control, LPS alone, LPS + amitriptyline 20 μM, and LPS + duloxetine 5 μM uptake experiments. **(B)** Summary diagram of hemichannel activity in untreated astrocytes (CTRL) and after treatments with amitriptyline (AMIT), imipramine (IMI), fluoxetine (FLUOX), paroxetine (PAROX), reboxetine (REBO), duloxetine (DULOX), and venlafaxine (VENLA) were also added from 5 to 20 μM in cellular medium during 24 h. Carbenoxolone (CBX, 50 μM, 24 h) was used as a positive control for the inhibition of Cx43 hemichannels. Data are the means ± SEM $n = 3$–6 per group, $^{***}p < 0.001$ vs. control, $+++p < 0.001$ vs. LPS, One-way ANOVA followed by Dunn post test. Scale bar 20 μM.

results are different compared those reported in the few articles that so far have addressed these properties for some of the antidepressants used in the present study, i.e., amitriptyline, fluoxetine, and duloxetine (see **Tables 1** and **2**). Indeed, Sun et al. (2012) and Morioka et al. (2014) reported, respectively, an enhancement in Cx43 mRNA and protein levels in astrocytes from cultured rat astrocytes treated with amitriptyline and in the prefrontal cortex of rats chronically treated with duloxetine (Sun et al., 2012; Morioka et al., 2014). Additionally, two studies have also shown a rise in Cx43 protein levels in the prefrontal cortex after *in vivo* treatment with fluoxetine (Fatemi et al., 2008; Sun et al., 2012), as well as in human astrocytoma cultures (Mostafavi et al., 2008). However, no changes in Cx43 gap junctional communication have been found after *in vivo* treatments with both fluoxetine and duloxetine in rats (Sun et al., 2012) whereas this Cx43 function was increased after amitriptyline treatment in cultured rat astrocytes (Morioka et al., 2014). In our experiments (see **Table 2**) only paroxetine (5 mM) was found to increase gap junctional communication while duloxetine (5 μM) has a slight, but not statistically significant increasing effect. Imipramine (20 μM) and reboxetine (10 μM) had no effect and an inhibition was observed with amitriptyline (20 μM), fluoxetine (10 μM), and venlafaxine (5 μM). These effects were not specific to a defined class of antidepressants and allow for taking a step back regarding results from other models. Indeed, our study demonstrates that the link between antidepressants and Cx43-mediated intercellular communication in astrocyte is likely more complex than the literature consensual interpretation concerning an increase of Cx43 expression and function, and that opposed effects are observed within a same therapeutic class. However, when pointing out the differences between our results and the current literature, several parameters must be taken into account. First, the models of study differed: we used cultured mouse astrocytes, while Sun et al. (2012) and Fatemi et al. (2008) worked *in vivo* on rats, and others used cell culture models from various species and cellular types in culture (human astrocytoma and rat astrocytes). Second, dosage, time, and chronicity of the treatments also differ. Indeed, our *in vitro* tested concentrations were non-toxic and similar to what is found in the brain in pharmacokinetics studies addressing clinically relevant doses for these molecules, and ranged from 1 to 100 μM in the literature. In literature, *in vitro* treatments were administered for several minutes to 48 h (see **Table 1**). We treated for 24 h, as for example preliminary trials of 48-h treatments with amitriptyline (20 μM) induced cell toxicity. In addition, in the studies that were performed using *in vivo* models, animals received chronic treatment (see **Table 1**) that certainly involved more integrated and complex mechanisms. Taken as a whole these information complete and extend the knowledge about the effect of antidepressants on gap junctional communication in astrocytes. Finally, as no changes in Cx43 expression were detected after treatment with antidepressant, we suggested that they act at the post-translational level.

Up-to-now there was no indication about the effect of antidepressants on the other channel function of Cx43 in astrocytes, i.e., the hemichannel activity. This lack of information can be attributed to the fact that for astrocytes this activity has been established much latter than the gap junction channel function and that hemichannels are weakly opened in normal conditions hence requiring a pathological context to be activated (see Bennett et al., 2003; Giaume et al., 2013). We confirmed this feature on untreated primary cortical cultures as the Cx channel inhibitor carbenoxolone had no significant no effect (data not shown) on the uptake of EtBr (see Giaume et al., 2012). However, we took advantage that our LPS-stimulated cultures contained a non-negligible proportion of microglial cells (11%), a situation already identified to activated Cx43 hemichannels and

to reduce gap junctional communication in astrocytes (Retamal et al., 2007). Indeed, we previously showed that when LPS-stimulated microglia are co-cultured with astrocytes two pro-inflammatory cytokines are released, i.e., TNF-α and IL-1β, activating Cx43 hemichannel activity in astrocytes (Retamal et al., 2007; Abudara et al., 2015). Interestingly, LPS has been reported to cause time-dependent behavioral alterations with sickness behavior (Huang et al., 2008) and a depressive-like behavior observed 24 h after LPS challenge (Painsipp et al., 2011; Custódio et al., 2013); and it is noteworthy that this effect is reversed by fluoxetine (Yirmiya et al., 2001), imipramine (Tomaz et al., 2014), paroxetine and duloxetine (Ohgi et al., 2013). Besides, the level of TNF-α and IL-1β, are increased after LPS treatments in animal model, similarly to the increase observed in subjects with major depression disease (Seidel et al., 1995; Sluzewska et al., 1996; Dowlati et al., 2010; Hannestad et al., 2011). On this basis LPS has been proposed as an inducer of depressive-like context (Mello et al., 2013; Ohgi et al., 2013) confirming the interest of LPS in the evaluation of antidepressant mechanisms. Using the EtBr uptake assay we found that all tested antidepressants had an inhibitory effect on LPS-induced astroglial hemichannel activity. The effect on Cx43-based hemichannel function was correlated when considering the class of the antidepressants, the SSRIs (fluoxetine, paroxetine) induced a strong inhibition while the TCAs (amitriptyline, imipramine) and NRI (reboxetine) had mild effect, the treatment with SNRI (duloxetine, venlafaxine) resulting in mixed inhibition efficiency. Interestingly, several of these antidepressants are known to have an inhibitory effect on the production of pro-inflammatory cytokines, in particular TNF-α and IL-1β, fluoxetine (Chiou et al., 2006; Tai et al., 2009; Valera et al., 2014), amitriptyline (Obuchowicz et al., 2006; Tai et al., 2009), paroxetine (Liu et al., 2014), and imipramine (Lee et al., 2012). Meanwhile venlafaxine, which presents the lowest inhibitory effect on LPS-induced hemichannel activity (23%), is the only tested compound that increased the level of TNF-α (Valera et al., 2014). Taken as a whole these results could support the idea that antidepressants may control Cx43 hemichannel activity through the production of TNF-a and/or IL-1β as the result of a reduction of microglial activation. However, just paroxetine and reboxetine modestly reversed the inhibition of gap junctional communication induced by LPS, it could consequently imply that antidepressant effects are not targeting microglia-released interleukins. It could also suggest that the antidepressant inhibitory effects on hemichannels are acting downstream to the microglial step and/or more directly on the Cx43 hemichannel function. Nevertheless, more work is needed to decipher the specific mechanisms involved in the regulation of astroglial Cx channels by antidepressants, an aim that is beyond the scope of the present study.

Interestingly, hemichannel activity in astrocytes has been shown to provide a pathway for glutamate release (Ye et al., 2003; Abudara et al., 2015). Consequently, the antidepressant inhibitory action on hemichannel activity could support the current hypothesis of the action of these drugs on glutamine/glutamate metabolic cycle (Garakani et al., 2013) and glutamate transmission (Gorman and Docherty, 2010; Sanacora and Banasr, 2013) in the pathophysiology of major depression (Etiévant et al., 2013). Finally, glutamate gliotransmission mediated by Cx43 hemichannels has been recently reported to occur in a model of chronic restraint stress (Orellana et al., 2015) which is admitted as a model inducing depressive-like symptoms in rodents (Levinstein and Samuels, 2014).

The present study reflects the need to re-evaluate the statement according to which an alternative strategy for antidepressive treatments is to target astroglial Cx43 and to increase gap junctional communication. This proposition was solely based on reports indicating that several antidepressants favor Cx43 expression levels while a few have investigated their effect on the functional aspect, i.e., gap junctional communication. Based on the present results the effect of antidepressant drugs on astroglial gap junctions appears more complex than initially thought and suggests that Cx43 hemichannel activity in astrocytes may be part of the mode of action of these drugs. Finally, our observation, and in particular those related to hemichannel activity, could benefit to the understanding of the mode of action of antidepressants in other pathologies treated by antidepressants such as neuropathic pain (Dworkin et al., 2010; Finnerup et al., 2015) and for which the involvement of glial Cx43 hemichannel activity has been proposed (Chen et al., 2014).

AUTHOR CONTRIBUTIONS

TJ and CG contributed to the study design, TJ and AP conducted the experiments, CG wrote the initial draft; TJ, CG, FM, MC contributed to the writing of the manuscript.

ACKNOWLEDGMENTS

This study was supported by the Collège de France and the INSERM. The authors wish to thank Dr. A. Koulakoff for constant and helpful discussions along this work.

REFERENCES

Abudara, V., Roux, L., Dallérac, G., Matias, I., Dulong, J., Mothet, J. P., et al. (2015). Activated microglia impairs neuroglial interaction by opening Cx43 hemichannels in hippocampal astrocytes. *Glia* 63, 795–811. doi: 10.1002/glia.22785

Araque, A., Parpura, V., Sanzgiri, R. P., and Haydon, P. G. (1999). Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* 22, 208–215. doi: 10.1016/S0166-2236(98)01349-6

Bennett, M. V., Contreras, J. E., Bukauskas, F. F., and Sáez, J. C. (2003). New roles for astrocytes: gap junction hemichannels have something to communicate. *Trends Neurosci.* 26, 610–617. doi: 10.1016/j.tins.2003.09.008

Bennett, M. V., Garré, J. M., Orellana, J. A., Bukauskas, F. F., Nedergaard, M., Giaume, C., et al. (2012). Connexin and pannexin hemichannels in inflammatory responses of glia and neurons. *Brain Res.* 1487, 3–15. doi: 10.1016/j.brainres.2012.08.042

Bernard, R., Kerman, I. A., Thompson, R. C., Jones, E. G., Bunney, W. E., Barchas, J. D., et al. (2011). Altered expression of glutamate signaling, growth factor,

and glia genes in the locus coeruleus of patients with major depression. *Mol. Psychiatry* 16, 634–646. doi: 10.1038/mp.2010.44

Chen, G., Park, C. K., Xie, R. G., Berta, T., Nedergaard, M., and Ji, R. R. (2014). Connexin-43 induces chemokine release from spinal cord astrocytes to maintain late-phase neuropathic pain in mice. *Brain* 137(Pt 8), 2193–2209. doi: 10.1093/brain/awu140

Chever, O., Lee, C. Y., and Rouach, N. (2014). Astroglial connexin43 hemichannels tune basal excitatory synaptic transmission. *J. Neurosci.* 34, 11228–11232. doi: 10.1523/JNEUROSCI.0015-14.2014

Chiou, S. H., Chen, S. J., Peng, C. H., Chang, Y. L., Ku, H. H., Hsu, W. M., et al. (2006). Fluoxetine up-regulates expression of cellular FLICE-inhibitory protein and inhibits LPS-induced apoptosis in hippocampus-derived neural stem cell. *Biochem. Biophys. Res. Commun.* 343, 391–400. doi: 10.1016/j.bbrc.2006.02.180

Colangelo, A. M., Alberghina, L., and Papa, M. (2014). Astrogliosis as a therapeutic target for neurodegenerative diseases. *Neurosci. Lett.* 565, 59–64. doi: 10.1016/j.neulet.2014.01.014

Cotrina, M. L., and Nedergaard, M. (2012). Brain connexins in demyelinating diseases: therapeutic potential of glial targets. *Brain Res.* 1487, 61–68. doi: 10.1016/j.brainres.2012.07.003

Custódio, C. S., Mello, B. S., Cordeiro, R. C., de Araújo, F. Y., Chaves, J. H., Vasconcelos, S. M., et al. (2013). Time course of the effects of lipopolysaccharide on prepulse inhibition and brain nitrite content in mice. *Eur. J. Pharmacol.* 713, 31–38. doi: 10.1016/j.ejphar.2013.04.040

Dermietzel, R., Hertberg, E. L., Kessler, J. A., and Spray, D. C. (1991). Gap junctions between cultured astrocytes: immunocytochemical, molecular, and electrophysiological analysis. *J. Neurosci.* 11, 1421–1432.

Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E. K., et al. (2010). A meta-analysis of cytokines in major depression. *Biol. Psychiatry* 67, 446–457. doi: 10.1016/j.biopsych.2009.09.033

Dworkin, R. H., O'Connor, A. B., Audette, J., Baron, R., Gourlay, G. K., Haanpää, M. L., et al. (2010). Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. *Mayo Clin. Proc.* 85, S3–S14. doi: 10.4065/mcp.2009.0649

Ernst, C., Nagy, C., Kim, S., Yang, J. P., Deng, X., Hellstrom, I. C., et al. (2011). Dysfunction of astrocyte connexins 30 and 43 in dorsal lateral prefrontal cortex of suicide completers. *Biol. Psychiatry* 70, 312–319. doi: 10.1016/j.biopsych.2011.03.038

Etiévant, A., Lambás-Señas, L., Scarna, H., Lucas, G., and Haddjeri, N. (2013). Astrocytes and gliotransmitters: new players in the treatment of major depression? *Curr. Drug Targets* 14, 1295–1307.

Fatemi, S. H., Folsom, T. D., Reutiman, T. J., and Lee, S. (2008). Expression of astrocytic markers aquaporin 4 and connexin 43 is altered in brains of subjects with autism. *Synapse* 62, 501–507. doi: 10.1002/syn.20519

Finnerup, N. B., Attal, N., Haroutounian, S., McNicol, E., Baron, R., Dworkin, R. H., et al. (2015). Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. *Lancet Neurol.* 14, 162–173. doi: 10.1016/S1474-4422(14)70251-0

Freitas-Andrade, M., and Naus, C. C. (2015). Astrocytes in neuroprotection and neurodegeneration: the role of connexin43 and pannexin1. *Neuroscience* doi: 10.1016/j.neuroscience.2015.04.035 [Epub ahead of print].

Froger, N., Orellan, J. A., Calvo, C. F., Amigou, E., Kozoriz, M. G., Naus, C. C., et al. (2010). Inhibition of cytokine-induced connexin43 hemichannel activity in astrocytes is neuroprotective. *Mol. Cell. Neurosci.* 45, 37–46. doi: 10.1016/j.mcn.2010.05.007

Garakani, A., Martinez, J. M., Yehuda, R., and Gorman, J. M. (2013). Cerebrospinal fluid levels of glutamate and corticotropin releasing hormone in major depression before and after treatment. *J. Affect. Disord.* 146, 262–265. doi: 10.1016/j.jad.2012.06.037

Giaume, C., Fromaget, C., el Aoumari, A., Cordier, J., Glowinski, J., and Gros, D. (1991). Gap junctions in cultured astrocytes: single-channel currents and characterization of channel-forming protein. *Neuron* 6, 133–143. doi: 10.1016/0896-6273(91)90128-M

Giaume, C., Kirchhoff, F., Matute, C., Reichenbach, A., and Verkhratsky, A. (2007). Glia: the fulcrum of brain diseases. *Cell Death Differ.* 14, 1324–1335. doi: 10.1038/sj.cdd.4402144

Giaume, C., Leybaert, L., Naus, C. C., and Sáez, J. C. (2013). Connexin and pannexin hemichannels in brain glial cells: properties, pharmacology, and roles. *Front. Pharmacol.* 4:88. doi: 10.3389/fphar.2013.00088

Giaume, C., Orellana, J. A., Abudara, V., and Sáez, J. C. (2012). Connexin-based channels in astrocytes: how to study their properties. *Methods Mol. Biol.* 814, 283–303. doi: 10.1007/978-1-61779-452-0_19

Gorman, J. M., and Docherty, J. P. (2010). A hypothesized role for dendritic remodeling in the etiology of mood and anxiety disorders. *J. Neuropsychiatry Clin. Neurosci.* 22, 256–264. doi: 10.1176/appi.neuropsych.22.3.256

Halassa, M. M., and Haydon, P. G. (2010). Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu. Rev. Physiol.* 72, 335–355. doi: 10.1146/annurev-physiol-021909-135843

Hannestad, J., DellaGioia, N., and Bloch, M. (2011). The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology* 36, 2452–2459. doi: 10.1038/npp.2011.132

Harris, A. L. (2007). Connexin channel permeability to cytoplasmic molecules. *Prog. Biophys. Mol. Biol.* 94, 120–143. doi: 10.1016/j.pbiomolbio.2007.03.011

Henry, M. E., Schmidt, M. E., Hennen, J., Villafuerte, R. A., Butman, M. L., Tran, P., et al. (2005). A comparison of brain and serum pharmacokinetics of R-fluoxetine and racemic fluoxetine: a 19-F MRS study. *Neuropsychopharmacology* 30, 1576–1583. doi: 10.1038/sj.npp.1300749

Huang, Y., Henry, C. J., Dantzer, R., Johnson, R. W., and Godbout, J. P. (2008). Exaggerated sickness behavior and brain proinflammatory cytokine expression in aged mice in response to intracerebroventricular lipopolysaccharide. *Neurobiol. Aging* 29, 1744–1753. doi: 10.1016/j.neurobiolaging.2007.04.012

Kang, J., Kang, N., Lovatt, D., Torres, A., Zhao, Z., Lin, J., et al. (2008). Connexin 43 hemichannels are permeable to ATP. *J. Neurosci.* 28, 4702–4711. doi: 10.1523/JNEUROSCI.5048-07.2008

Karlsson, L., Hiemke, C., Carlsson, B., Josefsson, M., Ahlner, J., Bengtsson, F., et al. (2011). Effects on enantiomeric drug disposition and open-field behavior after chronic treatment with venlafaxine in the P-glycoprotein knockout mice model. *Psychopharmacology (Berl.)* 215, 367–377. doi: 10.1007/s00213-010-2148-5

Kawasaki, A., Hayashi, T., Nakachi, K., Trosko, J. E., Sugihara, K., Kotake, Y., et al. (2009). Modulation of connexin 43 in rotenone-induced model of Parkinson's disease. *Neuroscience* 160, 61–68. doi: 10.1016/j.neuroscience.2009.01.080

Kielbasa, W., and Stratford, R. E. Jr. (2012). Exploratory translational modeling approach in drug development to predict human brain pharmacokinetics and pharmacologically relevant clinical doses. *Drug Metab. Dispos.* 40, 877–883. doi: 10.1124/dmd.111.043554

Koulakoff, A., Ezan, P., and Giaume, C. (2008). Neurons control the expression of connexin 30 and connexin 43 in mouse cortical astrocytes. *Glia* 56, 1299–1311. doi: 10.1002/glia.20698

Koulakoff, A., Mei, X., Orellana, J. A., Sáez, J. C., and Giaume, C. (2012). Glial connexin expression and function in the context of Alzheimer's disease. *Biochim. Biophys. Acta* 1818, 2048–2057. doi: 10.1016/j.bbamem.2011.10.001

Lee, Y. H., Kim, S. H., Kim, Y., Lim, Y., Ha, K., and Shin, S. Y. (2012). Inhibitory effect of the antidepressant imipramine on NF-κB-dependent CXCL1 expression in TNFα-exposed astrocytes. *Int. Immunopharmacol.* 12, 547–555. doi: 10.1016/j.intimp.2012.01.011

Levinstein, M. R., and Samuels, B. A. (2014). Mechanisms underlying the antidepressant response and treatment resistance. *Front. Behav. Neurosci.* 8:208. doi: 10.3389/fnbeh.2014.00208

Liu, R. P., Zou, M., Wang, J. Y., Zhu, J. J., Lai, J. M., Zhou, L. L., et al. (2014). Paroxetine ameliorates lipopolysaccharide-induced microglia activation via differential regulation of MAPK signaling. *J. Neuroinflammation* 11, 47. doi: 10.1186/1742-2094-11-47

Lundgaard, I., Osório, M. J., Kress, B. T., Sanggaard, S., and Nedergaard, M. (2014). White matter astrocytes in health and disease. *Neuroscience* 276, 161–173. doi: 10.1016/j.neuroscience.2013.10.050

Mello, B. S., Monte, A. S., McIntyre, R. S., Soczynska, J. K., Custódio, C. S., Cordeiro, R. C., et al. (2013). Effects of doxycycline on depressive-like behavior in mice after lipopolysaccharide (LPS) administration. *J. Psychiatr. Res.* 47, 1521–1529. doi: 10.1016/j.jpsychires.2013.06.008

Meme, W., Calvo, C. F., Froger, N., Ezan, P., Amigou, E., Koulakoff, A., et al. (2006). Proinflammatory cytokines released from microglia inhibit gap junctions in astrocytes: potentiation by b -amyloid. *FASEB J.* 20, 494–496.

Miguel-Hidalgo, J. J., Wilson, B. A., Hussain, S., Meshram, A., Rajkowska, G., and Stockmeier, C. A. (2014). Reduced connexin 43 immunolabeling in the orbitofrontal cortex in alcohol dependence and depression. *J. Psychiatr. Res.* 55, 101–109. doi: 10.1016/j.jpsychires.2014.04.007

Morioka, N., Suekama, K., Zhang, F. F., Kajitani, N., Hisaoka-Nakashima, K., Takebayashi, M., et al. (2014). Amitriptyline up-regulates connexin43-gap junction in rat cultured cortical astrocytes via activation of the p38 and c-Fos/AP-1 signalling pathway. *Br. J. Pharmacol.* 171, 2854–2867. doi: 10.1111/bph.12614

Mostafavi, H., Khaksarian, M., Joghataei, M. T., Hassanzadeh, G., Soleimani, M., Eftekhari, S., et al. (2008). Fluoxetin upregulates connexin 43 expression in astrocyte. *Basic Clin. Neurosci.* 5, 74–79.

Mylvaganam, S., Ramani, M., Krawczyk, M., and Carlen, P. L. (2014). Roles of gap junctions, connexins, and pannexins in epilepsy. *Front. Physiol.* 5:172. doi: 10.3389/fphys.2014.00172

Nagy, J. I., Dudek, F. E., and Rash, J. E. (2004). Update on connexins and gap junctions in neurons and glia in the mammalian nervous system. *Brain Res. Brain Res. Rev.* 47, 191–215. doi: 10.1016/j.brainresrev.2004.05.005

Naus, C. C., and Laird, D. W. (2010). Implications and challenges of connexin connections to cancer. *Nat. Rev. Cancer* 10, 435–441. doi: 10.1038/nrc2841

Obuchowicz, E., Kowalski, J., Labuzek, K., Krysiak, R., Pendzich, J., and Herman, Z. S. (2006). Amitriptyline and nortriptyline inhibit interleukin-1 release by rat mixed glial and microglial cell cultures. *Int. J. Neuropsychopharmacol.* 9, 27–35. doi: 10.1017/S146114570500547X

Ohgi, Y., Futamura, T., Kikuchi, T., and Hashimoto, K. (2013). Effects of antidepressants on alternations in serum cytokines and depressive-like behavior in mice after lipopolysaccharide administration. *Pharmacol. Biochem. Behav.* 103, 853–859. doi: 10.1016/j.pbb.2012.12.003

Orellana, J. A., Avendaño, B. C., and Montero, T. D. (2014). Role of connexins and pannexins in ischemic stroke. *Curr. Med. Chem.* 21, 2165–2182. doi: 10.2174/09298673216613228191714

Orellana, J. A., Moraga-Amaro, R., Díaz-Galarce, R., Rojas, S., Maturana, C. J., Stehberg, J., et al. (2015). Restraint stress increases hemichannel activity in hippocampal glial cells and neurons. *Front. Cell. Neurosci.* 9:102. doi: 10.3389/fncel.2015.00102

Orellana, J. A., Shoji, K. F., Abudara, V., Ezan, P., Amigou, E., Sáez, P. J., et al. (2011). Amyloid β-induced death in neurons involves glial and neuronal hemichannels. *J. Neurosci.* 31, 4962–4977. doi: 10.1523/JNEUROSCI.6417-10.2011

Painsipp, E., Köfer, M. J., Sinner, F., and Holzer, P. (2011). Prolonged depression-like behavior caused by immune challenge: influence of mouse strain and social environment. *PLoS ONE* 6:e20719. doi: 10.1371/journal.pone.0020719

Pannasch, U., Vargová, L., Reingruber, J., Ezan, P., Holcman, D., Giaume, C., et al. (2011). Astroglial networks scale synaptic activity and plasticity. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8467–8472. doi: 10.1073/pnas.1016650108

Parpura, V., Heneka, M. T., Montana, V., Oliet, S. H., Schousboe, A., Haydon, P. G., et al. (2012). Glial cells in (patho)physiology. *J. Neurochem.* 121, 4–27. doi: 10.1111/j.1471-4159.2012.07664.x

Pérez-Alvarez, A., and Araque, A. (2013). Astrocyte-neuron interaction at tripartite synapses. *Curr. Drug Targets* 14, 1220–1224. doi: 10.2174/13894501113149990203

Rajkowska, G., and Stockmeier, C. A. (2013). Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Curr. Drug Targets* 14, 1225–1236. doi: 10.2174/13894501113149990156

Rana, S., and Dringen, R. (2007). Gap junction hemichannel-mediated release of glutathione from cultured rat astrocytes. *Neurosci. Lett.* 415, 45–48. doi: 10.1016/j.neulet.2006.12.043

Ransom, B. R., and Giaume, C. (2013). "Gap junctions, hemichannels," in *Neuroglia*, 3rd Edn, eds H. Kettenmann and B. R. Ransom (Oxford: Oxford University Press), 292–305.

Retamal, M. A., Froger, N., Palacios-Prado, N., Ezan, P., Sáez, P. J., Sáez, J. C., et al. (2007). Cx43 Hemichannels and Gap Junction Channels in astrocytes are regulated oppositely by proinflammatory cytokines released from activate microglia. *J. Neurosci.* 27, 13781–13792. doi: 10.1523/JNEUROSCI.2042-07.2007

Sanacora, G., and Banasr, M. (2013). From pathophysiology to novel antidepressant drugs: glial contributions to the pathology and treatment of mood disorders. *Biol. Psychiatry* 73, 1172–1179. doi: 10.1016/j.biopsych.2013.03.032

Seidel, A., Arolt, V., Hunstiger, M., Rink, L., Behnisch, A., and Kirchner, H. (1995). Cytokine production and serum proteins in depression. *Scand. J. Immunol.* 41, 534–538. doi: 10.1111/j.1365-3083.1995.tb03604.x

Sluzewska, A., Rybakowski, J., Bosmans, E., Sobieska, M., Berghmans, R., Maes, M., et al. (1996). Indicators of immune activation in major depression. *Psychiatry Res.* 64, 161–167. doi: 10.1016/S0165-1781(96)02783-7

Stehberg, J., Moraga-Amaro, R., Salazar, C., Becerra, A., Echeverría, C., Orellana, J. A., et al. (2012). Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. *FASEB J.* 26, 3649–3657. doi: 10.1096/fj.11-198416

Sun, J. D., Liu, Y., Yuan, Y. H., Li, J., and Chen, N. H. (2012). Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. *Neuropsychopharmacology* 37, 1305–1320. doi: 10.1038/npp.2011.319

Tai, Y. H., Tsai, R. Y., Lin, S. L., Yeh, C. C., Wang, J. J., Tao, P. L., et al. (2009). Amitriptyline suppresses neuroinflammation-dependent interleukin-10-p38 mitogen-activated protein kinase-heme oxygenase-1 signaling pathway in chronic morphine-infused rats. *Anesthesiology* 110, 1379–1389. doi: 10.1097/ALN.0b013e31819fccd5

Takeuchi, H., and Suzumura, A. (2014). Gap junctions and hemichannels composed of connexins: potential therapeutic targets for neurodegenerative diseases. *Front. Cell. Neurosci.* 8:189. doi: 10.3389/fncel.2014.00189

Tomaz, V. S., Cordeiro, R. C., Costa, A. M., de Lucena, D. F., Nobre Júnior, H. V., de Sousa, F. C., et al. (2014). Antidepressant-like effect of nitric oxide synthase inhibitors and sildenafil against lipopolysaccharide-induced depressive-like behavior in mice. *Neuroscience* 268, 236–246. doi: 10.1016/j.neuroscience.2014.03.025

Valera, E., Ubhi, K., Mante, M., Rockenstein, E., and Masliah, E. (2014). Antidepressants reduce neuroinflammatory responses and astroglial alpha-synuclein accumulation in a transgenic mouse model of multiple system atrophy. *Glia* 62, 317–337. doi: 10.1002/glia.22610

Vardjan, N., Verkhratsky, A., and Zorec, R. (2015). Pathologic potential of astrocytic vesicle traffic: new targets to treat neurologic diseases? *Cell Transplant.* 24, 599–612. doi: 10.3727/096368915X687750

Verkhratsky, A., Rodríguez, J. J., and Parpura, V. (2012). Neurotransmitters and integration in neuronal-astroglial networks. *Neurochem. Res.* 37, 2326–2338. doi: 10.1007/s11064-012-0765-6

Ye, B., Shen, H., Zhang, J., Zhu, Y. G., Ransom, B. R., Chen, X. C., et al. (2015). Dual pathways mediate β-amyloid stimulated glutathione release from astrocytes. *Glia* 63, 2208–2219. doi: 10.1002/glia.22886

Ye, Z. C., Wyeth, M. S., Baltan-Tekkok, S., and Ransom, B. R. (2003). Functional hemichannels in astrocytes: a novel mechanism of glutamate release. *J. Neurosci.* 23, 3588–3596.

Yirmiya, R., Pollak, Y., Barak, O., Avitsur, R., Ovadia, H., Bette, M., et al. (2001). Effects of antidepressant drugs on the behavioral and physiological responses to lipopolysaccharide (LPS) in rodents. *Neuropsychopharmacology* 24, 531–544. doi: 10.1016/S0893-133X(00)00226-8

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Jeanson, Pondaven, Ezan, Mouthon, Charvériat and Giaume. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

frontiers in Cellular Neuroscience

OPINION
published: 12 January 2016
doi: 10.3389/fncel.2015.00509

The Role of Astroglia in the Antidepressant Action of Deep Brain Stimulation

Adeline Etiévant[1,2], *Guillaume Lucas*[3], *Ouria Dkhissi-Benyahya*[4,5] *and Nasser Haddjeri*[4,5]*

[1] Integrative and Clinical Neurosciences EA481, University of Bourgogne Franche-Comté, Besançon, France, [2] CHRU Besançon, Besançon, France, [3] Institut François Magendie, Institut National de la Santé et de la Recherche Médicale U862, University of Bordeaux, Bordeaux, France, [4] Stem Cell and Brain Research Institute, Institut National de la Santé et de la Recherche Médicale U1208, Bron, France, [5] University of Lyon, University of Lyon I, Lyon, France

Keywords: antidepressant, DBS mechanism of action, astrocytes, gliotransmission, adenosine

With more than 350 million depressed individuals worldwide, major depressive disorder is one of the most common psychiatric illnesses. Although, the pathophysiology of depression is far from being fully understood, five decades of development of different classes of antidepressants targeting central monoaminergic systems (serotonin, noradrenaline and dopamine) has led to the emergence of the monoaminergic hypothesis. However, despite a growing number of available pharmacotherapies, treatment of major depression nevertheless remains unsatisfactory.

ROLE OF GLIA IN THE PHYSIOPATHOLOGY OF DEPRESSION AND THE MECHANISMS OF ACTION OF ANTIDEPRESSANTS

Ten years ago it was postulated that abnormal functioning of glial cells, particularly of astrocytes, contribute to the physiopathology of depression (for review, Rajkowska and Miguel-Hidalgo, 2007). Structural and functional abnormalities of glial cells were found in brains of post-mortem depressed patients and in animal models of depression (Czéh et al., 2006, 2007; Banasr et al., 2007; Sun et al., 2012). Moreover, it has been shown that a loss or a functional alteration of astrocytes in the prefrontal cortex is sufficient to induce depressive-like behaviors in rodents (Banasr and Duman, 2008; Sun et al., 2012; Kong et al., 2014).

Given the intimate anatomical and functional relationships between astrocytes and neurons, a tempting hypothesis has emerged proposing that the effects of antidepressant therapies can be, at least in part, mediated by direct astrocytic modulations of neuronal networks. In support of this idea, increasing experimental evidence suggests that antidepressants induce functional changes in astrocytes (Czéh and Di Benedetto, 2012). In addition, it is becoming increasingly clear that the astrocytic network is able to regulate neuronal activity and synaptic transmission through the release of gliotransmitters at what is now called "the tripartite synapse" (Halassa and Haydon, 2010; Panatier et al., 2011). Astrocytes can be directly modulated by an antidepressant treatment or indirectly activated by antidepressant-induced increases in neurotransmitter concentrations in the synaptic cleft, leading to the activation of G protein–coupled receptors (including serotonergic, adrenergic and dopaminergic receptors) on astrocyte membranes. Once "activated," astrocytes release gliotransmitters and modulate neuronal communication and antidepressant responses. The concept of gliotransmission precisely refers to the process by which astrocytes release chemical factors in the vicinity of synapses thus modulating the activity of neighboring cells (for review, Volterra and Meldolesi, 2005; Santello et al., 2012). Although, a large number of such molecules have already been identified (e.g., glutamate, ATP, D-serine, GABA, neurotrophins), our focus here will be specifically on adenosine since it acts directly on the process of neuronal communication and is implicated in pseudo-depressive-like behavior and antidepressant response.

OPEN ACCESS

Edited by:
Luisa Pinto,
University of Minho, Portugal

Reviewed by:
Inbal Goshen,
The Hebrew University, Israel

***Correspondence:**
Nasser Haddjeri
nasser.haddjeri@inserm.fr

Received: *21 October 2015*
Accepted: *18 December 2015*
Published: *12 January 2016*

Citation:
Etiévant A, Lucas G, Dkhissi-Benyahya O and Haddjeri N (2016) The Role of Astroglia in the Antidepressant Action of Deep Brain Stimulation. Front. Cell. Neurosci. 9:509. doi: 10.3389/fncel.2015.00509

ATP is mainly considered as an excitatory transmitter (Gordon et al., 2005) but it is also rapidly hydrolyzed into adenosine by ectonucleotidases present in the synaptic cleft. Adenosine acts as a powerful inhibitor of excitatory transmission through the stimulation of adenosine A_1 receptors (Newman, 2003; Pascual et al., 2005). Adenosine or adenosine agonists induce depressive-like behaviors in two experimental paradigms, namely inescapable shocks (Minor et al., 1994; Hunter et al., 2003) and forced swimming tests (Kulkarni and Mehta, 1985; Cao et al., 2013), an effect that can be prevented by specific adenosine antagonists or antidepressants. Specific A_{2A} receptor antagonists also reverse synaptic changes induced by stress in the hippocampus, which is considered as a preclinical marker of antidepressant responses (Cunha et al., 2006). However, these interpretations are further complicated by the findings observed after selective manipulations of the adenosine A_1 transmission. Thus, central administration of an A_1 receptor agonist mimics the antidepressant effect of sleep deprivation, an effect absent in $A_1^{-/-}$ KO mice (Hines et al., 2013). Sleep deprivation is also associated with pronounced increases of adenosine levels and an up-regulation of glial adenosine A_1-receptors in the brains of both depressed patients (Van Calker and Biber, 2005) and rodents (Hines et al., 2013). The apparent discrepancy between the "depressiogenic" influence of adenosine, and some antidepressant-like actions of A_1 agonists, could be due to the complex modulation exerted by A_1 receptors on axonal firing that appears to depend on the degree of activity of the related networks (see below).

ASTROCYTES ARE DEEPLY INVOLVED IN THE NEUROBIOLOGICAL EFFECTS OF DEEP BRAIN STIMULATION

As observed during pharmacological treatments, recent data shows that astrocyte function can be modulated by deep brain stimulation (DBS), a non-pharmacological antidepressant intervention. DBS is an invasive brain stimulation technique considered as a new hope in the treatment of several intractable psychiatric diseases such as major depression (Mayberg et al., 2005; Puigdemont et al., 2015). Current research is mainly focused on the effects of DBS on neurons, i.e., how myelinated and unmyelinated axons, dendrites and neuronal cell bodies respond to DBS (Mcintyre et al., 2004; Gubellini et al., 2009). However, the role of astrocytes in this context has not yet been addressed. Several arguments support the view that the effects of DBS can, at least in part, be mediated by astrocytes acting on neuronal networks (for review, Vedam-Mai et al., 2012). First, it is well known that DBS modulates regional blood flow in the stimulated area, an effect that can be considered as a direct manifestation of changes in astrocytic activity (Kefalopoulou et al., 2010). Second, astrocytes can be directly activated by high frequency stimulation, leading to a rapid Ca^{2+} increase (Kang et al., 1998; Serrano et al., 2006, 2008). Third, high frequency stimulation of primary astrocytes *in vitro* results in calcium waves and release of glutamate and ATP (Tawfik et al., 2010). Accordingly, Bekar et al. (2008) have shown *in vitro* that DBS was associated with an increase of ATP outflow within the thalamus, resulting in an accumulation of adenosine, which in turn depressed excitatory transmission through A_1 receptors activation. The authors proposed that, once present in the synaptic cleft, adenosine would activate post-synaptic A_1 receptors positively coupled to K^+ channels and pre-synaptic A_1 receptors negatively associated with Ca^{2+} channels. Both actions would result in the inhibition of neuronal communication (Pascual et al., 2005).

Our recent investigations suggest that astrocytes are deeply involved in the antidepressant-like effects of DBS in rats. The antidepressant response induced by DBS in humans can be modeled in rats by stimulating the infralimbic part of the prefrontal cortex (IL-PFC). It has been shown that acute DBS produced an antidepressant-like effect in the forced swim test (Etiévant et al., 2015) and that chronic DBS is able to reverse the depressive-like states observed in Flinders sensitive Line rats (Rea et al., 2014) or induced by chronic mild stress (Hamani et al., 2012). Therefore, the antidepressant-like effect of DBS is associated with the occurrence of *in vivo* pre-clinical markers (Etiévant et al., 2015). We showed that acute DBS induced a rapid increase of hippocampal neurogenesis, reversed the effects of stress on hippocampal synaptic metaplasticity, increased spontaneous IL-PFC low-frequency oscillations and both raphe 5-HT firing activity and synaptogenesis. Significantly, we demonstrated that DBS-induced neural adaptations are strongly altered by pharmacological ablation of astrocytes within the site of stimulation (IL-PFC). Glial lesion with the gliotoxin L-alpha amino-adipic acid (L-AAA) counteracted the behavioral effect of high frequency DBS in the forced swim test and all above cited markers of the antidepressant response. We also found that DBS-induced antidepressant-like response was prevented by IL-PFC neuronal lesion and gap junction blockade as well as by adenosine A_1 receptor antagonists including caffeine.

An elegant review discussing the role of astrocytes in the effects of DBS (Vedam-Mai et al., 2012) recently raised the hypothesis that astrocytes, once activated by electrical stimulation, would release ATP and glutamate leading to an inhibition or an excitation of synaptic transmission, respectively. Recent data partially supports this hypothesis and offers further insights. Our *in vivo* electrophysiological results revealed that the astroglial modulation of DBS involved mechanisms related to changes in adenosine A_1 receptor function, together with the elevation of extracellular K^+ concentration (Etiévant et al., 2015). Our results further showed that the enhancing effect of bilateral DBS on 5-HT neuronal activity was potentiated by a selective A_1 receptor agonist, unilaterally infused during the stimulation. This result, together with the fact that the A_1 receptor antagonist DPCPX prevents the antidepressant-like effect of DBS in the forced swim test, indicates that the efficacy of DBS partially depends on adenosine A_1 receptor stimulation. Interestingly, recent studies aimed to characterize the role played by A_1 receptors in the shape of action potentials and the regulation of axonal conductance report that the administration of an adenosine antagonist increases the width of axonal action potentials. This result suggests that astrocytes, through the release of adenosine and subsequent A_1 receptor stimulation,

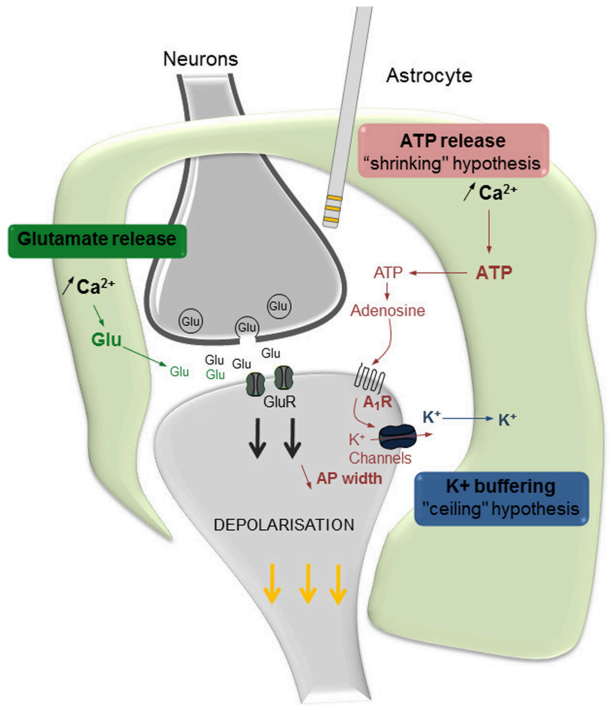


FIGURE 1 | Proposed hypotheses regarding the involvement of astrocytes in the effects of DBS. Once activated by DBS, astrocytes communicate with neurons at the synapse level and regulate the effects of DBS. Astrocytes, by releasing glutamate (Glu), stimulate neuronal synaptic release and contribute to the activation of post-synaptic receptors (in green). ATP is rapidly hydrolyzed into adenosine, which increases the stimulation of adenosine A_1 receptors (A_1R) and, in turn, results in a K^+ channel-mediated reduction of the late hyperpolarization phase of action potentials (in red). Ultimately, the resulting temporal shrinkage of action potentials (AP width) may help the neuron to sustain the high frequency demand related to IL-DBS. Astrocytes also maintain K^+ homeostasis, by actively pumping K^+ ions from the extracellular level thus preventing their accumulation due to neuronal activity (in blue). Modified from Etiévant et al. (2015).

are able to modulate the shape of axonal action potentials, shortening the total duration of the spike and "shrinking" its shape (Sasaki et al., 2011). This latter effect could be due to a modulation of the voltage-activated K^+ channels responsible for neuronal after hyperpolarization. It has been proposed that such a "temporal shrinking" of action potentials can be beneficial when the neuron is solicited in response to high-frequency stimulations, allowing to sustain bursting activity that requires very short inter-spike intervals (Sasaki et al., 2011). Thus, we have proposed that a loss of astrocytes within the site of stimulation induces a drop of adenosine extracellular concentrations and an altered temporal shrinking of action potentials responsible for the alteration of the neurobiological effects of DBS (**Figure 1**).

Since astrocytes are able to maintain the potassium homeostasis by actively pumping K^+ ions from the extracellular space (Kofuji and Newman, 2004), we hypothesized that an alteration of astrocyte function within the lesioned site leads to an accumulation of extracellular K^+ which, in turn, would produce a depolarization of neuron membrane and a blockade of DBS-mediated effects. Hence in our *in vivo* study, a K^+-enriched aCSF was perfused within the IL-PFC using reverse dialysis while recording 5-HT neurons. The obtained results confirmed our hypothesis of a "ceiling effect," related to a K^+-dependent depolarization of pyramidal neurons, since high frequency DBS is unable to further affect 5-HT activity in the presence of high [K^+]. Thus, the depolarizing action of an elevated extracellular [K^+] potentially impairs the ability of pyramidal cells to respond to the phasic, high-frequency solicitation demands of sustained electrical stimulations of 130 Hz. This effect is frequency-dependent since both the 5-HT-activating and the behavioral effectiveness in the forced swim test of a 30 Hz DBS remained unaltered in glial-lesioned rats and in high [K^+] conditions. This result strongly suggests that, in the absence of astrocytes, the depolarization of neuronal membrane related to K^+ accumulation fails to reach a supra-threshold, "depolarization block-like" level, and that pyramidal neurons are still able to follow a low frequency DBS (30 Hz; Etiévant et al., 2015).

CONCLUSION

The astroglial system plays a crucial role in the mechanisms of action of DBS. Accordingly, the antidepressant-like response induced by DBS is counteracted by a pharmacological lesion of astrocytes in the stimulated area. Two mechanistic hypotheses have been proposed to explain the astrocytic modulation of the neuronal response induced by DBS (**Figure 1**). First, the "shrinking hypothesis" suggests that astrocytes, by releasing adenosine in response to DBS, activate neuronal A_1 receptors resulting in a shortening of the width of action potentials. Second, the "ceiling hypothesis" proposes that astrocytes, by actively pumping K^+ ions from the extracellular spaces, prevent the establishment of the "depolarization-like blockade" of the neuronal membrane. Both events are directed to an optimal functioning of pyramidal neurons that are still capable of following high frequency stimulations induced by DBS. Lastly as a translational outcome, we have proposed that an unaltered neuronal–glial system constitutes a major prerequisite to optimize antidepressant DBS efficacy, and that decreasing the frequency of DBS would increase the antidepressant response of partial responders.

AUTHOR CONTRIBUTIONS

All the authors participated to the conception and the content of the opinion. AE wrote the opinion with the help of GL, OD, and NH. All the authors revised critically the manuscript and gave their approval for publication.

REFERENCES

Banasr, M., and Duman, R. S. (2008). Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol. Psychiatry* 64, 863–870. doi: 10.1016/j.biopsych.2008.06.008

Banasr, M., Valentine, G. W., Li, X. Y., Gourley, S. L., Taylor, J. R., and Duman, R. S. (2007). Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. *Biol. Psychiatry* 62, 496–504. doi: 10.1016/j.biopsych.2007.02.006

Bekar, L., Libionka, W., Tian, G. F., Xu, Q., Torres, A., Wang, X., et al. (2008). Adenosine is crucial for deep brain stimulation-mediated attenuation of tremor. *Nat. Med.* 14, 75–80. doi: 10.1038/nm1693

Cao, X., Li, L. P., Wang, Q., Wu, Q., Hu, H. H., Zhang, M., et al. (2013). Astrocyte-derived ATP modulates depressive-like behaviors. *Nat. Med.* 19, 773–777. doi: 10.1038/nm.3162

Cunha, G. M., Canas, P. M., Oliveira, C. R., and Cunha, R. A. (2006). Increased density and synapto-protective effect of adenosine A2A receptors upon sub-chronic restraint stress. *Neuroscience* 141, 1775–1781. doi: 10.1016/j.neuroscience.2006.05.024

Czéh, B., and Di Benedetto, B. (2012). Antidepressants act directly on astrocytes: evidences and functional consequences. *Eur. Neuropsychopharmacol.* 23, 171–185. doi: 10.1016/j.euroneuro.2012.04.017

Czéh, B., Müller-Keuker, J. I., Rygula, R., Abumaria, N., Hiemke, C., Domenici, E., et al. (2007). Chronic social stress inhibits cell proliferation in the adult medial prefrontal cortex: hemispheric asymmetry and reversal by fluoxetine treatment. *Neuropsychopharmacology* 32, 1490–1503. doi: 10.1038/sj.npp.1301275

Czéh, B., Simon, M., Schmelting, B., Hiemke, C., and Fuchs, E. (2006). Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. *Neuropsychopharmacology* 31, 1616–1626. doi: 10.1038/sj.npp.1300982

Etiévant, A., Oosterhof, C. A., Bétry, C., Abrial, E., Novo-Perez, M., Rovera, R., et al. (2015). Astroglial control of the antidepressant-like effects of prefrontal cortex deep brain stimulation. *EBiomedicine* 2, 896–906. doi: 10.1016/j.ebiom.2015.06.023

Gordon, G. R., Baimoukhametova, D. V., Hewitt, S. A., Rajapaksha, W. R., Fisher, T. E., and Bains, J. S. (2005). Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. *Nat. Neurosci.* 8, 1078–1086. doi: 10.1038/nn1498

Gubellini, P., Salin, P., Kerkerian-Le Goff, L., and Baunez, C. (2009). Deep brain stimulation in neurological diseases and experimental models: from molecule to complex behavior. *Prog. Neurobiol.* 89, 79–123. doi: 10.1016/j.pneurobio.2009.06.003

Halassa, M. M., and Haydon, P. G. (2010). Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu. Rev. Physiol.* 72, 335–355. doi: 10.1146/annurev-physiol-021909-135843

Hamani, C., Machado, D. C., Hipólide, D. C., Dubiela, F. P., Suchecki, D., Macedo, C. E., et al. (2012). Deep brain stimulation reverses anhedonic-like behavior in a chronic model of depression: role of serotonin and brain derived neurotrophic factor. *Biol. Psychiatry* 71, 30–35. doi: 10.1016/j.biopsych.2011.08.025

Hines, D. J., Schmitt, L. I., Hines, R. M., Moss, S. J., and Haydon, P. G. (2013). Antidepressant effects of sleep deprivation require astrocyte-dependent adenosine mediated signaling. *Transl. Psychiatry* 3, e212. doi: 10.1038/tp.2012.136

Hunter, A. M., Balleine, B. W., and Minor, T. R. (2003). Helplessness and escape performance: glutamate-adenosine interactions in the frontal cortex. *Behav. Neurosci.* 117, 123–135. doi: 10.1037/0735-7044.117.1.123

Kang, J., Jiang, L., Goldman, S. A., and Nedergaard, M. (1998). Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nat. Neurosci.* 1, 683–692. doi: 10.1038/3684

Kefalopoulou, Z., Paschali, A., Markaki, E., Ellul, J., Chroni, E., Vassilakos, P., et al. (2010). Regional cerebral blood flow changes induced by deep brain stimulation in secondary dystonia. *Acta Neurochir. (Wien.)* 152, 1007–1014. doi: 10.1007/s00701-010-0612-y

Kofuji, P., and Newman, E. A. (2004). Potassium buffering in the central nervous system. *Neuroscience* 129, 1045–1056. doi: 10.1016/j.neuroscience.2004.06.008

Kong, H., Zeng, X. N., Fan, Y., Yuan, S. T., Ge, S., Xie, W. P., et al. (2014). Aquaporin-4 knockout exacerbates corticosterone-induced depression by inhibiting astrocyte function and hippocampal neurogenesis. *CNS Neurosci. Ther.* 20, 391–402. doi: 10.1111/cns.12222

Kulkarni, S. K., and Mehta, A. K. (1985). Purine nucleoside–mediated immobility in mice: reversal by antidepressants. *Psychopharmacology (Berl.)* 85, 460–463. doi: 10.1007/BF00429665

Mayberg, H. S., Lozano, A. M., Voon, V., Mcneely, H. E., Seminowicz, D., Hamani, C., et al. (2005). Deep brain stimulation for treatment-resistant depression. *Neuron* 45, 651–660. doi: 10.1016/j.neuron.2005.02.014

Mcintyre, C. C., Savasta, M., Kerkerian-Le Goff, L., and Vitek, J. L. (2004). Uncovering the mechanism(s) of action of deep brain stimulation: activation, inhibition, or both. *Clin. Neurophysiol.* 115, 1239–1248. doi: 10.1016/j.clinph.2003.12.024

Minor, T. R., Winslow, J. L., and Chang, W. C. (1994). Stress and adenosine: II. Adenosine analogs mimic the effect of inescapable shock on shuttle-escape performance in rats. *Behav. Neurosci.* 108, 265–276. doi: 10.1037/0735-7044.108.2.265

Newman, E. A. (2003). Glial cell inhibition of neurons by release of ATP. *J. Neurosci.* 23, 1659–1666.

Panatier, A., Vallée, J., Haber, M., Murai, K. K., Lacaille, J. C., and Robitaille, R. (2011). Astrocytes are endogenous regulators of Basal transmission at central synapses. *Cell* 146, 785–798. doi: 10.1016/j.cell.2011.07.022

Pascual, O., Casper, K. B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J. Y., et al. (2005). Astrocytic purinergic signaling coordinates synaptic networks. *Science* 310, 113–116. doi: 10.1126/science.1116916

Puigdemont, D., Portella, M., Pérez-Egea, R., Molet, J., Gironell, A., De Diego-Adeliño, J., et al. (2015). A randomized double-blind crossover trial of deep brain stimulation of the subcallosal cingulate gyrus in patients with treatment-resistant depression: a pilot study of relapse prevention. *J. Psychiatry Neurosci.* 40, 224–231. doi: 10.1503/jpn.130295

Rajkowska, G., and Miguel-Hidalgo, J. J. (2007). Gliogenesis and glial pathology in depression. *CNS Neurol. Disord. Drug Targets* 6, 219–233. doi: 10.2174/187152707780619326

Rea, E., Rummel, J., Schmidt, T. T., Hadar, R., Heinz, A., Mathé, A. A., et al. (2014). Anti-anhedonic effect of deep brain stimulation of the prefrontal cortex and the dopaminergic reward system in a genetic rat model of depression: an intracranial self-stimulation paradigm study. *Brain Stimul.* 7, 21–28. doi: 10.1016/j.brs.2013.09.002

Santello, M., Calí, C., and Bezzi, P. (2012). Gliotransmission and the tripartite synapse. *Adv. Exp. Med. Biol.* 970, 307–331. doi: 10.1007/978-3-7091-0932-8_14

Sasaki, T., Matsuki, N., and Ikegaya, Y. (2011). Action-potential modulation during axonal conduction. *Science* 331, 599–601. doi: 10.1126/science.1197598

Serrano, A., Haddjeri, N., Lacaille, J. C., and Robitaille, R. (2006). GABAergic network activation of glial cells underlies hippocampal heterosynaptic depression. *J. Neurosci.* 26, 5370–5382. doi: 10.1523/JNEUROSCI.5255-05.2006

Serrano, A., Robitaille, R., and Lacaille, J. C. (2008). Differential NMDA-dependent activation of glial cells in mouse hippocampus. *Glia* 56, 1648–1663. doi: 10.1002/glia.20717

Sun, J. D., Liu, Y., Yuan, Y. H., Li, J., and Chen, N. H. (2012). Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. *Neuropsychopharmacology* 37, 1305–1320. doi: 10.1038/npp.2011.319

Tawfik, V. L., Chang, S. Y., Hitti, F. L., Roberts, D. W., Leiter, J. C., Jovanovic, S., et al. (2010). Deep brain stimulation results in local glutamate and adenosine release: investigation into the role of astrocytes. *Neurosurgery* 67, 367–375. doi: 10.1227/01.NEU.0000371988.73620.4C

Van Calker, D., and Biber, K. (2005). The role of glial adenosine receptors in neural resilience and the neurobiology of mood disorders. *Neurochem. Res.* 30, 1205–1217. doi: 10.1007/s11064-005-8792-1

Vedam-Mai, V., Van Battum, E. Y., Kamphuis, W., Feenstra, M. G., Denys, D., Reynolds, B. A., et al. (2012). Deep brain stimulation and the role of astrocytes. *Mol. Psychiatry* 17, 124–131. doi: 10.1038/mp.2011.61

Volterra, A., and Meldolesi, J. (2005). Astrocytes, from brain glue to communication elements: the revolution continues. *Nat. Rev. Neurosci.* 6, 626–640. doi: 10.1038/nrn1722

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2016 Etiévant, Lucas, Dkhissi-Benyahya and Haddjeri. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read, for greatest visibility

COLLABORATIVE PEER-REVIEW
Designed to be rigorous – yet also collaborative, fair and constructive

FAST PUBLICATION
Average 85 days from submission to publication (across all journals)

COPYRIGHT TO AUTHORS
No limit to article distribution and re-use

TRANSPARENT
Editors and reviewers acknowledged by name on published articles

SUPPORT
By our Swiss-based editorial team

IMPACT METRICS
Advanced metrics track your article's impact

GLOBAL SPREAD
5'100'000+ monthly article views and downloads

LOOP RESEARCH NETWORK
Our network increases readership for your article

Frontiers
EPFL Innovation Park, Building I • 1015 Lausanne • Switzerland
Tel +41 21 510 17 00 • Fax +41 21 510 17 01 • info@frontiersin.org
www.frontiersin.org

Find us on