Brain, Behavior, and Immunity 58 (2016) 261–271

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

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

Full-length Article

Fluoxetine treatment affects the inflammatory response and microglial function according to the quality of the living environment

Silvia Alboni [a,1], Silvia Poggini [b,1], Stefano Garofalo [c], Giampaolo Milior [c,d], Hassan El Hajj [e], Cynthia Lecours [e], Isabelle Girard [e], Steven Gagnon [e], Samuel Boisjoly-Villeneuve [e], Nicoletta Brunello [a], David P. Wolfer [f], Cristina Limatola [g,h], Marie-Ève Tremblay [e,2], Laura Maggi [c,2], Igor Branchi [b,f,*,2]

[a] Department of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy
[b] Section of Behavioural Neurosciences, Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy
[c] Department of Physiology and Pharmacology, Istituto Pasteur-Fondazione Cenci Bolognetti, Sapienza University of Rome, Italy
[d] Inserm U1127, CNRS UMR7225, Sorbonne Universités, UPMC UMR S1127, Institut du Cerveau et de la Moelle épinière, Paris 75013, France
[e] Axe Neurosciences, Centre de recherche du CHU de Québec, 2705, boulevard Laurier, Québec, Canada
[f] Institute of Anatomy, University of Zurich, Zurich, Switzerland
[g] Pasteur Institute Rome-Department of Physiology and Pharmacology, Sapienza University of Rome, Italy
[h] IRCCS Neuromed, Pozzilli, IS, Italy

ARTICLE INFO

Article history:
Received 23 March 2016
Received in revised form 25 July 2016
Accepted 25 July 2016
Available online 27 July 2016

Keywords:
Antidepressant
SSRI
Environment
Major depression
Plasticity
Serotonin
Undirected susceptibility to change hypothesis
Microglia
Inflammation
Cytokines

ABSTRACT

It has been hypothesized that selective serotonin reuptake inhibitors (SSRIs), the most common treatment for major depression, affect mood through changes in immune function. However, the effects of SSRIs on inflammatory response are contradictory since these act either as anti- or pro-inflammatory drugs. Previous experimental and clinical studies showed that the quality of the living environment moderates the outcome of antidepressant treatment. Therefore, we hypothesized that the interplay between SSRIs and the environment may, at least partially, explain the apparent incongruence regarding the effects of SSRI treatment on the inflammatory response. In order to investigate such interplay, we exposed C57BL/6 mice to chronic stress to induce a depression-like phenotype and, subsequently, to fluoxetine treatment or vehicle (21 days) while being exposed to either an enriched or a stressful condition. At the end of treatment, we measured the expression levels of several anti- and pro-inflammatory cytokines and inflammatory mediators in the whole hippocampus and in isolated microglia. We also determined microglial density, distribution, and morphology to investigate their surveillance state. Results show that the effects of fluoxetine treatment on inflammation and microglial function, as compared to vehicle, were dependent on the quality of the living environment. In particular, fluoxetine administered in the enriched condition increased the expression of pro-inflammatory markers compared to vehicle, while treatment in a stressful condition produced anti-inflammatory effects. These findings provide new insights regarding the effects of SSRIs on inflammation, which may be crucial to devise pharmacological strategies aimed at enhancing antidepressant efficacy by means of controlling environmental conditions.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed drugs for the treatment of major depression (MD), which constitutes an enormous medical, individual, societal and economical challenge and afflicts up to 10–15% of the population worldwide. However, the efficacy of SSRIs is variable and incomplete: 60–70% of patients do not experience remission and 30–40% do not show a significant response (Trivedi et al., 2006). One of the main reasons for such limited efficacy is the poor comprehension of their mechanisms of action at cellular and molecular levels.

In recent decades, the crosstalk between the innate and adaptive immune systems and the brain has been suggested to represent a key factor in antidepressant drug action (Carvalho et al., 2013; Eller et al., 2008; Lanquillon et al., 2000; Tuglu et al., 2003). Indeed, treatment with SSRIs has been shown to decrease

* Corresponding author at: Section of Behavioural Neurosciences, Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy.
E-mail address: igor.branchi@iss.it (I. Branchi).
[1] These authors equally contributed as first author.
[2] These authors equally contributed as last author.

http://dx.doi.org/10.1016/j.bbi.2016.07.155
0889-1591/

MD associated cytokine elevations. In particular, the levels of inflammatory cytokines IL-1β, IL-6 and TNF-α have been reported to be reduced following SSRI treatment (Basterzi et al., 2005; Kagaya et al., 2001; Lanquillon et al., 2000; Leo et al., 2006; Tuglu et al., 2003; Yoshimura et al., 2009). In addition, elevated baseline levels of TNF-α and IL-6 correlate with treatment failure (Carvalho et al., 2013; Eller et al., 2008; Lanquillon et al., 2000). However, other clinical studies obtained opposite results, demonstrating no or even pro-inflammatory effects of antidepressant treatment (Chen et al., 2010; Haastrup et al., 2012; Hannestad et al., 2011; Jazayeri et al., 2010; Kim et al., 2013; Song et al., 2009). Experimental studies reflect the incongruence of clinical findings. Though many studies attributed anti-inflammatory effects to antidepressant drugs (Bielecka et al., 2010; Kenis and Maes, 2002; Obuchowicz et al., 2006; Tynan et al., 2012; Xia et al., 1996), pro-inflammatory effects were reported as well (Diamond et al., 2006; Horikawa et al., 2010; Horowitz et al., 2015; Kubera et al., 2005; Tynan et al., 2012). Such discrepancy suggests that SSRIs may not have a univocal effect on inflammatory processes and additional factors may moderate the complex interplay between antidepressants and inflammation (Kraemer et al., 2006).

Recently, a number of preclinical studies have identified the living environment as a key moderator of the outcome of SSRI treatment (Alboni et al., 2016; Branchi, 2011; Branchi et al., 2013). In particular, since the increase in serotonin levels induced by SSRIs enhances neural plasticity, rendering individuals more susceptible to environmental conditions, the outcome of SSRI administration is not univocal but depends on the quality of the environment. This view, named the undirected susceptibility to change hypothesis, is supported by clinical studies showing that antidepressants are more effective in patients with a good quality of life, while having no or even detrimental consequences in patients experiencing stressful conditions (Cohen et al., 2006; Trivedi et al., 2006). Accordingly, the quality of the environment has been shown to determine the outcome of SSRI treatment on the vulnerability to obesity (Mastronardi et al., 2011; Wong and Licinio, 2001). Though the influence of the living environment in driving SSRI effects on depressive symptomatology starts to be unraveled, no information on its role in moderating SSRI effects on the inflammatory response is yet available.

The aim of the present study was to determine whether fluoxetine treatment, as compared to vehicle, affects the inflammatory response, which notably involves microglial cells within the brain, according to the quality of the living environment. To this purpose, we exposed C57BL/6 mice first to 14 days of stress, in order to induce a depression-like phenotype and, subsequently, to 21 days of either (i) an enriched or (ii) a stressful condition, while receiving fluoxetine or vehicle. We assessed the expression levels of several key inflammatory markers in the hippocampus, a highly plastic brain region that is deeply involved in MD and antidepressant effects (MacQueen and Frodl, 2011). In addition, in order to investigate possible changes in microglial function, we measured the expression levels of several inflammatory markers in freshly isolated hippocampal microglial cells, as well as microglial density, distribution and morphology. Our prediction was that the trajectories of inflammatory and microglial modifications induced by fluoxetine treatment depend on the living environment.

The results concerning the neural and behavioral response to fluoxetine treatment displayed in the different environmental conditions by the experimental subjects used in this study have been published elsewhere (Alboni et al., 2016). These show that the exposure to 14 days of stress before treatment induced a depression-like phenotype and the neurobehavioral profile was affected by treatment according to the quality of the environment.

2. Materials and methods

2.1. Animals and housing conditions

C57BL/6 male mice 12–15 week old were used and kept under 12-h light-dark cycle at 22–25 °C. Animals were housed in the Intellicage system (TSE-system, NewBehavior AG, Zürich, Switzerland), which is an apparatus designed for the automatic monitoring of mouse behavior (Branchi et al., 2013). Food was freely available. Animals were examined for signs of discomfort as indicated by the animal care and use guidelines [National Academy of Sciences. Guide for the care and use of laboratory animals, 1998, "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research" (National Research Council 2003)]. All procedures were carried out in accordance with the EC guidelines (EEC Council Directive 2010/63/UE86/609 1987), Italian legislation on animal experimentation (Decreto Legislativo 26/2014).

The animals were gradually habituated to the Intellicage environment during a 14-days period. Five days before being moved to the Intellicage, each animal was injected with a subcutaneous transponder (T-IS 8010 FDX-B; Datamars SA, Switzerland). Independent experiments were performed for each environmental condition. In each experiment, animals were housed in the enriched, stressful or standard condition, and received fluoxetine or vehicle.

2.1.1. Enriched condition

The Intellicage provides an enriched environment because mice are socially housed and exposed to Plexiglas shelters of different colors and shapes (four red transparent Tecniplast plastic nest boxes and four white opaque boxes), and to tissue paper. New paper was provided every 5 days and the plastic shelters were cleaned every week.

2.1.2. Stressful condition

The mice were exposed each day to a different stressor, randomly chosen among social stress and other stressful procedures provided by the Intellicage. Exposing mice to different stressors was used to prevent habituation to each of these. The stressful procedures used are: *Social stress*: moving animals from one Intellicage into another, creating new social groups hence forcing mice to re-establish their social hierarchy; *Short open door*: door to access water or saccharin remains open for only 1.5 s; *Open door* 25%: door opens only following 25% of nosepokes; *Air puff*: when the mouse enters the corner, it has a 20% chance of receiving an air puff; *Delayed door*: door opens 2.5 s after the first nosepoke. In addition, in the stressful condition, no shelter or tissue paper was provided.

2.1.3. Standard condition

Mice were housed in a standard laboratory condition, two individuals per cage. Each cage was 33 × 13 × 14 cm Plexiglas box with metal tops and sawdust as bedding. Pellet food and tap water were provided *ad libitum*.

2.2. Treatment

Fluoxetine (Fluoxetine HCl, SantaCruz, USA) was dissolved in water or saccharin solution and delivered *ad libitum* in the drinking bottles for 3 weeks. Compared to injection, this administration method avoids stress due to the handling. The solutions were prepared according to the mouse average weight and daily water consumption in order to provide an average daily intake of 30 mg/kg. The average amount of fluoxetine administered to each