



The vagus nerve role in antidepressants action: Efferent vagal pathways participate in peripheral anti-inflammatory effect of fluoxetine

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ABSTRACT

The mechanisms responsible for the anti-inflammatory effects of antidepressants are only partially understood. Published data indicate that the vagal anti-inflammatory pathway could be involved in mediating this effect. Therefore, we investigated the influence of subdiaphragmatic vagotomy on the anti-inflammatory effect of fluoxetine in rats injected with lipopolysaccharide (LPS) to induce an inflammatory response. The extent of this response was determined by measurement of TNF- α , IL-1 β , and IL-6 plasma levels, along with gene expression of TNF- α , IL-1 β , and IL-6 in the spleen and selected structures of the brain. To evaluate possible central mechanisms, *c-fos* mRNA levels were determined in the nucleus of the solitary tract, dorsal motor nucleus of the vagus, paraventricular hypothalamic nucleus, basolateral amygdala, central nucleus of the amygdala, hippocampus, and frontal cortex. We found that pretreatment with fluoxetine substantially prevented LPS-induced increases of pro-inflammatory cytokines in plasma and gene expression in the spleen and brain in animals with an intact vagus nerve. However, in vagotomized animals, fluoxetine pretreatment only partially attenuated the LPS-induced increase in these markers of peripheral inflammation. Our data has shown that fluoxetine exerts potent anti-inflammatory effects in both the periphery and brain. Moreover, we found that the peripheral anti-inflammatory action of fluoxetine is mediated, at least partially, by activation of a vagal anti-inflammatory pathway. The role of the vagus nerve in mediating the anti-inflammatory effects of antidepressants has been marginally explored and our findings highlight its potential contribution to this mechanism of action of antidepressants.

1. Introduction

Depression, mainly conceptualized as the clinical form of Major Depressive Disorder, is a prevalent mental disorder and one of the leading cause of burden for individuals and societies (Ferrari et al., 2013; Vos et al., 2015). Broad evidence of the role of inflammation as an important factor for both the onset and maintenance of depression has resulted in the establishment of a cytokine theory of depression (Connor and Leonard, 1998; Maes et al., 2012). The possibility of using anti-inflammatory agents for the treatment of depression (Kohler et al., 2016) as well as potential anti-inflammatory mechanisms of antidepressants have been evaluated (Galecki et al., 2018; Walker, 2013), although data from clinical studies are heterogeneous (Dahl et al., 2014; Hannestad et al., 2011). In support to this theory, the different pathways enabling antidepressants to affect immune function, including a direct effect on immune cells (particularly macrophages) and

altering neurotransmission-related processes, were proposed (Nazimek et al., 2017; O'Brien et al., 2004; Roumestan et al., 2007).

In the periphery, immune homeostasis is maintained by several factors, including immune, endocrine, and nervous system regulation (Di Comite et al., 2007). In the last two decades, significant progress has been achieved in understanding the process of nervous system regulation of immune activity. Besides the effects of the neuroendocrine system and sympathetic nerves on immune cells (Elenkov et al., 2000), a profound anti-inflammatory effect of the efferent vagal pathways on immune function has also been described (Pavlov and Tracey, 2017).

The anti-inflammatory effects of antidepressants have been described in the central nervous system as well as in the periphery (Galecki et al., 2018; Tynan et al., 2012). Based on published data, we hypothesize that antidepressants may, at least partially, exert their anti-inflammatory effect indirectly by activating the vagal anti-inflammatory pathway. Activation of this pathway may attenuate

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peripheral inflammation and consequently reduce inflammation in the brain, thereby participating in the restoration of brain's milieu with subsequent reduction of symptomatology of depression (for review see (Ondicova et al., 2010). To evaluate this hypothesis, we chose fluoxetine (Flx), a Selective Serotonin Reuptake Inhibitor (SSRI) class antidepressant broadly used in clinical practice (Wong et al., 1995) that possesses anti-inflammatory properties (Liu et al., 2011; Waiskopf et al., 2014).

In our experiments, we evaluated the anti-inflammatory effect of Flx as measured by changes in plasma tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , and IL-6 as a marker of peripheral inflammation in vagotomized rats exposed to intraperitoneal injection of LPS, an inflammatory stimulus. Moreover, gene expression of TNF- α , IL-1 β , and IL-6 was determined in the spleen as well as brain structures associated with immune functions (nucleus of the solitary tract, NTS), behavior modulation (basolateral amygdala, BLA; central nucleus of the amygdala, CeA; hippocampus, Hip; frontal cortex, FCx), and vagal efferent pathway activities (dorsal motor nucleus of the vagus, DMNV; paraventricular hypothalamic nucleus, PVN). Gene expression of *c-fos* as a marker for neuronal activity was also determined in the above-mentioned brain structures. Sham-operated animals were used as controls.

2. Experimental procedures

2.1. Experimental animals

A total of 56 adult male Sprague Dawley rats (225–250 g) were purchased from the AnLab s.r.o. (Prague, Czech Republic). Rats were housed under standard laboratory conditions under a 12/12 h light/dark cycle with free access to food and water. All experimental procedures were approved in accordance with institutional guidelines of the Animal Health and Animal Welfare Division of the State Veterinary and Food Administration of the Slovak Republic (permission no Ro 331/16–221) and in accordance with the Council Directive 2010/63EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes.

2.2. Experimental design

After an acclimatization period of 14 days, either a vagotomy or sham operation was performed in two randomly divided subgroups of animals (Fig. 1A). Then, animals were fed with liquid diet for the first 9 days after surgery, and then fed with pelleted food up to the 33rd day while being weighed regularly to monitor post-surgical recovery (Fig. 1B). At 33rd day animals were injected intraperitoneally by Flx or saline (the first injection), followed 30 min later by LPS or saline (second injection). Three hours after the second injection, the animals were killed by rapid decapitation, after which trunk blood as well as the above-mentioned tissues were removed for analysis (Fig. 1C).

2.3. Experimental procedures

2.3.1. Subdiaphragmatic vagotomy

The subdiaphragmatic vagotomy was performed as previously described (Khasar et al., 2003). Briefly, the animals were randomly assigned to the sham-operated (SHAM) and vagotomized (VGT) groups. Following an overnight food deprivation while allowed access to water *ad libitum*, rats were anesthetized with a mixture containing ketamine-xylazine (Narkamon 5%; 1.2 mL/kg body weight; Rometar 2%; 0.4 mL/kg body weight; Spofa, Czech Republic; i.m.). After a middle upper laparotomy, both ventral and dorsal trunks of the vagus nerve running along the esophagus, including their branches, were dissected free and cut with an electrocautery. In the sham-operated rats, the abdominal vagus nerve was similarly exposed without cutting. To avoid gastrointestinal complications (e.g. gastrostasis) and possible mortality in the vagotomized rats, along with preventing excessive loss of body mass

(Ootsuka et al., 2008), all rats received a palatable liquid diet (Fresubin, Bad Homburg, Germany) for 9 days, after which they were fed with regular pelleted food for the remainder of the study. Animals were allowed 33 days for recovery, which is similar to what was used in a prior vagotomy study (Romanovsky et al., 1997). By day 33, both groups of rats had fully recovered, as inferred from their body weight as a proxy measure (Fig. 1B).

2.3.2. Drug administration

On the final day of the experiment, the rats received a single intraperitoneal (i.p.) injection of either saline (Sal; 0.9% NaCl) or fluoxetine hydrochloride (Flx; 10 mg/kg, dissolved in 0.9% of saline) (Sigma-Aldrich) in a volume of 2 mL/kg 30 min after these injections, an i.p. injection of a lipopolysaccharide (LPS; *Escherichia coli* serotype 055:B5, Sigma-Aldrich) was applied at a dose of 50 μ g/kg of body weight to subgroups randomly created from both vagotomized and sham-operated animals (Fig. 1C). Doses of both Flx and LPS for this experiment were chosen according to published data (Roumestan et al., 2007; Sanchez-Lemus et al., 2009) and suspensions for injections were freshly prepared. Administration of LPS in dose of 50 μ g/kg of body weight induces sickness behavior, neuroendocrine and immune effects, but not endotoxic shock. Previously it was found that acute treatment with above-mentioned dose of Flx is able to attenuate LPS-induced neuro-immune changes, sickness behavior, neuroendocrine and immune effects (Yirmiya et al., 2001).

Three hours after LPS injection, the animals were sacrificed by rapid decapitation. This time interval is sufficient to allow for a rise in pro-inflammatory cytokine levels in plasma along with mRNA levels in the spleen and the brain (Sanchez-Lemus et al., 2009), while also allowing for sufficient levels of Flx to be reached (Unceta et al., 2007). Following decapitation, trunk blood was collected and the brain and spleen were quickly removed, frozen on dry ice, and then stored at -70°C .

2.3.3. Microdissection of brain structures

After decapitation, the brains were quickly removed and frozen on dry ice to avoid tissue autolytic processes prior to cryosectioning. Frozen brains were then sliced at -10°C into 300 μ m thick serial coronal sections using a Reichert-Jung cryostat. Sections were then placed on histological slides (3–6 sections per slide), removed from the cryostat for a few seconds to allow the brain sections to partially thaw so they would adhere to slides, and then placed on dry ice until brain areas were dissected using micropunch sampling according to the original punching guide atlas (Palkovits and Brownstein, 1988).

Selected brain structures from both hemispheres were obtained on a cold plate (Leica Biosystems) under a dissection microscope by specialized hard stainless-steel punching needles with a diameter 600–1200 μ m (see Table 1). Isolated samples were then placed in an Eppendorf tubes, again flash frozen on dry ice, and then stored at -70°C until further analysis.

2.3.4. RNA isolation and Real Time PCR

The mRNA levels of cytokines and the immediate early gene *c-fos* in the rat brain were measured by real-time PCR. Total RNA was isolated from the frozen tissue samples of the microdissected brain structures and from the spleen using the TRI Reagent[®] RT (MRC, Inc.) according to the manufacturers' protocol with concentrations quantified using the NanoDrop 2000 (Thermo Fisher Scientific). Reverse transcription of total RNA was performed with the RevertAid H minusFirst Strand cDNA Synthesis kit (Thermo Fisher Scientific) according to the manufacturer's protocol, using an oligo dT primer. Semi-quantitative Real Time PCR was set up in a total volume of 25 μ L containing 30 ng of template cDNA mixed with 12.5 μ L of FastStart Universal SYBR Green Master Rox (Roche Diagnostics), 1 μ L of the specific primer pair set, and 9.5 μ L of water. Sequences of specific primers are shown in Table 2. Each sample was analyzed in duplicate on a ABI7900HT Fast Real-Time PCR instrument (Applied Biosystems) under the following conditions: 1 cycle

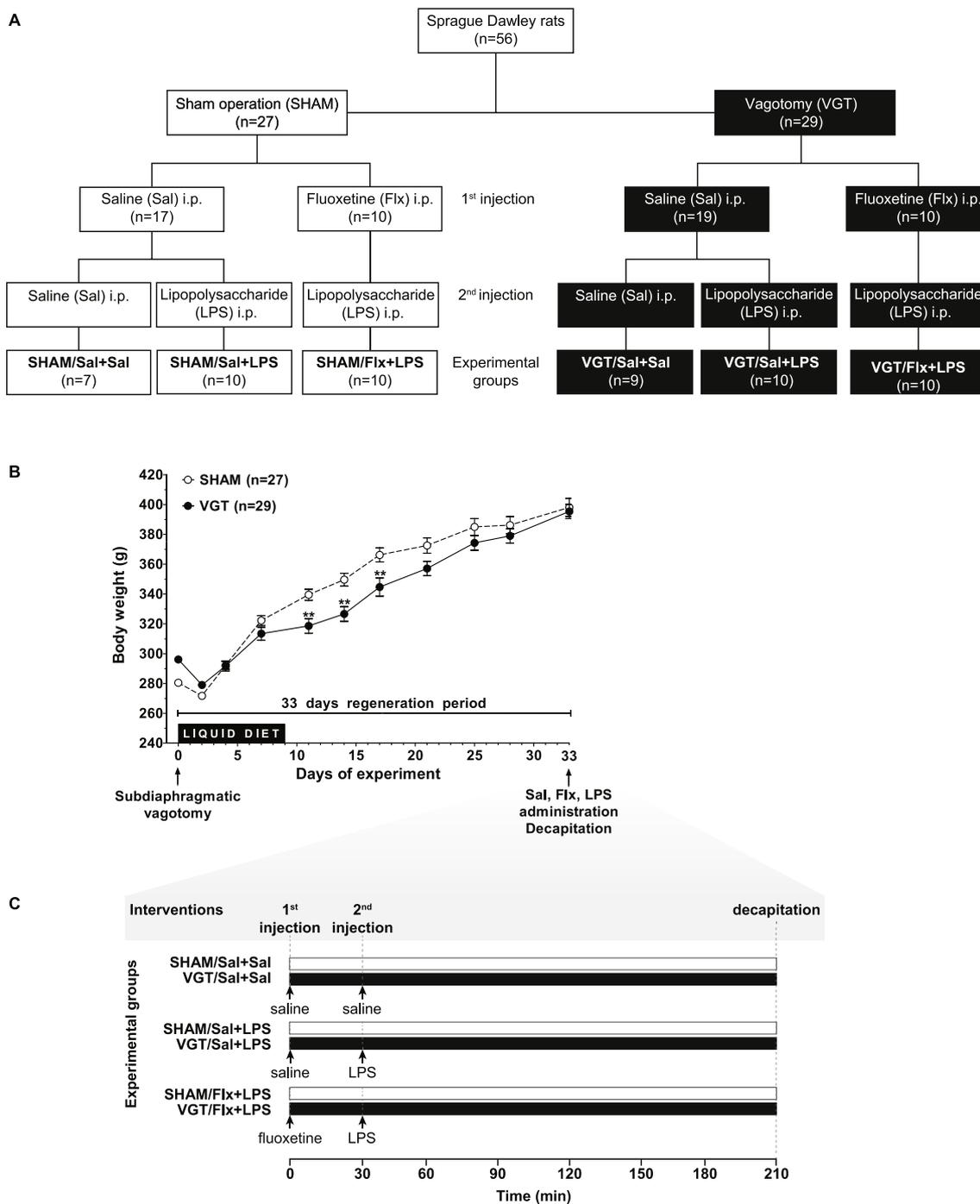


Fig. 1. Schematic illustration of the experimental design for animals who underwent a sham operation or subdiaphragmatic vagotomy (A, C) and a graph charting the changes of body weight for 4 weeks after surgery (B). Sham-operated (SHAM; n = 27; ○) and vagotomized (VGT; n = 29; ●) rats were fed with a liquid diet for the first 9 days after surgery (B) to prevent gastrointestinal complications and mortality in the vagotomized rats. After this critical time, rats were fed with regular pelleted food for the remainder of the study. The body weight of the animals was measured three times a week to determine recovery after surgery. On days 11, 14, and 17, the body mass difference between SHAM and VGT rats was ± 22 g. On day 33, the body weight was same in both groups and animals received a single intraperitoneal (i.p.) injection of either fluoxetine (Flx) or saline (Sal) as indicated at (A) and (C) as the 1st injection. Thirty minutes later, the rats received an i.p. injection of a lipopolysaccharide (LPS) or saline (Sal), as indicated at (A) and (C) as the 2nd injection. Three hours after the second i.p. injection, rats were killed by decapitation (C). Statistical significance of the difference between SHAM and VGT groups at the same time point: ***P* < 0.01, as measured by a two-way ANOVA. Data are expressed as mean ± SEM.

of 2 min at 50 °C, followed by 1 cycle of 10 min at 95 °C, and then 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Data were normalized to GAPDH levels and expressed as the relative fold change, calculated using the $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001). A melting curve analysis was performed to confirm the specificity of the amplified products. Sample values were calculated as x-fold difference from

control sample (SHAM/Sal + Sal, value determined as 1) within the same experiment.

2.3.5. Determination of plasma cytokine levels

Immediately after decapitation, trunk blood was collected in polyethylene tubes containing EDTA (1 mg/mL blood) and centrifuged at

Table 1

The diameters of punching needles used for microdissections of selected brainstem and forebrain structures and nuclei in the rat brain.

Brain structures	Abbreviations	Punching needles diameter (μm)
Nucleus of the solitary tract	NTS	600
Dorsal motor nucleus of the vagus	DMNV	600
Paraventricular hypothalamic nucleus	PVN	600
Basolateral amygdala	BLA	900
Central nucleus of the amygdala	CeA	900
Hippocampus	Hip	900
Frontal cortex	FCx	1200

Table 2

Specific primers used for gene expression measurements of *c-fos*, *c-Jun*, *Fra-2*, tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 1beta (IL-1β).

Target gene	Primer set
<i>c-fos</i>	F: 5'-GGCAGAAGGGGCAAAGTAGA -3' R: 5'-AGTTGATCTGTCTCCGCTTGG -3'
TNFα	F: 5'-GATCGGTCCCAACAAGGAGG -3' R: 5'-GTTTGCTACGACGTGGGCTA -3'
IL-6	F: 5'-ATACCACCACAACAGACCAGT-3' R: 5'-GATGAGTTGGATGGTCTTGGT-3'
IL-1β	F: 5'-CAGCTTTCGACAGTGAGGAGA -3' R: 5'-TGTCGAGATGCTGCTGTGAG -3'
<i>c-Jun</i>	F: 5'-TGGGCACATCACCACACTACAC-3' R: 5'-GGGCAGCGTATTCTGGCTAT-3'
<i>Fra-2</i>	F: 5'-TACCTCCATGTCCAACCCCT-3' R: 5'-GACAGCTGCTCATCTCTCCT-3'
GAPDH	F: 5'-TGGACCACCCAGCCAGCAAG-3' R: 5'-GGCCCTCCTGTTTATGGGGT-3'

10,000 × g for 10 min at 4 °C to separate the plasma, which was stored at –20 °C until analyzed. Plasma samples were assayed for IL-6, TNF-α, and IL-1β using commercial rat IL-6, TNF-α, and IL-1β Quantikine ELISA kits according to the manufacturer's instructions (R&D Systems Europe, Abingdon, UK). Assays were sensitive to 21 pg/mL of IL-6, less than 5 pg/mL of TNF-α, and 5 pg/mL of IL-1β, with inter- and intra-assay coefficients of variation less than 10%.

2.4. Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 program (GraphPad Software, San Diego CA, USA). Statistical differences among the groups were determined by one- or two-way analyses of variance (ANOVA) followed by post hoc pair wise comparisons using Bonferroni's correction. Differences were considered statistically significant at $P < 0.05$. Data are expressed as mean ± SEM and representing the mean for 7–10 rats.

3. Results

3.1. Effect of vagotomy and fluoxetine pretreatment on lipopolysaccharide-induced peripheral inflammation

Efferent pathways of the vagus nerve exert potent anti-inflammatory effects (Pavlov and Tracey, 2015). To investigate the role of these pathways in Flx peripheral anti-inflammatory effect, animals with an intact or transected vagus nerve were pretreated by saline or Flx and then exposed to the immune stimulus LPS.

3.1.1. Response to saline administration

Plasma cytokine levels in animals injected with saline (Sal + Sal) were below detection limits, except for the TNF-α level found in

vagotomized animals (1.33 ± 0.23 pg/mL; Fig. 2A).

3.1.2. Response to LPS administration

In both sham-operated and vagotomized groups, intraperitoneal administration of LPS (Sal + LPS groups at Fig. 2A and B, C) induced profound increases in the plasma levels of all three assessed cytokines. However, the response in vagotomized animals was lower than what was found in sham-operated animals, with a statistically significant difference only found for TNF-α ($t_{19} = 5.954$; $P < 0.001$; Fig. 2A). Therefore, it could be concluded that vagotomy may attenuate some components of the inflammatory response induced by LPS injection.

3.1.3. Response to LPS in animals pretreated with fluoxetine

Pretreatment with Flx 30 min before injection of LPS (groups Flx + LPS at Fig. 2) brought about a different response. Fluoxetine administration caused a complete inhibition of the IL-1β cytokine response to the LPS challenge in both sham-operated and vagotomized animals (Fig. 2B), and an almost complete inhibition of TNF-α in the SHAM operated group (Fig. 2A). The increases in TNF-α and IL-6 after LPS injection in animals pretreated with fluoxetine were higher in VGT groups compared to sham-operated animals with a significant difference only detected for IL-6 ($t_{19} = 5.212$, $P < 0.001$; Fig. 2C).

When comparing cytokine plasma levels between vagotomized rats exposed to LPS and pretreated by either Flx or saline (VGT/Flx + LPS - SHAM/Flx + LPS vs. SHAM/Sal + LPS), it can be estimated that the normal function of vagus nerve contributes 18% of Flx observed attenuation effect on TNF-α and 42% to the effect on IL-6.

3.1.4. Reaction to LPS challenge within the spleen

Because the spleen represents a crucial organ in the modulation of peripheral inflammation and stimulation of efferent vagal pathways exerts potent suppression of pro-inflammatory cytokine production in the spleen (Reardon, 2016), we investigated the effect of Flx pretreatment on LPS-induced peripheral inflammation by measuring the expression of specific genes within the spleen by assessment of corresponding mRNA levels.

Administration of LPS induced significant increases in the mRNA levels of all examined cytokines when compared to the Sal + Sal group in both sham-operated and vagotomized animals: TNF-α (SHAM: $t_{11} = 6.408$, $P < 0.001$; VGT: $t_{11} = 5.869$, $P < 0.001$), IL-1β (SHAM: $t_{11} = 5.285$, $P < 0.001$; VGT: $t_{11} = 4.132$, $P < 0.01$), and IL-6 (SHAM: $t_{11} = 7.264$, $P < 0.001$; VGT: $t_{11} = 4.603$, $P < 0.01$). That there were no significant differences in the increase of cytokines between sham-operated and vagotomized groups indicates that the vagus nerve is not extensively involved in splenic TNF-α, IL-1β, and IL-6 production in response to intraperitoneal LPS exposure (Fig. 2D–F).

Fluoxetine pretreatment significantly abolished LPS-induced increases in splenic mRNA levels of pro-inflammatory cytokines in sham-operated rats in comparison with saline pretreatment for all three cytokines (TNF-α: $t_{10} = 5.946$, $P < 0.001$; IL-1β: $t_{10} = 3.772$, $P < 0.05$; IL-6: $t_{10} = 6.906$, $P < 0.001$; Fig. 2D–F). However, in vagotomized rats a significant difference was only found for TNF-α mRNA expression ($t_{10} = 3.311$, $P < 0.05$; Fig. 2D). When comparing gene expression of pro-inflammatory cytokines in the spleen between sham-operated and vagotomized rats exposed to LPS following either Flx or saline pretreatment (VGT/Flx + LPS - SHAM/Flx + LPS vs. SHAM/Sal + LPS), it can be estimated that the vagus nerve contributes 37% of Flx attenuation effect on TNF-α mRNA levels, 72% of the effect on IL-1β mRNA levels, and 21% of the effect on IL-6 mRNA levels (Fig. 2D–F).

3.2. Effect of vagotomy and fluoxetine on lipopolysaccharide-induced changes in neuronal activity and pro-inflammatory cytokine mRNA levels in selected brain structures

Peripheral inflammatory stimuli can trigger central nervous system inflammation (Benicky et al., 2009). Therefore, we investigated anti-

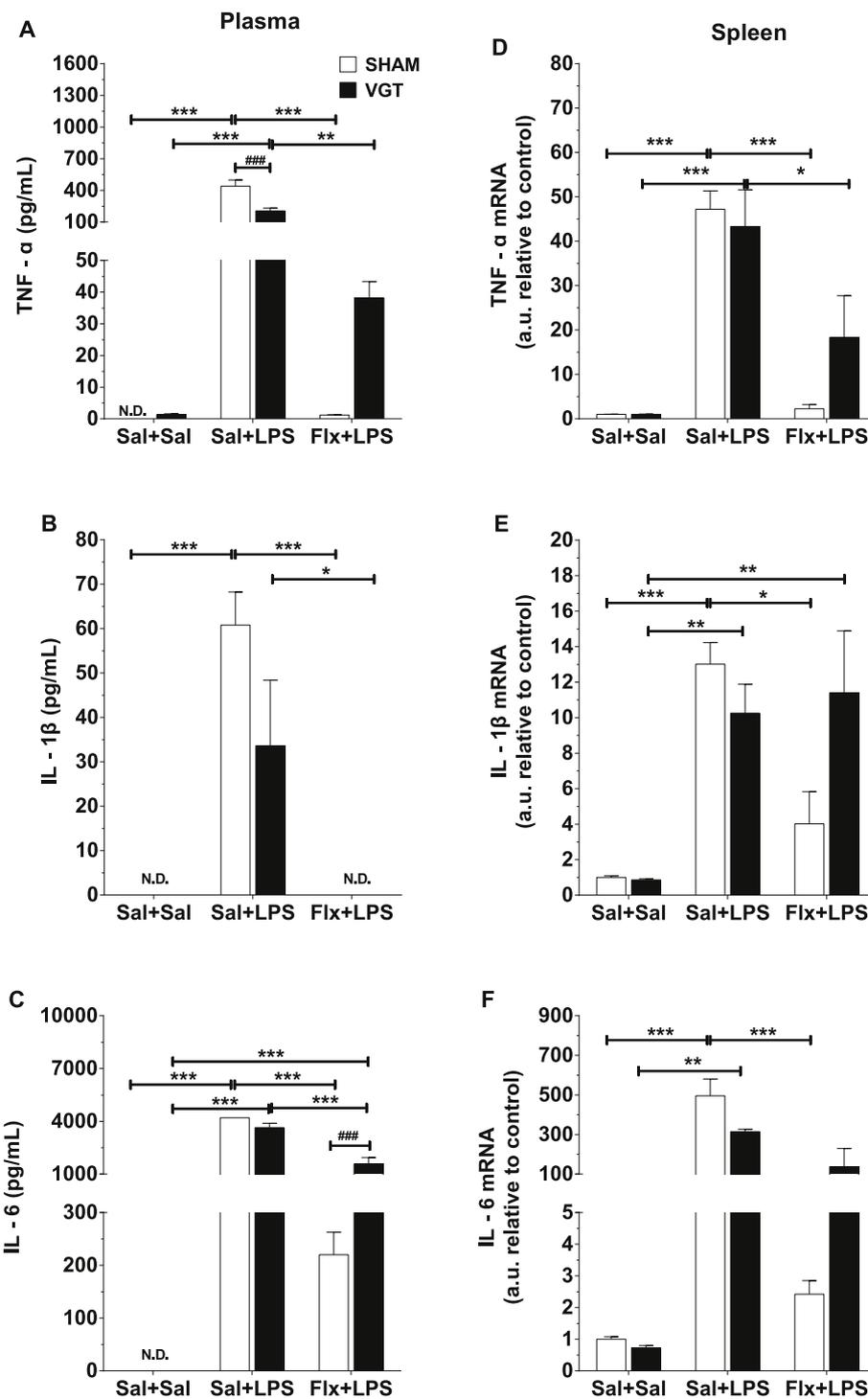


Fig. 2. Effect of an intraperitoneal injection (i.p.) of fluoxetine (Flx) or saline (Sal) on lipopolysaccharide (LPS)-induced changes in plasma levels of the pro-inflammatory cytokines (A–C) and of gene expression of the proinflammatory cytokines in the spleen (D–F) in sham-operated (SHAM; □) or vagotomized (VGT; ■) rats. Data are expressed as mean ± SEM and were analyzed using a one-way ANOVA. Significance is given as: * differences with group, **P* < 0.05; ***P* < 0.01; ****P* < 0.001; # differences between SHAM and VGT animals undergone the same treatment; ###*P* < 0.001. N.D. not detected.

inflammatory effect of Flx in combination with either an intact or transected vagus nerve in brain structures that: 1) process inflammatory signals from the periphery (NTS), 2) activate the anti-inflammatory response (PVN, DMNV; (Jänig, 2006), or 3) regulate behavioral and psychopathological changes in depression (BLA, CeA, Hip, FCx; (Drevets et al., 2008; Pandya et al., 2012). The activity at selected structures was assessed by measurement of the expression of *c-fos* and pro-inflammatory cytokine genes.

Intraperitoneal administration of LPS induced a significant increase of *c-fos* gene expression in sham-operated rats only in the NTS ($t_{11} = 3.719, P < 0.05$) and PVN ($t_{11} = 9.264, P < 0.001$), whereas Flx pretreatment prevented this effect as shown by comparing the Sal + LPS vs. Flx + LPS groups (NTS: $t_{11} = 4.484, P < 0.01$; PVN: $t_{10} = 9.198, P < 0.001$) (Fig. 3A and B). Neither administration of LPS alone nor pretreatment with Flx significantly affected *c-fos* mRNA levels in the remaining investigated brain structures (Fig. 3C–G).

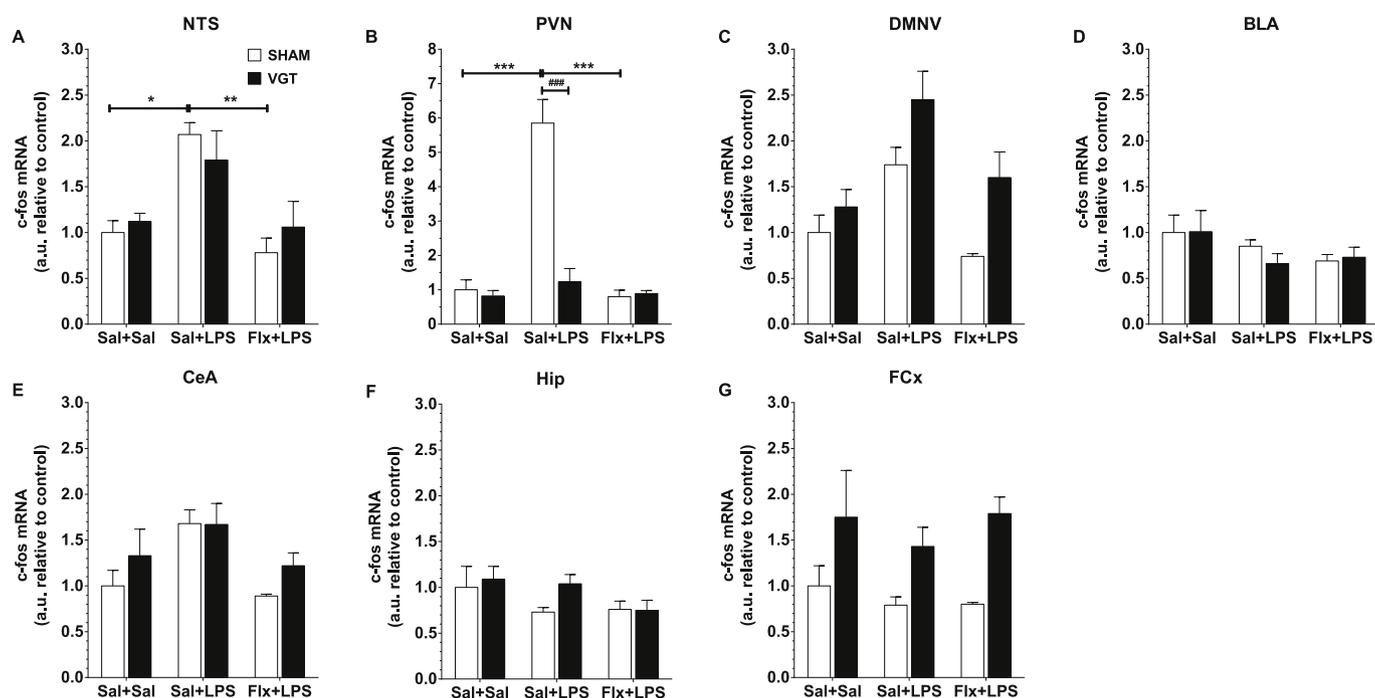


Fig. 3. Effect of an intraperitoneal injection (i.p.) of fluoxetine (Flx) or saline (Sal) on lipopolysaccharide (LPS)-induced changes in the expression of the immediate-early gene *c-fos* mRNA in brain structures of sham-operated (SHAM; □) or vagotomized (VGT; ■) rats. Data are expressed as mean \pm SEM and were analyzed using a one-way ANOVA. Significance is given as: * differences between SHAM and VGT groups with different treatments, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; # differences between SHAM and VGT animals with identical treatment; ### $P < 0.001$. NTS nucleus of the solitary tract; DMNV dorsal motor nucleus of the vagus; PVN paraventricular hypothalamic nucleus, BLA basolateral amygdala, CeA central nucleus of the amygdala; Hip hippocampus; FCx frontal cortex.

Based on our knowledge of the relationship between central and peripheral regulation of inflammatory processes (Miller et al., 2009; Perry, 2004) we investigated members of the Fos family (*c-fos* and its smaller splice variants, *Fra-2*) in combination with *c-Jun*, as early markers of neuronal activation in the DMNV, which contains neurons that transmit anti-inflammatory signals via efferent vagal pathways (Pavlov et al., 2003). Administration of LPS in both sham-operated and vagotomized animals induced a significant increase of *Fra-2* mRNA levels in the DMNV (SHAM: $t_{11} = 4.884$, $P < 0.001$; VGT: $t_{11} = 4.994$, $P < 0.001$; Supplementary Fig. 1B), however, gene expression of *c-fos* and *c-Jun* was not affected in this brain area (Fig. 3C; Supplementary Fig. 1A). Additionally, Flx pretreatment significantly reduced LPS-induced increases of *Fra-2* mRNA levels in the DMNV of sham-operated animals ($t_{11} = 3.599$, $P < 0.05$) and there was a trend suggesting an attenuated increase of *c-fos* mRNA levels in vagotomized rats ($t_{10} = 2.674$; Fig. 3C) and *Fra-2* ($t_{10} = 2.581$; Supplementary Fig. 1B).

Administration of LPS induced significant increases in gene expression of TNF- α and IL-1 β mRNA in all investigated brain structures of sham-operated animals (Figs. 4 and 5). Vagotomy significantly reduced the LPS-induced expression of TNF- α in the NTS ($t_{11} = 3.878$, $P < 0.01$), DMNV ($t_{10} = 2.410$, $P < 0.05$), PVN ($t_{11} = 4.904$, $P < 0.001$), BLA ($t_{11} = 4.827$, $P < 0.001$), CeA ($t_{11} = 3.302$, $P < 0.05$), and FCx ($t_{11} = 4.838$, $P < 0.001$) compared to the SHAM/Sal + LPS group. Similarly, while vagotomy significantly reduced LPS-induced expression of IL-1 β in the DMNV ($t_{10} = 3.488$, $P < 0.05$), PVN ($t_{11} = 5.090$, $P < 0.001$), BLA ($t_{11} = 5.515$, $P < 0.001$), and CeA ($t_{11} = 3.757$, $P < 0.05$) compared to the SHAM/Sal + LPS group, differences in the expression of IL-1 β in the NTS (Fig. 4B), Hip, and FCx (Fig. 5F, H) were not significant.

Fluoxetine pretreatment completely abolished LPS-induced increases of TNF- α and IL-1 β mRNA levels in all brain structures of sham-operated animals (Figs. 4 and 5). Furthermore, there were significant differences between the VGT/Sal + LPS and VGT/Flx + LPS groups in expression of the TNF- α gene in the NTS ($t_{11} = 3.214$, $P < 0.05$; Fig. 4A), BLA ($t_{11} = 3.471$, $P < 0.05$; Fig. 5A), Hip ($t_{11} = 3.552$,

$P < 0.05$; Fig. 5E), and FCx ($t_{11} = 6.269$, $P < 0.001$; Fig. 5G). Additionally, TNF- α and IL-1 β mRNA levels in vagotomized animals pretreated by Flx were similar to levels detected in Flx treated, sham-operated animals with the exception of IL-1 β mRNA levels within the PVN ($t_{11} = 4.036$, $P < 0.01$; Fig. 4F) and Hip ($t_{11} = 3.355$, $P < 0.05$; Fig. 5F), which were significantly higher.

4. Discussion

The cytokine theory of depression indicates that anti-inflammatory drugs may be useful for the treatment of this disease (Kohler et al., 2016). Importantly, it has been proven that several antidepressants exert anti-inflammatory effects in the brain as well as in the periphery by different mechanisms (Galecki et al., 2018; Walker, 2013). These mechanisms are attributed to their direct effect on immune cells in the brain, in addition to peripheral tissues and circulation (Galecki et al., 2018; Nazimek et al., 2017; Tynan et al., 2012). The peripheral anti-inflammatory effects of antidepressants from the Selective Serotonin Re-uptake Inhibitor (SSRI) class are attributed to serotonin-dependent as well as serotonin-independent mechanisms (Di Rosso et al., 2016). Serotonin has complex effects on various components of the immune system, with different immune cells expressing a variety of serotonin receptors and serotonin transporter (SERT) with consequent activation or inhibition of homeostatic regulatory processes (for review see (Herr et al., 2017)).

Although Flx is classified as a SSRI antidepressant, interpreting its mechanism of action should not be limited to the effect of serotonin only. The blockade of SERT and increased availability of neurotransmitter in the synaptic cleft causes a net effect of involvement of other neurotransmitter systems and circuits (Blieher and El Mansari, 2013). In addition to the nanomolar affinity to SERT, Flx has micromolar affinities to the noradrenaline transporter, serotonin 5-HT $_2C$ receptors (Wong et al., 1995), and muscarinic M $_1$ receptors (Owens et al., 2001). There are also reports about direct activity of Flx on the immune system independent from its effect on serotonin turnover. This

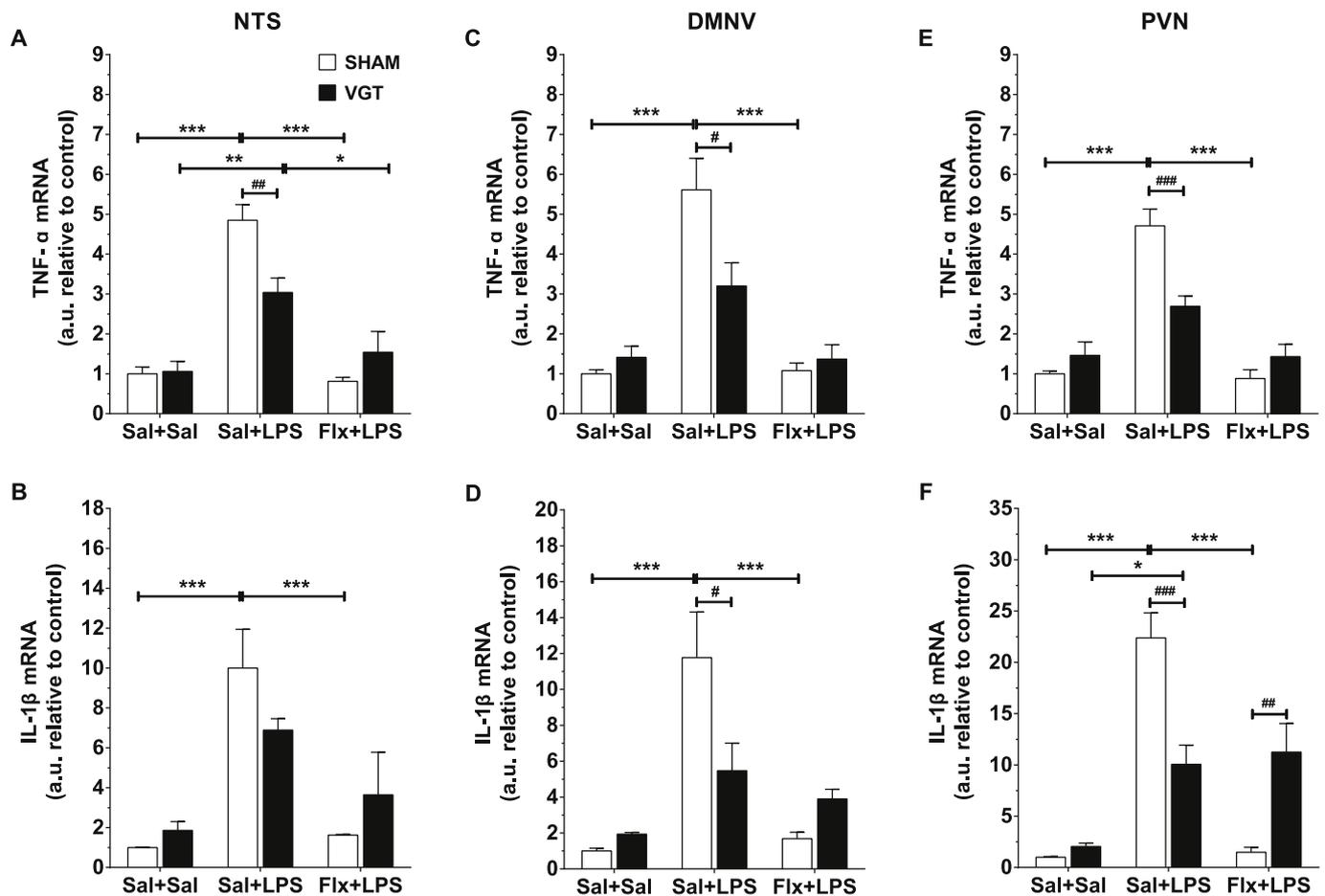


Fig. 4. Effect of an intraperitoneal injection (i.p.) of fluoxetine (Flx) or saline (Sal) on lipopolysaccharide (LPS)-induced changes in gene expression of pro-inflammatory cytokines in rat brain structures in sham-operated (SHAM; □) or vagotomized (VGT; ■) rats. Data are expressed as mean \pm SEM and are analyzed using a one-way ANOVA. Significance is given as: * differences between SHAM and VGT groups with different treatments, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; # differences between SHAM and VGT animals with same treatment; $\#P < 0.05$; $\#\#P < 0.01$; $\#\#\#P < 0.001$. NTS nucleus of the solitary tract; DMNV dorsal motor nucleus of the vagus; PVN paraventricular hypothalamic nucleus.

encompasses an inhibitory effect on T lymphocytes and the expression of the cytokines TNF- α and interferon gamma (INF- γ) (Frick et al., 2008). Moreover, a possible interaction with Toll-like-receptor 4 (TLR4) at the same site as bacterial LPS, along with cholinergic signaling through neuronal $\alpha 2\beta 4$ or $\alpha 3\beta 4$ nicotinic acetylcholine receptors (nAChRs), could result in decreased production of pro-inflammatory cytokines (Waiskopf et al., 2014). Importantly, several data indicate that the peripheral anti-inflammatory effect of antidepressants may be, at least partially, mediated by efferent vagal pathways employing cholinergic signaling (for review (see (Ondicova et al., 2010).

Based on the above-mentioned facts we investigated the anti-inflammatory effect of Flx in rats exposed to an immune stimulus after transmission of immune-related signals via the vagus nerve was interrupted by subdiaphragmatic vagotomy. We found that intraperitoneal administration of LPS induced huge increases in plasma TNF- α , IL-1 β , and IL-6 levels in both sham-operated and vagotomized rats, and also significantly enhanced the splenic mRNA levels of these cytokines. These findings confirm results from previous studies (Benicky et al., 2009) and indicate that we were using an adequate dose of LPS to induce intensive activation of the peripheral immune system. Intraperitoneal injection of LPS activates afferent vagal nerves projecting to the central nervous system and their subsequent induction of efferent signals that suppresses cytokine levels in the periphery. This effect is mediated at least partially by activation of the splenic nerve (Olofsson et al., 2012). In contrast to the above-mentioned vagal anti-

inflammatory effect, we found that subdiaphragmatic vagotomy did not potentiate LPS-induced increases in plasma levels of IL-1 β and IL-6, as well as mRNA levels of TNF- α , IL-1 β , and IL-6 in the spleen. Interestingly, we found that vagotomy significantly decreased plasma TNF- α levels when compared to sham-operated rats. These findings are supported by previously published data indicating that the peripheral inflammatory response to LPS is not dependent solely on the transmission of signals by afferent or efferent vagal pathways running through the subdiaphragmatic portion of the vagus nerve innervating the abdominal cavity (Hansen et al., 2000; Zielinski et al., 2013). Our findings may reflect the fact that afferent signalization related to LPS injection was interrupted by vagotomy. We suggest that processing LPS-related signalization transmitted by the vagus nerve to the brain is necessary for a fully developed inflammatory response to LPS in animals with interrupted efferent vagal pathways.

The pretreatment of sham-operated animals with Flx almost completely abolished the LPS-induced increase of some investigated markers of inflammation, which underscores what is known about the anti-inflammatory action of this antidepressant (Lu et al., 2017; Reardon, 2016; Roumestan et al., 2007). So, our findings indicate that Flx exerts potent anti-inflammatory effects on the periphery in this animal model. However, a crucial result of our study found that vagotomy lowers the effect of pretreatment with Flx on LPS-induced increases of inflammatory markers in plasma and spleen. This finding strongly indicates that the peripheral anti-inflammatory effect of Flx is at least partially mediated by the vagus nerve. The proportional extent of how

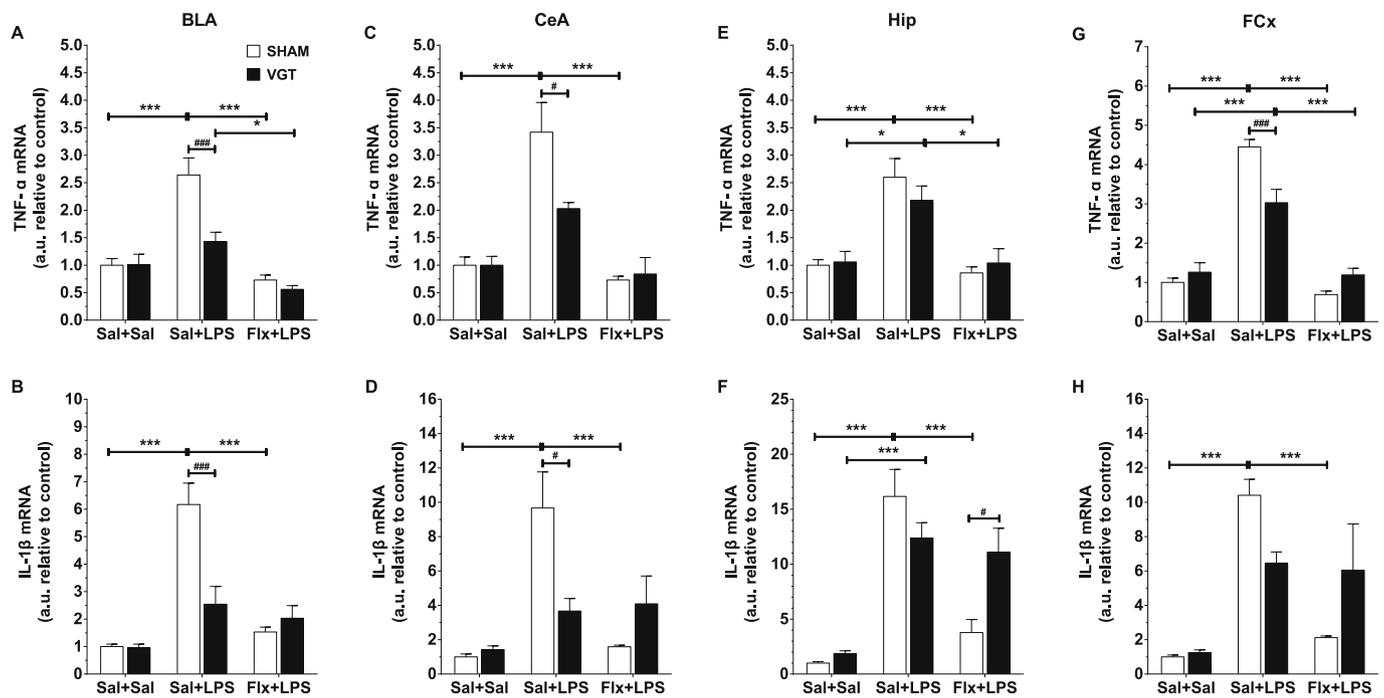


Fig. 5. Effect of an intraperitoneal injection (i.p.) of fluoxetine (Flx) or saline (Sal) on lipopolysaccharide (LPS)-induced changes in gene expression of the pro-inflammatory cytokines in the rat brain structures in sham-operated (SHAM; □) or vagotomized (VGT; ■) rats. Data are expressed as mean \pm SEM and analyzed using a one-way ANOVA. Significance is given as: * differences between SHAM and VGT groups with different treatments, * $P < 0.05$; *** $P < 0.001$; # differences between SHAM and VGT animals with same treatment; # $P < 0.05$; ### $P < 0.001$. BLA basolateral amygdala, CeA central nucleus of the amygdala, Hip hippocampus; FCx frontal cortex.

the peripheral anti-inflammatory effect of Flx is dependent on efferent vagal pathways can be estimated from differences in cytokine mRNA levels between vagotomized and sham-operated animals treated by Flx (Fig. 2). Because subdiaphragmatic vagotomy interrupts transmission of signals by both afferent and efferent vagal pathways, the question arises of whether afferent and/or efferent vagal pathways participate in this effect. It is known that the vagus nerve exerts its anti-inflammatory effects via cholinergic signaling (Rosas-Ballina et al., 2011) and that antidepressants are able to suppress inflammation by direct action on peripheral immune cells, with at least part of these effects not dependent on serotonin (Nazimek et al., 2017). Importantly, interaction between the serotonin and cholinergic systems has been described for different functional neural systems (Scarr et al., 2013). For example, serotonin released from the gastrointestinal system activates vagal afferent nerves resulting in activation of neurons in the NTS, area postrema, PVN, and supraoptic nucleus. This activation seems to be mediated by 5-HT₃ receptors and is blocked by truncal vagotomy or perivagal application of capsaicin (Mazda et al., 2004). It is possible that a similar mechanism could underlie the mediation of inflammatory response studied in our current work.

However, if Flx anti-inflammatory effect in the periphery depended solely on its direct effect on peripheral immune cells, then vagotomy would have little or no influence on this process. The absence of potentiation of LPS-induced increases of either inflammatory markers in plasma or spleen by vagotomy indicates that there is a missing additional activation of the immune response in the periphery induced by brain structures regulating immune homeostasis. Based on these findings, we suggest that Flx anti-inflammatory effect is not mediated solely by its direct effect on peripheral immune cells, but also by its effect on efferent vagal pathways known for their potent anti-inflammatory influence on peripheral tissues.

Based on the above-mentioned findings we also investigated the neuronal activity of the 3 crucial brain structures: 1) NTS in the brainstem as the main relay station for signals transmitted via afferent vagal pathways; 2) DMNV as the final regulator of the anti-

inflammatory effect of efferent vagal pathways, which is interconnected with NTS; 3) PVN as the main integration center for neuroendocrine and autonomic responses to peripheral immune stimuli. Furthermore, the other brain structures (Hip, FCx, BLA, and CeA) were studied as they are related to regulation of inflammation related sickness behavior and emotions.

In the sham-operated rats, vagal afferent signaling in the NTS and PVN was demonstrated by an increase in the expression of the early gene *c-fos* in response to intraperitoneally administered LPS. Because no differences were found between NTS neuronal activity in sham-operated and vagotomized animals injected with LPS we suggest that immune-related signals may not utilize abdominal vagal pathways and may be transmitted to the brain exclusively via humoral or cellular pathways (Capuron and Miller, 2011). Suppression of LPS-induced increases of *c-fos* gene expression in the NTS of sham-operated animals pretreated by Flx indicates that this antidepressant also modulates afferent transmission of inflammation-related signals from the NTS to other brain structures. Furthermore, complete inhibition of LPS-induced increases of *c-fos* mRNA levels in the PVN of sham-operated animals by Flx indicates that this antidepressant also modulates activity of brain structures orchestrating the neuroendocrine, autonomic, and immune response to inflammatory stimuli. Additionally, LPS injection did not activate the immediate early genes *c-fos* and *c-Jun* in the DMNV, which modulates activity of vagal preganglionic neurons, even though *c-fos* mRNA expression is generally induced within 15 min of stimulation and *c-Jun* is activated by extracellular stimuli, including pro-inflammatory cytokines (Brenner et al., 1989; Wisdom et al., 1999). Therefore, we examined the activation of *Fra-2* expression, which is delayed and more stable. Previous studies have demonstrated that *Fra-2* expression in the lungs or osteoblasts plays a key role in systematic inflammation via an enhanced inflammatory response to LPS, while molecular changes in the microenvironment can influence the inflammatory response (Luo et al., 2018; Mishra et al., 2016). We found that LPS induced *Fra-2* mRNA expression in the DMNV in both SHAM and VGT groups and that pretreatment with Flx reduced this activity,

predominantly in sham-operated animals. These data, although somewhat contradictory, may indicate that the general activity of vagal efferent pathways is suppressed by Flx, but it would be necessary to utilize additional methods, like patch clamp or direct electrophysiological detection of the firing of efferent vagal axons to determine the activity of the small population of vagal preganglionic neurons that are a part of the vagal anti-inflammatory pathway.

In accordance with data showing that peripheral immune stimuli are able to induce neuroinflammation (D'Mello and Swain, 2017), we found that intraperitoneal injection of LPS induced significant increases in gene expression of the pro-inflammatory cytokines involved in induction of sickness behavior and depressed mood (IL-1 β and TNF- α) in all investigated brain structures (Figs. 4 and 5). In line with other authors, we have shown that subdiaphragmatic vagotomy blunted the LPS-induced TNF- α and IL-1 β expression in the investigated brain structures independently from peripheral cytokine expression (Hansen et al., 1998; Laye et al., 1995). We also found an anti-inflammatory effect of vagotomy in selected brain structures independent from the systematic effect of vagal immune modulation. It has been suggested that neural communication pathways contribute to cytokine-to-brain communication when the route of administration and dose of injected LPS do not cause sepsis (Dantzer et al., 2008). Therefore, if the inflammation response is systematic and highly intensive, the humoral and cellular pathways could override the afferent pathways of the vagus nerve (Schweighofer et al., 2016). These findings may reflect the fact that under different conditions, peripheral cytokines use multiple routes of communication to the brain.

Suppression of neuroinflammation by Flx confirmed that this antidepressant exerts potent anti-inflammatory effects, not only at the level of peripheral tissues and circulation, but also in the brain (Lu et al., 2017). The potential of Flx and other antidepressants to change the LPS-induced inflammatory response has been shown via in vitro experiments that excluded the modulatory effect of neuronal signalization (Baumeister et al., 2016). In our experiment, Flx combined with vagotomy had no significant anti-inflammatory effects because vagotomy itself prevents LPS-induced neuroinflammation. Moreover, these findings emphasize the role of antidepressants in the attenuation or prevention of the induction of neuroinflammation caused by peripheral immune stimuli.

4.1. Caveats

It is necessary to note that our study has several limitations. First, we investigated the effect of a single Flx administration on acute inflammation. However, published data indicate that depression is associated with long-lasting pro-inflammatory activity in the periphery and in the brain. Therefore, it will be necessary to confirm the anti-inflammatory effect of Flx in an animal model of chronic inflammation. To generalize our findings, it will also be necessary to determine potential anti-inflammatory effect of other antidepressants. Moreover, it is necessary to take in consideration the fact that the loss of vagal signaling in vagotomized animals could have many effects on the immune system that could alter immune cell response to Flx. For example, it is possible that the loss of tonic vagal anti-inflammatory signaling, as previously described (Karimi et al., 2010; O'Mahony et al., 2009), accounts for the apparent reduction in the effectiveness of Flx. Immune responses could also be modulated through changes in catecholamine responses following vagotomy.

5. Conclusion

Our data indicate that the peripheral anti-inflammatory action of Flx depends, at least partially, on activation of vagal efferent pathways. These pathways represent crucial neuronal mechanisms regulating immune system activity (Bonaz et al., 2016). Therefore, it can be hypothesized that the therapeutic efficiency of Flx depends not only on

restoration of monoaminergic neurotransmission and neuroinflammation in the brain and direct suppression of the activity of immune cell in the periphery, but also on the attenuation of immune responses within the periphery through vagal efferent pathways. However, further studies with other antidepressants, along with studies involving long-term administration of antidepressants to animals with protractedly increased pro-inflammatory cytokine levels are necessary to generalize the involvement of these pathways in the clinical efficacy of antidepressant drugs as a group. Confirmation of this effect may extend our knowledge about mechanisms through which antidepressants, vagus nerve stimulation, regular exercise, and other vagus nerve activating approaches alleviate depressive symptoms.

Conflicts of interest

The authors declare that there is no conflict of interest in the publication of this study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neuint.2019.02.003>.

References

- Baumeister, D., Ciufolini, S., Mondelli, V., 2016. Effects of psychotropic drugs on inflammation: consequence or mediator of therapeutic effects in psychiatric treatment? *Psychopharmacology (Berlin)* 233, 1575–1589.
- Benicky, J., Sanchez-Lemus, E., Pavel, J., Saavedra, J.M., 2009. Anti-inflammatory effects of angiotensin receptor blockers in the brain and the periphery. *Cell. Mol. Neurobiol.* 29, 781–792.
- Blier, P., El Mansari, M., 2013. Serotonin and beyond: therapeutics for major depression. *Philos. Trans. R Soc Lond B Biol. Sci.* 368, 20120536.
- Bonaz, B., Sinniger, V., Pellissier, S., 2016. Anti-inflammatory properties of the vagus nerve: potential therapeutic implications of vagus nerve stimulation. *J. Physiol.* 594, 5781–5790.
- Brenner, D.A., O'Hara, M., Angel, P., Chojkier, M., Karin, M., 1989. Prolonged activation of jun and collagenase genes by tumour necrosis factor- α . *Nature* 337, 661–663.
- Capuron, L., Miller, A.H., 2011. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacol. Ther.* 130, 226–238.
- Connor, T.J., Leonard, B.E., 1998. Depression, stress and immunological activation: the role of cytokines in depressive disorders. *Life Sci.* 62, 583–606.
- D'Mello, C., Swain, M.G., 2017. Immune-to-Brain communication pathways in inflammation-associated sickness and depression. *Curr Top Behav Neurosci* 31, 73–94.
- Dahl, J., Ormstad, H., Aass, H.C., Malt, U.F., Bendz, L.T., Sandvik, L., Brundin, L., Andreassen, O.A., 2014. The plasma levels of various cytokines are increased during ongoing depression and are reduced to normal levels after recovery. *Psychoneuroendocrinology* 45, 77–86.
- Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat. Rev. Neurosci.* 9, 46–56.
- Di Comite, G., Grazia Sabbadini, M., Corti, A., Rovere-Querini, P., Manfredi, A.A., 2007. Conversation galante: how the immune and the neuroendocrine systems talk to each other. *Autoimmun. Rev.* 7, 23–29.
- Di Rosso, M.E., Palumbo, M.L., Genaro, A.M., 2016. Immunomodulatory effects of fluoxetine: a new potential pharmacological action for a classic antidepressant drug? *Pharmacol. Res.* 109, 101–107.
- Drevets, W.C., Price, J.L., Furey, M.L., 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct. Funct.* 213, 93–118.
- Elenkov, I.J., Wilder, R.L., Chrousos, G.P., Vizi, E.S., 2000. The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. *Pharmacol. Rev.* 52, 595–638.
- Ferrari, A.J., Charlson, F.J., Norman, R.E., Patten, S.B., Freedman, G., Murray, C.J., Vos, T., Whiteford, H.A., 2013. Burden of depressive disorders by country, sex, age, and year: findings from the global burden of disease study 2010. *PLoS Med.* 10 e1001547.
- Frick, L.R., Palumbo, M.L., Zappia, M.P., Brocco, M.A., Cremaschi, G.A., Genaro, A.M., 2008. Inhibitory effect of fluoxetine on lymphoma growth through the modulation of antitumor T-cell response by serotonin-dependent and independent mechanisms.

- Biochem. Pharmacol. 75, 1817–1826.
- Galecki, P., Mossakowska-Wojcik, J., Talarowska, M., 2018. The anti-inflammatory mechanism of antidepressants - SSRIs, SNRIs. *Prog. Neuro Psychopharmacol. Biol. Psychiatr.* 80, 291–294.
- Hannestad, J., DellaGioia, N., Bloch, M., 2011. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: a meta-analysis. *Neuropsychopharmacology* 36, 2452–2459.
- Hansen, M.K., Nguyen, K.T., Fleschner, M., Goehler, L.E., Gaykema, R.P., Maier, S.F., Watkins, L.R., 2000. Effects of vagotomy on serum endotoxin, cytokines, and corticosterone after intraperitoneal lipopolysaccharide. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 278, R331–R336.
- Hansen, M.K., Taishi, P., Chen, Z., Krueger, J.M., 1998. Vagotomy blocks the induction of interleukin-1beta (IL-1beta) mRNA in the brain of rats in response to systemic IL-1beta. *J. Neurosci.* 18, 2247–2253.
- Herr, N., Bode, C., Duerschmied, D., 2017. The effects of serotonin in immune cells. *Front Cardiovasc Med* 4, 48.
- Jänig, W., 2006. *The Integrative Action of the Autonomic Nervous System. Neurobiology of Homeostasis.* Cambridge University Press, Cambridge.
- Karimi, K., Bienenstock, J., Wang, L., Forsythe, P., 2010. The vagus nerve modulates CD4+ T cell activity. *Brain Behav. Immun.* 24, 316–323.
- Khasar, S.G., Green, P.G., Miao, F.J., Levine, J.D., 2003. Vagal modulation of nociception is mediated by adrenomedullary epinephrine in the rat. *Eur. J. Neurosci.* 17, 909–915.
- Kohler, O., Krogh, J., Mors, O., Benros, M.E., 2016. Inflammation in depression and the potential for anti-inflammatory treatment. *Curr. Neuropharmacol.* 14, 732–742.
- Laye, S., Bluthé, R.M., Kent, S., Combe, C., Medina, C., Parnet, P., Kelley, K., Dantzer, R., 1995. Subdiaphragmatic vagotomy blocks induction of IL-1 beta mRNA in mice brain in response to peripheral LPS. *Am. J. Physiol.* 268, R1327–R1331.
- Liu, D., Wang, Z., Liu, S., Wang, F., Zhao, S., Hao, A., 2011. Anti-inflammatory effects of fluoxetine in lipopolysaccharide(LPS)-stimulated microglial cells. *Neuropharmacology* 61, 592–599.
- Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25, 402–408.
- Lu, Y., Ho, C.S., Liu, X., Chua, A.N., Wang, W., McIntyre, R.S., Ho, R.C., 2017. Chronic administration of fluoxetine and pro-inflammatory cytokine change in a rat model of depression. *PLoS One* 12, e0186700.
- Luo, Y., Grottsch, B., Hannemann, N., Jimenez, M., Ipseiz, N., Uluckan, O., Lin, N., Schett, G., Wagner, E.F., Bozec, A., 2018. Fra-2 expression in osteoblasts regulates systemic inflammation and lung injury through osteopontin. *Mol. Cell Biol.* 38.
- Maes, M., Berk, M., Goehler, L., Song, C., Anderson, G., Galecki, P., Leonard, B., 2012. Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. *BMC Med.* 10, 66.
- Mazda, T., Yamamoto, H., Fujimura, M., Fujimiyama, M., 2004. Gastric distension-induced release of 5-HT stimulates c-fos expression in specific brain nuclei via 5-HT3 receptors in conscious rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 287, G228–G235.
- Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol. Psychiatry* 65, 732–741.
- Mishra, R.K., Potteti, H.R., Tamatam, C.R., Elangovan, I., Reddy, S.P., 2016. c-Jun is required for nuclear factor-kappaB-dependent, LPS-stimulated fos-related antigen-1 transcription in alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* 55, 667–674.
- Nazimek, K., Strobel, S., Bryniarski, P., Kozłowski, M., Filipczak-Bryniarska, I., Bryniarski, K., 2017. The role of macrophages in anti-inflammatory activity of antidepressant drugs. *Immunobiology* 222, 823–830.
- O'Brien, S.M., Scott, L.V., Dinan, T.G., 2004. Cytokines: abnormalities in major depression and implications for pharmacological treatment. *Hum. Psychopharmacol.* 19, 397–403.
- O'Mahony, C., van der Kleij, H., Bienenstock, J., Shanahan, F., O'Mahony, L., 2009. Loss of vagal anti-inflammatory effect: in vivo visualization and adoptive transfer. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297, R1118–R1126.
- Olofsson, P.S., Rosas-Ballina, M., Levine, Y.A., Tracey, K.J., 2012. Rethinking inflammation: neural circuits in the regulation of immunity. *Immunol. Rev.* 248, 188–204.
- Ondicova, K., Pecenek, J., Mravec, B., 2010. The role of the vagus nerve in depression. *Neuroendocrinol. Lett.* 31, 602–608.
- Ootsuka, Y., Blessing, W.W., Steiner, A.A., Romanovsky, A.A., 2008. Fever response to intravenous prostaglandin E2 is mediated by the brain but does not require afferent vagal signaling. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R1294–R1303.
- Owens, M.J., Knight, D.L., Nemeroff, C.B., 2001. Second-generation SSRIs: human monoamine transporter binding profile of escitalopram and R-fluoxetine. *Biol. Psychiatr.* 50, 345–350.
- Palkovits, M., Brownstein, M.J., 1988. *Maps and Guide to Microdissection of the Rat Brain.* Elsevier Science Publishing Co., New York.
- Pandya, M., Altinay, M., Malone Jr., D.A., Anand, A., 2012. Where in the brain is depression? *Curr. Psychiatr. Rep.* 14, 634–642.
- Pavlov, V.A., Tracey, K.J., 2015. Neural circuitry and immunity. *Immunol. Res.* 63, 38–57.
- Pavlov, V.A., Tracey, K.J., 2017. Neural regulation of immunity: molecular mechanisms and clinical translation. *Nat. Neurosci.* 20, 156–166.
- Pavlov, V.A., Wang, H., Czura, C.J., Friedman, S.G., Tracey, K.J., 2003. The cholinergic anti-inflammatory pathway: a missing link in neuroimmunomodulation. *Mol. Med.* 9, 125–134.
- Perry, V.H., 2004. The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative disease. *Brain Behav. Immun.* 18, 407–413.
- Reardon, C., 2016. Neuro-immune interactions in the cholinergic anti-inflammatory reflex. *Immunol. Lett.* 178, 92–96.
- Romanovsky, A.A., Simons, C.T., Szekely, M., Kulchitsky, V.A., 1997. The vagus nerve in the thermoregulatory response to systemic inflammation. *Am. J. Physiol.* 273, R407–R413.
- Rosas-Ballina, M., Olofsson, P.S., Ochani, M., Valdes-Ferrer, S.I., Levine, Y.A., Reardon, C., Tusche, M.W., Pavlov, V.A., Andersson, U., Chavan, S., Mak, T.W., Tracey, K.J., 2011. Acetylcholine-synthesizing T cells relay neural signals in a vagus nerve circuit. *Science* 334, 98–101.
- Roumestan, C., Michel, A., Bichon, F., Portet, K., Detoc, M., Henriquet, C., Jaffuel, D., Mathieu, M., 2007. Anti-inflammatory properties of desipramine and fluoxetine. *Respir. Res.* 8, 35.
- Sanchez-Lemus, E., Benicky, J., Pavel, J., Larrayoz, I.M., Zhou, J., Baliava, M., Nishioku, T., Saavedra, J.M., 2009. Angiotensin II AT1 blockade reduces the lipopolysaccharide-induced innate immune response in rat spleen. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1376–R1384.
- Scarr, E., Gibbons, A.S., Neo, J., Udawela, M., Dean, B., 2013. Cholinergic connectivity: its implications for psychiatric disorders. *Front. Cell Neurosci.* 7, 55.
- Schweighofer, H., Rummel, C., Roth, J., Rosengarten, B., 2016. Modulatory effects of vagal stimulation on neurophysiological parameters and the cellular immune response in the rat brain during systemic inflammation. *Intensive Care Med Exp* 4, 19.
- Tynan, R.J., Weidenhofer, J., Hinwood, M., Cairns, M.J., Day, T.A., Walker, F.R., 2012. A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. *Brain Behav. Immun.* 26, 469–479.
- Unceta, N., Barrondo, S., Ruiz de Azua, I., Gomez-Caballero, A., Goicolea, M.A., Salles, J., Barrio, R.J., 2007. Determination of fluoxetine, norfluoxetine and their enantiomers in rat plasma and brain samples by liquid chromatography with fluorescence detection. *J. Chromatogr. B: Analytical Technologies in the Biomedical and Life Sciences* 852, 519–528.
- Vos, T., Barber, R.M., 2013 Collaborators, 2015. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet* 386, 743–800.
- Waiskopf, N., Ofek, K., Gilboa-Geffen, A., Bekenstein, U., Bahat, A., Bennett, E.R., Podoly, E., Livnah, O., Hartmann, G., Soreq, H., 2014. AChE and RACK1 promote the anti-inflammatory properties of fluoxetine. *J. Mol. Neurosci.* 53, 306–315.
- Walker, F.R., 2013. A critical review of the mechanism of action for the selective serotonin reuptake inhibitors: do these drugs possess anti-inflammatory properties and how relevant is this in the treatment of depression? *Neuropharmacology* 67, 304–317.
- Wisdom, R., Johnson, R.S., Moore, C., 1999. c-Jun regulates cell cycle progression and apoptosis by distinct mechanisms. *EMBO J.* 18, 188–197.
- Wong, D.T., Bymaster, F.P., Engleman, E.A., 1995. Prozac (fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. *Life Sci.* 57, 411–441.
- Yirmiya, R., Pollak, Y., Barak, O., Avitsur, R., Ovadia, H., Bette, M., Weihe, E., Weidenfeld, J., 2001. Effects of antidepressant drugs on the behavioral and physiological responses to lipopolysaccharide (LPS) in rodents. *Neuropsychopharmacology* 24, 531–544.
- Zielinski, M.R., Dunbrasky, D.L., Taishi, P., Souza, G., Krueger, J.M., 2013. Vagotomy attenuates brain cytokines and sleep induced by peripherally administered tumor necrosis factor-alpha and lipopolysaccharide in mice. *Sleep* 36, 1227–1238.