Antipsychotic Drugs Attenuate Interleukin-6 Secretion by Microglia Via Dopamine Inhibition

CURRENT STATUS: UNDER REVIEW

BMC Neurology

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DOI: 10.21203/rs.2.18640/v1

SUBJECT AREAS   Neurology   Clinical Pharmacology

KEYWORDS
microglia, antipsychotics, inflammation, interleukin 6, dopamine, clozapine, haloperidol
Abstract

Background: Antipsychotic drugs are commonly used for various neuropsychiatric disorders, such as psychotic disorders, mood disorders, and neuropsychiatric symptoms in dementia. Their mechanism of action is thought to be by modulation of neurotransmitter activity in the brain, mainly dopamine. It has been suggested that antipsychotic drugs may also exert anti-inflammatory properties. This study aimed to examine whether the modulating effect of antipsychotic drugs on neurotransmitters attenuates the inflammatory response of microglia cells.

Methods: Levels of interleukin 6 (IL-6) were measured following activation of microglia cultures with lipopolysaccharides and treatment with antipsychotic drugs (risperidone, haloperidol, and clozapine), neurotransmitters (dopamine, serotonin, and acetylcholine), or a combination of dopamine and either haloperidol or clozapine.

Results: Haloperidol and clozapine decreased IL-6 secretion by microglia cells when treated at a concentration of 10-5M. Interestingly, dopamine at a concentration of 1 μM increased IL-6 secretion by the microglia cells, while a concentration of 100 μM decreased it. The combination of dopamine (from 0.001 μM to 100 μM) with either haloperidol (10-5M or 10-8M) or clozapine (10-5M or 10-7M) attenuated IL-6 secretion in a bell-shaped curve with a peak at 1 μM. High concentrations of both haloperidol and clozapine decreased IL-6 secretion, while low concentrations modestly increased IL-6 levels.

Conclusions: Our findings support anti-inflammatory properties of antipsychotic drugs, and suggest that their action is mediated via the inhibition of dopaminergic activity in microglia cells. The bell-shaped curve of IL-6 secretion by microglia might
suggest the presence of an “optimal zone” of operation for these cells that is mediated by dopamine.

BACKGROUND

Antipsychotic drugs are commonly used in the treatment of psychotic disorders, such as schizophrenia, as well as in the management of mood disorders [1, 2], behavioral disturbances in children and adolescents [3], and neuropsychiatric symptoms in dementia patients (NPSD) [4]. The first generation of antipsychotic drugs (also referred to as conventional or “typical” antipsychotics) has mainly antagonistic properties to the type two dopamine (D2) receptor in the brain, thus lowering dopaminergic activity that entails both therapeutic as well as adverse effects [5]. Newer (or “atypical”) antipsychotic drugs were developed in order to decrease the rates of adverse effects, and they are mainly antagonistic to the D2 receptor as well as to the 5HT\textsubscript{2A} serotonin receptors, although they also affect the muscarinic, adrenergic, and histaminergic receptors [5].

Neuroinflammation is a relatively new hypothesis for the pathophysiology of neuropsychiatric disorders. It suggests that aberrant immune activation is an additional key component for the development of these disorders (e.g., schizophrenia [6], mood disorders [7], autism [8], and dementia [9]). Various components of the immune system have been studied in humans, animal models, and in-vitro. Microglia and pro-inflammatory cytokines were suggested as key players in several studies [10–12]. Interleukin 6 (IL-6) has been often studied in this context, and it was suggested to play a central role in neuroinflammation [13–15]. Preliminary studies with an IL-6 antagonist (tocilizumab) as a treatment for schizophrenia have also been carried out [16, 17].
Of great interest are studies that have shown that antipsychotic drugs have anti-inflammatory properties [18–20], thus raising the possibility that their therapeutic effects are not based solely on their anti-dopaminergic-serotonergic properties [21].

It is not yet clear, however, how antipsychotic drugs influence the underlying biological mechanism by which neuroinflammation appears to be driven [22]. The aim of this study was to examine whether there is a common pathway for the neurotransmitter blocking capacity of antipsychotic drugs and their immune-modulating abilities. We hypothesized that the anti-dopaminergic properties of the drugs will modulate the inflammatory response of microglia cells, as measured by their secretion of IL-6.

METHODS

Microglia Cell Cultures

The N9 cell line (RRID CVCL_0452) was received from the laboratory Prof. Dr. Michael T. Heneka from the Clinical Research Center for Neurodegenerative Diseases (KBFZ) at the University Hospital, Bonn, Germany. These murine microglial cells, which display features similar to primary microglial cells [23], were maintained in a Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with L-Glu (Biological Industries, Kibbutz Beit Haemek, Israel, 01-100-1A) with an additional 10% fetal bovine serum HI (Biological Industries, 04-127-1A), and 1% pen-strep-nystatin (Biological Industries, 03-032-1B) in 5% CO2 atmosphere at 37 °C.

Antipsychotic Medication and Neurotransmitters

Haloperidol, clozapine, and serotonin were kindly donated by Professor Moshe Rehavi of the Sackler Faculty of Medicine at the Tel Aviv University, Israel.
Risperidone was kindly donated by Janssen-Cilag Pharmaceuticals. Dopamine and acetylcholine were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

**Cytokine Assessment**

Quantitative ELISA analyses for IL-6 were performed using paired antibodies and recombinant cytokines according to the manufacturer’s recommendations (R&D Systems, Abingdon, United Kingdom, #DY406)

**MTT Assay**

To assess cell viability, we used the colorimetric MTT (3-[4,5-dimethylthiazol – 2-yl]-2,5-diphenyl tetrazolium bromide) assay based on conversion of MTT, as described in detail elsewhere [24]. Cell viability was measured by the absorbance at 570/650 nm.

**Cell Treatments**

N9 cells were plated on 48-well tissue culture plates at a concentration of $1 \times 10^5$ cells/ml in all experiments. After 24 hours, neurotransmitters and/or antipsychotics were added to the medium with $0.1\mu$g/ml lipopolysaccharide (LPS; Sigma-Aldrich). After an additional 24 hours, the supernatants were removed and used for analysis of the IL-6 levels. Three experiments were conducted. In the first one, the N9 cells were treated with either haloperidol, risperidone, or clozapine at decreasing concentrations ($10^{-5}$, $10^{-6}$, $10^{-8}$, $10^{-10}$, and $10^{-12}$M). In the second one, the cells were treated with dopamine, serotonin, or acetylcholine in doses of 1, 10, and 100 $\mu$M. In the third experiment, the cells were treated by a combination of dopamine at increasing concentrations (0.001, 0.1, 1, 10, and 100 $\mu$M), as well as clozapine ($10^{-5}$ or $10^{-7}$M) or haloperidol ($10^{-5}$M or $10^{-8}$M). A control consisting of LPS alone was used in all three experiments.
Data Analyses

Statistical analyses were conducted using IBM SPSS Statistics for Windows v.20 (IBM Corp, Armonk, New York). Groups means were compared using one-way ANOVA followed by Scheffe post-hoc test.

RESULTS

Impact of Antipsychotic Drugs on IL-6 Secretion by Microglia

Based on the hypothesis that IL-6 plays a central role in neuroinflammation, we assessed the effect of various antipsychotics drugs on microglia cells in the neuroinflammatory microenvironment by activating the cells with LPS together with selected drugs. Although an overall significant difference was found for risperidone ($F(5,11) = 4.07, P = 0.025$), post-hoc analyses did not reveal any significant differences in IL-6 levels for specific concentrations of the drug (Table 1, Fig. 1A). In contrast, both haloperidol and clozapine significantly lowered IL-6 levels ($F(5,11) = 14.30, P < 0.001$ and $F(5,12) = 13.99, P < 0.001$ respectively). Both caused a significant decrease of IL-6 secretion by the N9 cells at a dose of $10^{-5}$M (equivalent to $375.9 \times 10^{-5}$ mg/ml for haloperidol and $326.8 \times 10^{-5}$ mg/ml for clozapine), an effect that disappeared in lower doses ($57.27 \pm 9.02$ vs. $103.15 \pm 20.77, P = 0.012$ for haloperidol and $67.27 \pm 0.67$ vs. $103.15 \pm 20.77, P = 0.020$ for clozapine. Table 1, Fig. 1B and 1C).
Table 1
- Interleukin 6 Secretion Mediated by Drugs

| Drug                  | 10^{-5} (pg/ml ± SD) | 10^{-6} (pg/ml ± SD) | 10^{-8} (pg/ml ± SD) | 10^{-10} (pg/ml ± SD) | 10^{-12} (pg/ml ± SD) |
|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Risperidone           | 106.11 ± 6.67         | 124.73 ± 3.05         | 138.1 ± 7.92          | 127.70 ± 13.04        | 128.80 ± 2.36         |
| Haloperidol           | 57.27 ± 9.02          | 115.60 ± 7.21         | 114.63 ± 1.58         | 130.00 ± 3.74         | 115.57 ± 2.90         |
| Clozapine             | 67.27 ± 0.67          | 109.53 ± 8.92         | 117.83 ± 2.11         | 124.93 ± 5.81         | 120.43 ± 6.49         |
| LPS                   |                       |                       |                       | 103.15 ± 20.77        |                       |

\( ^\dagger \) Compared to a concentration of 10^{-5}M of the same drug.

The Impact of Neurotransmitters on IL-6 Secretion by Microglia

Next, we assessed the effect on microglia-secreted IL-6 of neurotransmitters that are attenuated by antipsychotics drugs. Dopamine had an overall significant effect on IL-6 secretion that was also evident in the post-hoc analysis (\( F(3,9) = 41.17, P < 0.001 \), Fig. 2). IL-6 levels were significantly higher compared to LPS control at the lowest dopamine concentration of 1 \( \mu \)M (427.90 ± 42.24 vs. 295.03 ± 23.05, \( P = 0.001 \)). No difference was found for dopamine at a concentration of 10 \( \mu \)M.

Interestingly, we found decreased IL-6 levels at a dopamine concentration of 100 \( \mu \)M (218.13 ± 5.47 vs. 295.03 ± 23.05, \( P = 0.023 \)). Notably, the rise in IL-6 levels induced by dopamine 1 \( \mu \)M was also significantly higher than dopamine 10 \( \mu \)M and 100 \( \mu \)M (\( P < 0.001 \) for both comparisons). ANOVA was significant for the overall serotonin results (\( F(3,11) = 3.76, P = 0.044 \)), but the post-hoc analysis did not reveal any significant differences. Finally, the ANOVA finding for acetylcholine was not significant (\( F(3,8) = 0.73, P = 0.564 \)).

Antipsychotics and Neurotransmitters’ Impact on IL-6 Secretion

We then sought to determine whether different concentrations of dopamine influence the effect of clozapine on microglial IL-6 secretion. The results showed that IL-6 secretion was significantly lower compared to the LPS control for most
dopamine concentrations (0.001, 0.1 and 10 \( \mu \)M. \( F(6,12) = 11.47, P < 0.001 \)) at a high dose of clozapine (\( 10^{-5} \)M or \( 326.8 \times 10^{-5} \) mg/ml), while the 1 \( \mu \)M and 100 \( \mu \)M doses yielded no significant differences (Table 2 and Fig. 3A).

| Dopamine Concentration | 0\( \mu \)M | 0.001\( \mu \)M | 0.1\( \mu \)M | 1\( \mu \)M | 10\( \mu \)M | 100\( \mu \)M |
|------------------------|-------------|----------------|-------------|-----------|---------|---------|
| Clozapine 10\(^{-5}\)M (pg/ml ± SD) | 30.96 ± 9.88 | 42.59 ± 11.70 | 52.14 ± 29.98 | 72.83 ± 13.32 | 39.92 ± 13.48 | 78.81 ± 13.28 |
| \( P \) compared to LPS only | 0.001 | 0.005 | 0.032 | 0.181 | 0.008 | 0.355 |
| Clozapine 10\(^{-7}\)M (pg/ml ± SD) | 113.63 ± 4.95 | 134.63 ± 8.34 | 132.00 ± 18.79 | 166.13 ± 24.56 | 149.87 ± 24.57 | 172.80 ± 12.72 |
| \( P \) compared to LPS only | 1.000 | 0.736 | 0.819 | 0.047 | 0.250 | 0.047 |
| Haloperidol 10\(^{-5}\)M (pg/ml ± SD) | 0.21 ± 0.36 | 7.15 ± 7.24 | 16.91 ± 2.59 | 69.23 ± 13.59 | 28.57 ± 8.72 | 20.01 ± 6.38 |
| \( P \) compared to LPS only | < 0.001 | < 0.001 | < 0.001 | 0.001 | < 0.001 | < 0.001 |
| \( P \) compared to Dopamine 1\( \mu \)M | < 0.001 | < 0.001 | < 0.001 | NA | 0.001 | < 0.001 |
| Haloperidol 10\(^{-8}\)M (pg/ml ± SD) | 94.93 ± 14.09 | 126.23 ± 9.10 | 151.93 ± 4.98 | 195.13 ± 13.17 | 145.23 ± 12.04 | 118.03 ± 6.18 |
| \( P \) compared to LPS only | 0.773 | 0.650 | 0.009 | < 0.001 | 0.033 | 0.977 |
| \( P \) compared to Dopamine 1\( \mu \)M | < 0.001 | < 0.001 | 0.008 | NA | 0.002 | < 0.001 |
| LPS only (pg/ml ± SD) | 109.50 ± 3.96 |

When we tested the impact of low-dose clozapine (\( 10^{-7} \)M equivalent to \( 326.8 \times 10^{-7} \) mg/ml) on IL-6 secretion, it emerged that IL-6 levels were significantly higher compared to the LPS control (\( F(6,13) = 5.95, P = 0.004 \)) at dopamine concentrations of 1 \( \mu \)M and 100 \( \mu \)M (166.13 ± 24.56 vs 109.50 ± 3.96, \( P = 0.047 \) and 172.80 ± 12.72 vs. 109.50 ± 3.96, \( P = 0.047 \), respectively). Notably, IL-6 levels for the N9 cells treated with low-dose clozapine were higher than the LPS control for all dopamine concentrations, although not always to a level of significance (Table 2 and Fig. 3A).
Testing of high-dose haloperidol ($10^{-5}$M or $375.9 \times 10^{-5}$ mg/ml) revealed that all IL-6 measurements were significantly lower compared to the LPS control ($F(6,14) = 86.60, P < 0.001$) and that they spread in a bell-shaped curve (Table 2, Fig. 3B). Interestingly, IL-6 levels for the dopamine $1 \times 10^{-3}$M-treated cells were significantly higher compared to the cells treated at other dopamine concentrations (Table 2 and Fig. 3B).

Finally, we tested low-dose haloperidol ($10^{-8}$M equivalent to $375.9 \times 10^{-8}$ mg/ml) and found that three dopamine concentrations showed significant differences compared to the LPS control (0.1, 1 and $10 \times 10^{-4}$M), and that IL-6 levels were higher than the control ($F(6,14) = 33.45, P < 0.001$. Table 2 and Fig. 3B). Again, the cells treated with dopamine $1 \times 10^{-3}$M exhibited significantly higher levels of IL-6 compared to the other treated cells (Table 2, Fig. 3B).

**The Effects of Antipsychotics and Neurotransmitters on Microglial Viability**

We assessed the effect of antipsychotics and neurotransmitters on the viability of microglia cells to rule out confounding effects. The results for the MTT assay are detailed in the supplementary table. The ANOVA analysis of the antipsychotics alone (in all concentrations used) was significant only for clozapine ($F(5,12) = 3.62, P = 0.032$), but the differences were not significant in the post-hoc analysis. For the combination of clozapine and dopamine, clozapine at a high dose ($10^{-5}$M) produced an increase in microglial viability as observed in the MTT signal ($F(6,14) = 4.550, P = 0.009$), specifically, between dopamine $0.001 \times 10^{-3}$M and dopamine $0.1 \times 10^{-3}$M ($0.61 \pm 0.09$ vs. $0.77 \pm 0.06, P = 0.02$). There were no significant differences compared to LPS alone control. ANOVA was significant overall for low-dose clozapine ($10^{-8}$M)
(F(6,14) = 4.511, P = 0.01), but no comparable significance was seen on the post-hoc analyses. The ANOVAs for haloperidol were not significant for both tested concentrations (F(6,14) = 1.713, P = 0.190 and F(6,14) = 1.220, P = 0.353).

**DISCUSSION**

The aim of our study was to examine the hypothesis of there being a possible common pathway between the neurotransmitter-modulating and the anti-inflammatory effects of antipsychotic drugs. Our findings are in line with previous studies that showed an anti-inflammatory effect of antipsychotic drugs [18–20]. The biologic mechanism of this effect is not altogether clear, and our results suggest that dopamine plays a central role in this process. The importance of dopamine as a mediator of inflammation has been demonstrated in several studies [25, 26]. Investigations that aimed to identify the presence of dopamine receptors on various subpopulations of leukocytes found that the D2 receptor family is more prevalent than the D1 family and that monocytes have a higher density of D2 and D3 receptors compared to the other types [27]. Specifically, microglia cells were shown to have D1 and D2 receptors, and that the activation of these receptors regulates microglia cell activity[25]. In a study employing human monocyte-derived macrophages that were stimulated with different concentrations of dopamine (2μM and 20μM), an increase of IL-6, CCL2, CXCL8, and IL-10 secretion was found while the secretion of TNFα declined. Lower concentrations of dopamine in that study (20 nM and 200 nM) affected only TNFα, IL-6, and CCL2 secretion and they upregulated IL-10, albeit insignificantly. Therefore, the authors suggested that macrophages develop differential responses, depending upon their microenvironment, and that they are modulated by dopamine [28]. Similarly, our
findings suggest that various concentrations of dopamine trigger different responses from microglia cells, which seem to be most active when dopamine concentration is in its physiologic range of ~ 1×M [29]. The fact that haloperidol (an anti-psychotic drug with high affinity to the D2 receptor) had a much more significant effect on IL-6 secretion than clozapine (which has a lower affinity to the D2 receptor) supports the notion that the modulating effect of antipsychotic drugs on microglia is mediated by the D2 receptors.

IL-6 has been extensively studied in schizophrenia [13], NPSD [9], and other neuropsychiatric disorders [7, 8]. It is considered to be a pro-inflammatory cytokine, but current studies indicated that IL-6 has more complex properties than had been thought in the past (e.g., that it can be anti-inflammatory as well as pro-inflammatory), and that it has a variety of physiologic roles in the central nervous system [13]. Due to its complex and varied role, and the fact that IL-6 is a major cytokine produced by microglia cells, it may be more prudent to view it as a marker of microglial cell activation in the context of this study. Our finding of the bell-shaped curve of the resultant IL-6 levels support an “optimal” setpoint for microglia cell activity, and that a deviation from it might underlie pathologic processes.

Similarly, Yirmiya et al suggested that depression may be considered microgliopathies in which either microglial cell activation or decline underlies the depressive disorder [30]. Our findings propose that this hypothesis may also be applicable to other neuropsychiatric disorder, such as schizophrenia and dementia. We had previously reported that neurotoxic microglia showed increased sensitivity to dopamine and had increased IL-6 secreted levels in Parkinson’s disease [23, 31]. The possibility also arises that the therapeutic role of antipsychotic drugs is due to their ability to attenuate the neuroinflammatory properties of microglia. An
interesting finding in this context is that clozapine and haloperidol at low concentrations seemed to increase rather than decrease IL-6 secretion.

CONCLUSIONS

The findings of this study demonstrated that the interaction between dopamine and antipsychotic drugs mediates IL-6 secretion by microglia cells in a bell-shaped manner. Both dopamine and anti-psychotics drugs have pro- and anti-inflammatory properties, depending upon their concentrations and dosages, respectively. This points to the possibility that microglia cells have a “optimal zone” of functioning (at least from an inflammatory perspective), that can be influenced in either way by dopamine availability in the cell microenvironment. Our results warrant further investigation of the optimal dose of antipsychotic drugs needed in order to identify and activate the neuroimmunological properties of microglia cells. They also suggest the existence of a common pathway between various postulated pathophysiologic mechanisms of neuropsychiatric disorders (neurotransmitter imbalance vs. neuroinflammation). Further studies are needed to elucidate this mechanism and its implication in clinical practice.

Abbreviations

IL-6
Interleukin 6
NPSD
neuropsychiatric symptoms in dementia
RPMI
Roswell Park Memorial Institute
MTT
3-[4,5-dimethylthiazol – 2-yl]-2,5-diphenyl tetrazolium bromide
LPS
lipopolysaccharide

TNFα
Tumor Necrosis Factor alpha

Declarations

Acknowledgments
The authors would like to thank Mrs. Ronit Galron and Erik Jedenius PhD for their guidance and help.

Authors’ Contributions
This study was an academically-initiated study. Janssen-Cilag was not represented in the trial steering committee with regard to study design and provided only the risperidone preparations. EMD carried out the experiments and data analysis. YN and AR assisted with cytokines measurement and data analysis. DF and YFL assisted in planning the experiments and in critical reading and discussion. All authors have read and approved the manuscript.

Funding
This study was supported by grant from SAIA Fund for HIV and Parkinson’s Diseases Research, Tel Aviv University (DF). The funders had no role in design and conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

Availability of data and materials
The datasets used during the current study are available from the corresponding author on reasonable request.

Ethics approval and Consent to participate
Not applicable
Consent for publication

Not applicable

Competing interests

All the authors report having no Competing interests.

References

1. Gao K, Gajwani P, Elhaj O, Calabrese JR: **Typical and atypical antipsychotics in bipolar depression.** *The Journal of clinical psychiatry* 2005, **66**:1376-1385.

2. Medici CR, Kai LM, Kristensen SB, Kirkedal C, Munk-Jørgensen P, Straszek S: **Typical Versus Atypical Antipsychotics for Acute Mania.** *American journal of therapeutics* 2018.

3. Pillay J, Boylan K, Carrey N, Newton A, Vandermeer B, Nuspl M, MacGregor T, Jafri SHA, Featherstone R, Hartling L: **First-and second-generation antipsychotics in children and young adults: systematic review update.** 2017.

4. Carson S, McDonagh MS, Peterson K: **A Systematic Review of the Efficacy and Safety of Atypical Antipsychotics in Patients with Psychological and Behavioral Symptoms of Dementia.** *Journal of the American Geriatrics Society* 2006, **54**:354-361.

5. Stahl SM, Stahl SM: *Stahl's essential psychopharmacology: neuroscientific basis and practical applications.* Cambridge university press; 2013.

6. Feigenson KA, Kusnecov AW, Silverstein SM: **Inflammation and the two-hit hypothesis of schizophrenia.** *Neuroscience & Biobehavioral Reviews* 2014, **38**:72-93.

7. Goldsmith DR, Rapaport MH, Miller BJ: **A meta-analysis of blood cytokine...**
network alterations in psychiatric patients: comparisons between schizophrenia, bipolar disorder and depression. *Mol Psychiatry* 2016, **21**:1696-1709.

8. Xu N, Li X, Zhong Y: Inflammatory cytokines: potential biomarkers of immunologic dysfunction in autism spectrum disorders. *Mediators Inflamm* 2015, **2015**:531518.

9. Holmgren S, Hjorth E, Schultzberg M, Larksater M, Frenkel D, Tysen-Backstrom AC, Aarsland D, Freund-Levi Y: Neuropsychiatric symptoms in dementia-a role for neuroinflammation? *Brain Res Bull* 2014, **108**:88-93.

10. Prata J, Santos SG, Almeida MI, Coelho R, Barbosa MA: Bridging Autism Spectrum Disorders and Schizophrenia through inflammation and biomarkers - pre-clinical and clinical investigations. *J Neuroinflammation* 2017, **14**:179.

11. Rosenblat JD, Cha DS, Mansur RB, McIntyre RS: Inflamed moods: a review of the interactions between inflammation and mood disorders. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2014, **53**:23-34.

12. Shabab T, Khanabdali R, Moghadamtousi SZ, Kadir HA, Mohan G: Neuroinflammation pathways: a general review. *International Journal of Neuroscience* 2017, **127**:624-633.

13. Borovcanin MM, Jovanovic I, Radosavljevic G, Pantic J, Minic Janicijevic S, Arsenijevic N, Lukic ML: interleukin-6 in Schizophrenia—is There a Therapeutic Relevance? *Frontiers in psychiatry* 2017, **8**:221.

14. Wiener CD, Moreira FP, Portela LV, Strogulski NR, Lara DR, da Silva RA, de Mattos Souza LD, Jansen K, Oses JP: Interleukin-6 and Interleukin-10 in mood disorders: a population-based study. *Psychiatry Research* 2019.
15. Ng A, Tam WW, Zhang MW, Ho CS, Husain SF, McIntyre RS, Ho RC: **IL-1β, IL-6, TNF-α and CRP in elderly patients with depression or Alzheimer's disease: Systematic review and meta-analysis.** *Scientific reports* 2018, 8:12050.

16. Miller BJ, Dias JK, Lemos HP, Buckley PF: **An open-label, pilot trial of adjunctive tocilizumab in schizophrenia.** *The Journal of clinical psychiatry* 2016, 77:275.

17. Girgis RR, Ciarleglio A, Choo T, Haynes G, Bathon JM, Cremers S, Kantrowitz JT, Lieberman JA, Brown AS: **A randomized, double-blind, placebo-controlled clinical trial of tocilizumab, an interleukin-6 receptor antibody, for residual symptoms in schizophrenia.** *Neuropsychopharmacology* 2018, 43:1317.

18. Sugino H, Futamura T, Mitsumoto Y, Maeda K, Marunaka Y: **Atypical antipsychotics suppress production of proinflammatory cytokines and up-regulate interleukin-10 in lipopolysaccharide-treated mice.** *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2009, 33:303-307.

19. Shin H, Kim J, Song J-H: **Clozapine and olanzapine inhibit proton currents in BV2 microglial cells.** *European journal of pharmacology* 2015, 755:74-79.

20. Haring L, Koido K, Vasar V, Leping V, Zilmer K, Zilmer M, Vasar E: **Antipsychotic treatment reduces psychotic symptoms and markers of low-grade inflammation in first episode psychosis patients, but increases their body mass index.** *Schizophrenia Research* 2015, 169:22-29.

21. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B: **Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects.** *Biological psychiatry* 2011, 70:663-671.
22. Baumeister D, Ciufolini S, Mondelli V: Effects of psychotropic drugs on inflammation: consequence or mediator of therapeutic effects in psychiatric treatment? Psychopharmacology 2016, 233:1575-1589.

23. Trudler D, Weinreb O, Mandel SA, Youdim MB, Frenkel D: DJ-1 deficiency triggers microglia sensitivity to dopamine toward a pro-inflammatory phenotype that is attenuated by rasagiline. J Neurochem 2014, 129:434-447.

24. Kupershmidt L, Weinreb O, Amit T, Mandel S, Carri MT, Youdim MB: Neuroprotective and neuritogenic activities of novel multimodal iron-chelating drugs in motor-neuron-like NSC-34 cells and transgenic mouse model of amyotrophic lateral sclerosis. Faseb j 2009, 23:3766-3779.

25. Sarkar C, Basu B, Chakroborty D, Dasgupta PS, Basu S: The immunoregulatory role of dopamine: an update. Brain, behavior, and immunity 2010, 24:525-528.

26. Arreola R, Alvarez-Herrera S, Pérez-Sánchez G, Becerril-Villanueva E, Cruz-Fuentes C, Flores-Gutierrez EO, Garcés-Alvarez ME, de la Cruz-Aguilera DL, Medina-Rivero E, Hurtado-Alvarado G: Immunomodulatory effects mediated by dopamine. Journal of immunology research 2016, 2016.

27. McKenna F, McLaughlin P, Lewis B, Sibbring G, Cummerson J, Bowen-Jones D, Moots R: Dopamine receptor expression on human T-and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. Journal of neuroimmunology 2002, 132:34-40.

28. Gaskill PJ, Carvallo L, Eugenin EA, Berman JW: Characterization and function of the human macrophage dopaminergic system: implications for CNS
disease and drug abuse. Journal of neuroinflammation 2012, 9:203.

29. Yan Y, Jiang W, Liu L, Wang X, Ding C, Tian Z, Zhou R: Dopamine controls systemic inflammation through inhibition of NLRP3 inflammasome. Cell 2015, 160:62-73.

30. Yirmiya R, Rimmerman N, Reshef R: Depression as a microglial disease. Trends Neurosci 2015, 38:637-658.

31. Nash Y, Schmukler E, Trudler D, Pinkas-Kramarski R, Frenkel D: DJ-1 deficiency impairs autophagy and reduces alpha-synuclein phagocytosis by microglia. J Neurochem 2017, 143:584-594.

Figures
Figure 1

Interleukin 6 Secretion Mediated by Antipsychotic Drugs. The effect of antipsycho
Figure 2

Interleukin 6 Secretion Mediated by Dopamine. Dopamine mediates IL-6 secretion
3A. Clozapine and Dopamine

3B. Haloperidol and Dopamine

Figure 3

Interleukin 6 Mediated by Antipsychotic Drugs and Dopamine. The combined effec
Supplementary Files

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Microglia, IL-6 and Antipsychotics - Supplementary table.docx