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Immunomodulatory effects of fluoxetine. A new potential pharmacological action for a classic antidepressant drug?

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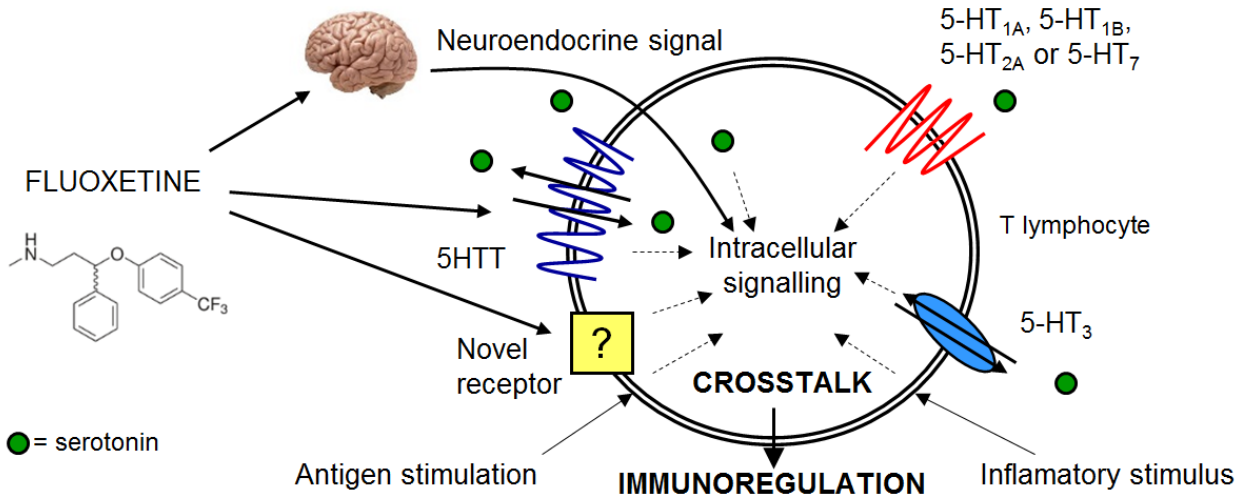
Graphical Abstract



Abstract

Selective serotonin reuptake inhibitors are frequently used antidepressants. In particular, fluoxetine is usually chosen for the treatment of the symptoms of depression, obsessive–compulsive, panic attack and *bulimia nervosa*. Antidepressant therapy has been associated with immune dysfunction. However, there is contradictory evidence about the effect of fluoxetine on the immune system. Experimental findings indicate that lymphocytes express the serotonin transporter. Moreover it has been shown that fluoxetine is able to modulate the immune function through a serotonin-dependent pathway and through a novel independent mechanism. In addition, several studies have shown that fluoxetine can alter tumor cell viability. Thus, it was recently demonstrated *in vivo* that chronic fluoxetine treatment inhibits tumor growth by increasing antitumor T-cell activity.

Here we briefly review some of the literature referring to how fluoxetine is able to modify, for better or worse, the functionality of the immune system. These results of our analysis point to the relevance of the novel pharmacological action of this drug as an immunomodulator helping to treat several pathologies in which immune deficiency and/or deregulation is present.

Keywords: Antidepressant; Fluoxetine; Serotonin; Immune System; Immunomodulation.

1. Introduction

Serotonin [5-hydroxytryptamine (5-HT)] and catecholamines are primitive biogenic amines essential to the regulation of central processes, like food and sexual appetite, mood, sleep, body temperature and breathing. Their bioavailability is exquisitely regulated through diverse mechanisms, such as vesicular sequestration mediated by selective transporters. In addition, these neurotransmitters and the corresponding transporter proteins are found in peripheral cells, mainly those of the immune–inflammatory axis. Also it has been described that they are able to modulate immune cell activity in an autocrine manner. These transporters have been used widely as pharmacological targets for the treatment of mood and anxiety-related disorders. Specifically, selective 5-HT reuptake inhibitors (SSRIs) have been demonstrated to be very efficient, safe and tolerable. In particular, fluoxetine is usually chosen for the treatment of the symptoms of depression, obsessive-compulsive disorder, panic attacks and *bulimia nervosa*. However, antidepressant therapy has been associated with alterations of the immune function.

Here we concisely review the literature pertaining to how fluoxetine is able to modify -for good or ill- the functionality of the immune system. Firstly, we describe the evidence on the presence and the role of 5-HT and of the transporters within the immune system. Secondly, we mention the findings about the alteration of 5-HT homeostasis in affective disorders and other pathologies such as cancer. Thirdly, the effect of fluoxetine on the immune response in both healthy subjects and in sickness conditions will be depicted.

2. Role of serotonin as a regulator of immune response

During the last twenty years experimental findings have indicated reciprocal communication between the immune and nervous systems, where 5-HT has been proven to have important modulatory effects [1–5]. Enterochromaffin cells of the gastrointestinal tract are the main producers of 5-HT. These cells release 5-HT, which is taken up by platelets that then act as "mobile storage units". The rapid release of platelet 5-HT is induced by inflammatory stimuli, such as C5a, platelet activating factor and IgE-containing immune complexes. Thus, at sites of inflammation, lymphocytes can be exposed to large amounts of platelet-derived 5-HT, reaching near 1 mM in the local milieu [3]. In addition, immune cells contain and synthesize 5-HT [6-9]. The content may be different between diverse subtypes of cells, in lymphocytes and monocytes, and even in subpopulations of T lymphocytes. These differences could indicate particular roles of 5-HT as an immunomodulatory factor acting as an autocrine transmitter. For example, dendritic cells are able to uptake and store 5-HT, and then release it by exocytosis, inducing proliferation and differentiation of naïve T cells [10]. Therefore autocrine and paracrine interplays might take place among lymphocytes.

The neuroimmune axis constitutes one more source of 5-HT for lymphocytes. It is known that lymphoid organs are extensively innervated by the autonomic nervous system. It has been proposed that nerve fibers in close contact with lymphoid tissues might take up 5-HT and then release it on following stimulation of the nerves [3]. Several studies have shown that 5-HT is able to regulate numerous immune responses by increasing of mitogen-stimulated lymphocyte proliferation, promoting natural killer

cell activation and macrophage/dendritic accessory function as well as contributing to the initiation of the delayed-type hypersensitivity response [3, 11, 12].

Within the immune system, 5-HT can be transformed through catabolic pathways in melatonin, a cyclooxygenase inhibitor called formyl-N-acetyl-5-methoxykynurenamine, formyl-5-hydroxykynurenamine, 5-hydroxyindoleacetic acid and N-methylserotonin. However, it is not thoroughly understood the extent by which these metabolites generated during catabolism may affect the immune system [for a review see 13].

Membrane receptors for 5-HT are, at present, grouped into 7 families, known as 5-HT1 to 5-HT7 [13]. In mammalian, 14 different 5-HT receptor subtypes have been found using molecular biological techniques. The 5-HT1 subgroup includes at least five subtypes: 5-HT1A, 5-HT1B, 5-HT1D, 5-HT1E, and 5-HT1F. The 5-HT2 consists of three subtypes: 5- HT2A, 5-HT2B, and 5-HT2C. For 5-HT5 two related receptor were identified: 5-HT5A and 5-HT5B. Excluding the 5-HT3 receptor that is a cation channel, all subtypes are G-protein-coupled receptors. 5-HT1, 5-HT4, 5-HT6, 5-HT7 subgroups are linked to adenylate cyclase and 5-HT2 to phospholipase C. The second messenger system coupled to the 5-HT5 receptors has not been identified yet; however, evidence exists indicating the functional coupling of the rat 5-HT5A receptor subtype to adenylate cyclase [14, 15].

The presence of 5-HT receptors in the immune system, such as 5-HT1, 5-HT2, 5-HT3 and 5-HT7, has mostly been studied by pharmacological methods [3]. Stefulj et al. [16] investigated the mRNA expression of 5-HT receptors in rat lymphoid tissue cells and in mitogen-stimulated spleen cells by RT-PCR methods. The authors found that 5-HT1B,

5-HT1F, 5-HT2A, 5-HT2B, 5-HT6, and 5-HT7 receptor mRNAs are expressed in *ex vivo* isolated thymus, spleen, and peripheral blood lymphocytes. Mitogen-stimulated cells also expressed 5-HT3 receptor subtype mRNA. However, mRNA corresponding to 5-HT1A, 5- HT1D, 5-HT2C, 5-HT4, 5-HT5A, and 5-HT5B subtypes were undetected in the examined lymphoid populations [16]. Other authors, however found 5-HT1A receptor to be expressed in lymphocytes, where it is involved in the promotion of T-cell proliferation, a property conserved between mammals and fish [8, 9]. In addition 5-HT1A receptor is expressed in B lymphocytes and, as with T cells, both mRNA and protein for this receptor are up-regulated by NF-kB–dependent mechanisms [17]. Moreover, 5-HT1A receptor seems to participate in the 5-HT–augmentation of rodent splenic B cells mitogenic responses induced by lipopolysaccharide and dextran sulfate [18].

Antagonism of 5-HT1B/D receptors inhibits proliferation of human and murine T cells [19] and agonism of 5-HT1A receptors increases proliferation of rat [20], mouse [21] and human [22] lymphocytes, indicating a crucial role of 5-HT as an immunomodulator [23]. Also, the expression of 5-HTR2A in PBMC has been investigated and linked to the regulation of tumor necrosis factor (TNF)-α and interleukin (IL)-1β secretion [24]. Furthermore, it was demonstrated that 5-HT3, 5-HT4 and 5-HT7 subtypes regulate the release of IL-1β, IL-6, IL-12, IL-8 and TNF-α in monocytes and dendritic cells [12, 25, 26]. It was reported that 5-HT3A is expressed in naive and activated B-lymphocytes [27], T lymphocytes, and human monocytes [28]. Inhibition of the 5-HT3 receptors with antagonists, such as ondasetron and tropisentrol, disrupts TNF-α and IL-1β production, suggesting that these receptors may activate the p38/MAPK pathway [29, 30]. Besides, 5-HT regulates migration, cytokine and chemokine release and T-cell priming capacity

of dendritic cells [12]. Table 1 shows the different receptor subtypes and the effect of 5-HT in different cell types.

High affinity uptake of 5-HT and its inhibition by SSRIs in peripheral-blood lymphocytes has been described [31]. In addition, the expression of the 5-HT transporter (SERT) has been demonstrated by specific saturable binding of the labeled SSRI paroxetine on lymphocytes [32]. It is important to note that the capacity to accumulate 5-HT seems to be evolutionarily conserved among lymphocytes suggesting that lymphoid SERT has a relevant role, if not a crucial function, within the periphery [6, 33].

Cytokine modulation of SERT mRNA expression *in vitro* has been reported [34-37]. Furthermore, TNF-α, IL-6, IL-10, IFN-γ production requires intracellular lymphocyte 5-HT, but this production may be suppressed by an increase in extracellular 5-HT concentration [38]. These observations suggest that SERT could be able to regulate the production of cytokines.

Experimental evidence shows that SERT does not act just as a transport protein but also as a substrate-dependent signal transducer [39]. In addition, SERT-delivered 5-HT has been probed to impact signal transduction directly by a novel mechanism, the "serotonylation" of small GTPases [40, 41]. Thus, SERT appears equipped to modify cell's functional behavior in potentially diverse ways [15].

3. Alteration of serotonin homeostasis in disease

Reports indicating alterations on 5-HT homeostasis in immune system are referred especially to psychiatric disorders, such as the major depressive disorder; immune dysfunction and malignant immune cells, like Burkitt lymphoma.

The major depressive disorder is defined as a pervasive and persistent low mood with a multi-factor cause. It has long been considered that dysfunction of the central serotonergic system is involved in the pathogenesis of this disorder. Several studies have shown that 5-HT manipulations alter subjective emotional state [42]. Kambeitz and Howes [43] performed a meta-analysis of *in vivo* and *post mortem* findings and conclude that SERT availability is diminished in crucial regions of the limbic system in depressed patients. However, it was reported that there are no variation in the plasma levels of circulating 5-HT and their metabolite 5-hydroxyindoleacetic acid (5-HIAA) [6, 44]. Nevertheless, in major depression patients a reduction was found in lymphoid SERT that was increased after fluoxetine [6] or mirtazapine treatment [44]. In addition, a decrease of the SERT mRNA was described in these patients [7]. SERT protein expression in leukocytes has, however, been found in other studies to be increased in depressive patients and reverted by antidepressant administration [45]. These different results may be linked to the type of cell preparation utilized or the type of patients evaluated in each study. These findings support the significant action of SERT on different circulating cells playing an important role for the maintenance of serotonergic homeostasis such as it happens in the brain.

Changes in lymphocyte proliferation have been described in depression. Thus, it has been reported a lower mitogen-induced T cell proliferation in depressed patients [46-48]. However, the probability of a basal immune activation in these patients that in turn

promote an autoimmune reaction cannot be discarded. In fact, in depression, it was reported an increased basal lymphocyte proliferation, an over-reactivity of 5-HT1A receptors [48] and a reduction of lymphocyte cAMP [49]. Moreover, other immune variations were evidenced, such as leukocytosis with lymphopenia and increased CD4+/CD8+ ratio [50]. Besides, some studies have shown an increase in the CD3+/CD8+ ratio without modification in CD3+CD4+ lymphocytes and a higher percentage of natural killer (NK) cells in major depression [51]. Finally, Fazzino et al. [52] found a decreased number of SERT in CD8+ lymphocytes without changes in CD4+/CD8+ relation in major depression. The presence of SERT in CD4+ and CD8+ cells may be indicative of the existence of differential serotonergic system regulation of cell activity in lymphocyte subpopulations.

Immune responses to viruses, bacteria, fungi, and parasites require 5-HT. Human immunodeficiency virus (HIV) infection is a primary model for the study of the action of 5-HT during infection. Numerous studies encourage a role of 5-HT in T-cell function of HIV-seropositive individuals. 5-HT induces a decrease of intracellular cAMP levels in lymphocytes and an increase of proliferative responses mediated by IL-2 production through 5-HT1A receptors [53]. In addition, 5-HT decreases the expression of the HIV co-receptor CCR5 on infected macrophages and reduces proviral synthesis by 50% [54].

The role of the serotonergic system in cancer patients is still under investigation. Increased levels of systemic 5-HT appears to correlate with advanced stages of breast cancer [55]. Serafin et al. [56] showed in established Burkitt lymphoma (BL) cell lines that 5-HT plays an important role in driving programmed cell death. Despite Burkitt lymphoma being a very aggressive tumor, it shows a propensity to apoptosis, as

indicated by its classical starry sky histology. The authors described that 5-HT induced a considerable suppression of DNA synthesis in parallel with an important apoptosis in BL cells culture. These effects were anteceded by early caspase activation and mitochondrial membrane potential disruption. However, 5-HT receptor antagonists (i.e. granisetron, methysergide, SDZ205-557) were not able to inhibit 5-HT-driven apoptosis, while fluoxetine, paroxetine, and citalopram extensively blocked the monoamine actions. Nevertheless, SSRIs directly signal for apoptosis in these cells [57]. The presence of SERT, as well as the active uptake of 5-HT indicated by transport assays in BL cells, suggest that 5-HT is able to drive apoptosis by an active transport mechanism.

4. Fluoxetine action on immune system.

Experimental findings suggest that antidepressants can modulate the proliferation of immune cells. However, there is conflicting evidence about the action of fluoxetine on the immune system. Pellegrino and Bayer [58, 59] described a reduced mitogen-induced T lymphocyte proliferation after acute, but not chronic fluoxetine therapy. In addition, the subchronic administration of fluoxetine in depressed patients regularized the initially increased plasma concentrations of pro-inflammatory cytokines IL-6 [60] and IL-1β [61]. Repeated treatment with fluoxetine and tricyclic antidepressants (TCAs) might suppress the acute phase response in major depression [62]. On the contrary, we described that after 4 weeks treatment with fluoxetine in normal mice, an augmentation of T cell mediated immunity occurs. Thus, an increase of T cell proliferation with no changes on CD4+/CD8+ ratio as well as an enhanced IFN-γ and TNF-α cytokines production was observed [63]. Experiments carried out with athymic mice -lacking of T

lymphocytes- indicated that fluoxetine effects are selective for T cells, not found in other lymphocyte subpopulations [63]. On the other hand, Fazzino et al. [64] found that chronic administration of fluoxetine in normal rats induces a significant reduction of CD4+/CD8+ ratio and an increase of IL-4/IL-2 ratio. It is important to note that the antidepressant route of administration influences the effects observed. For instance, Fazzino et al. [64] administered fluoxetine intraperitoneally and our group [63] orally. It is probable that intraperitoneal injection represents an extra stressor that leads to an opposite effect.

Fluoxetine was shown to revert the stress-induced suppression on T lymphocyte population [65-67] and activity of phagocytosis [68] without affecting them in control mice. In addition, chronic restraint stress provokes a reduction of the total number of T CD4+ cells affecting T helper immunity in BALB/c mice. Nevertheless, according to our observations, fluoxetine increases T cell reactivity with no changes in the total number of T lymphocytes. This differential effect could be indicating that fluoxetine exerts this modulatory action on T helper immunity through compensatory and/or specific mechanisms [69]. Thus, it has been proposed that fluoxetine can act by indirect effects through the activation of central 5-HT2 receptors [65], but that it may also mediate a direct regulation of peripheral cells [63, 69, 70]. In fact, *in vitro* results indicate that fluoxetine is able to modulate immune functions. It was shown that fluoxetine suppresses *in vitro* mitogen induced rat T- and B-lymphocyte proliferation in a dose-dependent manner [71]. Fazzino et al. [52] reported that elevated basal proliferation observed in patients with depression, as mentioned above, was reduced efficiently in the presence of fluoxetine. Besides, incubation of mitogen-stimulated human immune cells with fluoxetine *in vitro*, significantly reduces the production of the

pro-inflammatory cytokines TNF-α and IFN-γ, and the IFN-γ/IL-10 production ratio, with [72] or without [73] significant increase of the anti-inflammatory cytokine IL-10. Moreover, desipramine and fluoxetine were able to suppress lymphocyte proliferation [71] and to decrease the inflammatory reaction and mortality in rat and murine models [74]. In addition, Kubera et al. [75] showed that both fluoxetine and desipramine significantly ameliorate the contact hypersensitivity induced by a topical application of hapten 2,4-dinitrofluorobenzene (DNFB). This effect is mediated by the suppression of the T cytotoxic-cell-mediated antigen-specific type of skin inflammation by antidepressant drugs. These findings suggest that these drugs may be beneficial therapeutic tools in the treatment of contact allergy. Table 2 reports the available information on the immunomodulatory effect of fluoxetine in pathological situations in which immune alterations play a role.

It is probable that the effect of fluoxetine on T-cell activity is related to the degree of lymphocyte activation. We found [69,70] that when lymphocytes were stimulated with optimal Concanavalin A (Con A) concentration fluoxetine promotes an increase of intracellular Ca^{2+} levels that in turn induces proteolysis of protein kinase C (PKC) increasing cyclic-AMP levels, so impairing lymphocyte proliferation. However, when suboptimal Con A concentration was utilized, fluoxetine increased PKC translocation, without affecting cAMP levels, leading to T-cell proliferation. These results suggest that fluoxetine has a dual activity on T-cell proliferation through Ca^{2+} mobilization that influences PKC and protein kinase A pathways [69, 70]. Accordingly, we reported a similar dual effect of fluoxetine on B-cell receptor (BCR)-driven murine B-cell proliferation [76]. In addition, Frank et al. showed that *in vitro* exposure of mononuclear cells to fluoxetine and paroxetine directly increase NK-cell activity [77].

Blockade of SERT may be followed by an increment in 5-HT concentration close the lymphocytes, modulating proliferation through specific receptors. To evaluate the role of 5-HT in the effects of fluoxetine on T-cell activity we determined the mitogen-induced proliferation in the presence of 5-HT or fluoxetine alone and in combination with fluoxetine. We [63] found that 5-HT and fluoxetine have similar effects; both were capable of stimulating the proliferation induced by suboptimal Con A and of inhibiting the optimal one. However, for 5-HT, these effects were directly related to the concentration used. On the contrary, the stimulatory effect of fluoxetine was inversely linked to the concentration used, whereas the inhibitory effect was directly related to fluoxetine concentrations. When combinations of 5-HT and fluoxetine were used, 5-HT was able to decrease the inhibitory effect of fluoxetine at optimal Con A-induced proliferation and to increase the stimulatory effect of fluoxetine on suboptimal Con A stimulated proliferation. These results point out that the modulatory effects of fluoxetine are partially independent of its capacity to increase 5-HT extracellular levels. This mechanism might introduce the action of fluoxetine through a novel receptor or new intracellular pathway coupled to 5-HT transport [63, 67].

The evidence summarized above suggests that SERT cannot only remove the monoamine from the extracellular space, but also, under particular conditions pump 5-HT out of cells. Moreover, it is now perceived that intracellular 5-HT is not simply passive since the ‘serotonylation’ of small GTPases, like Rho and Rac, induce their degradation directly impacting on signal transduction [15].

It is important to note that fluoxetine *per se* has an immunomodulatory effect in normal animals. We demonstrated that fluoxetine alone increases mitogen-induced T cell

proliferation and IL-2, IFN-γ and TNF-α expression over control levels [63]. Moreover, we found that fluoxetine was able to inhibit tumor growth, retard its appearance, and prolong survival of mice. These effects were maximal in animals receiving continuously fluoxetine. However, if fluoxetine is administered only before or after tumor injection, a decrease in tumor progression and an increase in survival were also observed. An outstanding finding is the fact that the survival was increased when fluoxetine was administered after tumor injection, suggesting a potential pharmacological beneficial effect of fluoxetine on cancer therapy [63].

A relevant question is thus which dose of fluoxetine is to be used. It was reported that the oral intake of 10 and 25 mg/kg/day of fluoxetine in BALB/c mice yield a plasma concentration near 170–1780 g/ml, corresponding to 5 x 10^{-7} M to 5 x 10^{-6} M [78]. In addition the range of plasma levels reported in patients consuming 20–80 mg/day Prozac is near 100–700 ng/ml [79], equivalent to a fluoxetine concentration of 2 x10^{-7} to 3 x 10^{-6} M. Using these doses we demonstrated that fluoxetine improves the reduced T cell proliferation found in stressed mice both *in vivo* and *in vitro* [67]. These results emphasize that when the direct effects are analyzed by *in vitro* approaches, the antidepressant concentration used is crucial since the cytotoxic/apoptotic phenomena could be just due to high doses of these drugs, but not necessary an effect occurring *in vivo*.

5. Concluding Remarks

Based on the above described actions of fluoxetine on immune responses, it has been proposed that serotonergic and dopaminergic pathways offer an attractive target to treat human immune system disorders.

Despite some conflicting experimental findings, in general it appears that fluoxetine is able to modulate the immune response. Thus, when basal immune function is high, fluoxetine administration reduces lymphocyte activity. On the contrary, when immune function is deficient, fluoxetine administration improves it.

Fluoxetine can exert its effect by direct and indirect mechanisms. Indirect mechanisms would be 5HT-dependent or independent (Figure 1). It is relevant to note that the concentration used for analyzing the direct action of fluoxetine *in vitro* is crucial taking into account that cytotoxic / apoptotic effects can be due to the usage of high doses of this drug, not necessarily occurring at the concentration found when the drug is administered *in vivo*.

These results highlight the importance of the novel pharmacological action of this drug as an immunomodulator, helping to treat several pathologies that course with immune deficiency and/or deregulation.

Fluoxetine can exert immunomodulatory effects by its action on serotonergic neurons in the central nervous system regulating neuroendocrine signals. In addition, fluoxetine is also able to act directly on T lymphocytes modulating their proliferation in a dual manner depending on cellular activation. This mechanism could include: a) the action through a novel receptor or a novel intracellular pathway coupled to 5-HT transport, b) the increase of 5-HT in the lymphocyte milieu which in turn activates intracellular signals by binding to serotonergic receptors, and c) the increase of intracellular 5-HT

leading to “serotonylation” of small GTPases. The crosstalk of different events leads to the immunoregulatory effect in the presence of antigen or inflammatory stimulus.

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Figure Captions

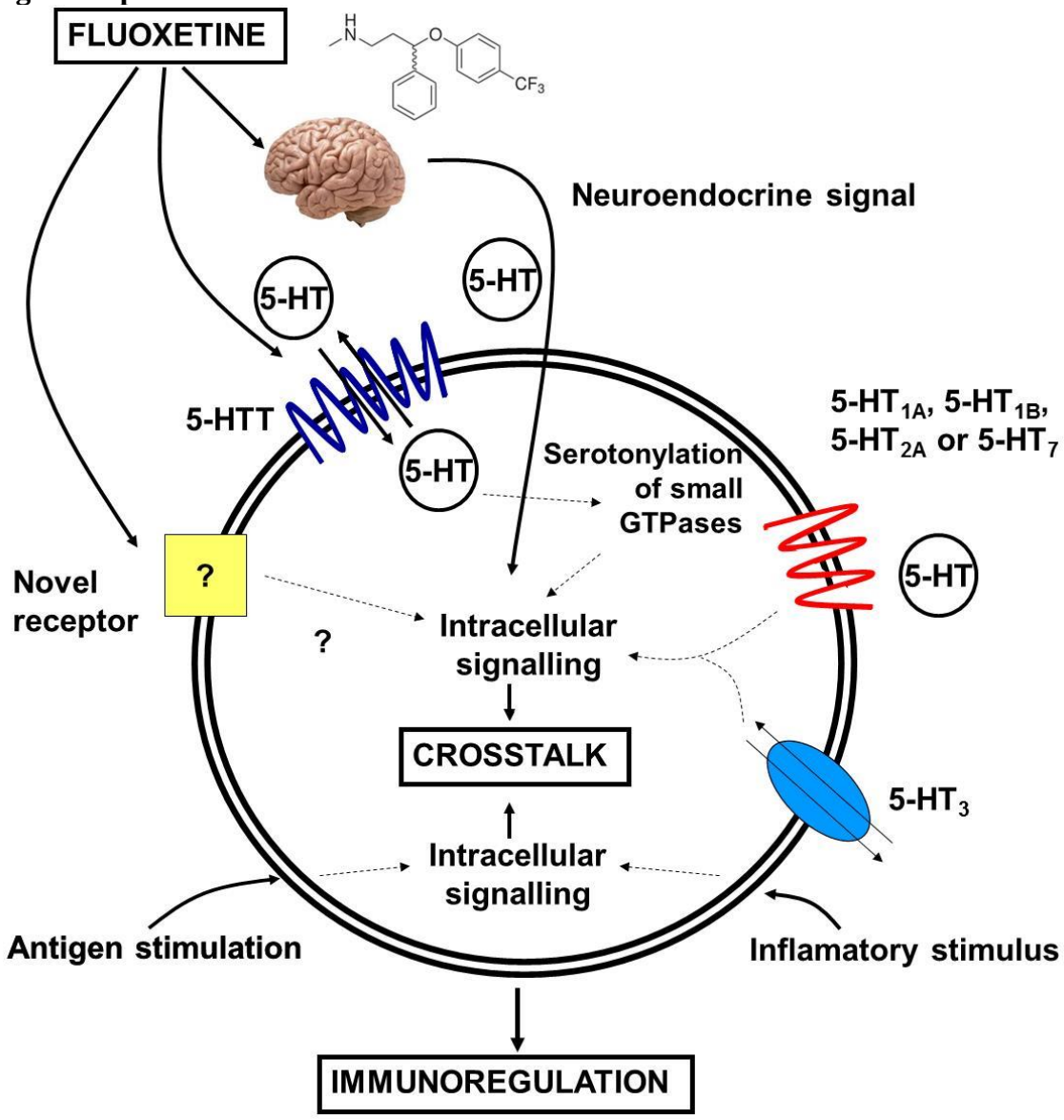

Figure 1: Hypothetical Pathways for Fluoxetine Immunomodulatory Effects.

Tables

Table 1. 5-HT receptor subtype and serotonin action in immune cells.

Immune cell type	5-HT receptor subtype	5-HT effect
T-cell	5-HT1A, 5-HT1B, 5HT2A, 5HT3, 5HT7 [13-15]	-Induction of Na^+ influx [22] -Induction of IL-16 secretion from $CD8^+$ T cell[22] -Enhancement of proliferation[22] -Potentiation of T-cell activation [22] -Increase of IL-2 and IFN-γ production[22]
B-cell	5-HT1A[17]	-Increase of proliferation[17]
Dendritic cell	5-HT1B, 5-HT2, 5-HT3, 5-HT4, 5-HT7[12]	-Induction of oriented migration in immature DC[12] -Induction of Th2-cell polarization capacity of mature DC[12] -Inhibition of CXCL10 production in mature DC[12] -Stimulation of CCL22 production in mature and immature DC[12] -Stimulation of IL-1β, IL-6 and IL-8 in mature DC[12, 26] -Inhibition of IL-12, TNF-α in mature DC [26] -Stimulation of IL-10 in LPS-stimulated mature DC[12] -Inhibition of IL-12p70 in LPS-stimulated mature DC[12]
Macrophage	5-HT1E, 5-HT2A, 5-HT3, 5-HT4, 5-HT7[23]	-Stimulation of IL-1β, IL-6, IL-8 and IL-12 [25] -Inhibition of TNF-α release[25]

*Abbreviation*s: DC: Dendritic Cells; CXCL10: Chemokine 10; CCL22: Chemokine 22; LPS: lipopolysaccharide.

Table 2 Immunomodulatory effect of fluoxetine in pathology.

Pathological situation	Immune alteration	Fluoxetine effect
Inflammation	-Increase of INF-γ and TNF-α	-Decrease TNF-α and INF-γ[73,74] -Increase of IL-10[72,75]
Stress	-Decrease of phagocytosis -Decrease of T cell response -Decreased production of TNF-α, and IFN-γ	-Restoration of phagocytosis[68] -Restoration of T cell response[65,66,67] -Enhance of TNF-α and IFN-γ production [63]
Depressive disorder	-Elevated basal and stimulated proliferation -High IL-6 levels and IL-1β	-Normalization of both basal and stimulated proliferation [52] -Reduction of IL-6[60] and IL-1β[61]