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Lymphocytes transport serotonin and dopamine: agony or ecstasy?

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Lymphocytes apparently carry active transport systems for the neurotransmitters serotonin and dopamine. Meanwhile, pharmacological substrates for the transporters have been claimed to impinge on immune function: these include, commonly used antidepressants [such as fluoxetine (Prozac®)], appetite suppressants and the recreational drugs MDMA [3,4-Methylenedioxymethamphetamine ('Ecstasy')] and cocaine. Data on these issues can be patchy. Given the widespread use – or, abuse – of these drugs, we propose that a concerted effort should be made towards a full description of biogenic amine transporters in the immune system and the potential impact of their substrates – both natural and artificial – on its functioning. Could such knowledge one day help to treat immune deficiency and/or dysregulation?

Neurotransmitters, such as serotonin [5-hydroxtryptamine (5-HT)] and the catecholamines, are primitive biogenic amines crucial to the regulation of central processes, such as mood, appetite (food and sexual), sleep and other body rhythms. Their bioavailability is tightly regulated through several mechanisms, for example, sequestration to synaptic vesicles through selective transporters. These neurotransmitters are also found systemically and peripheral cells, especially those of the immune–inflammatory axis, have variably been described as carrying corresponding transporter proteins. Within the nervous system, the transporters offer attractive targets for pharmacological intervention in the control of mood and anxiety-related disorders and selective serotonin re-uptake inhibitors (SSRIs) have proved a remarkable success story. Psychostimulants that influence neurotransmitter transport have also found widespread recreational use among certain sections of society. Although cocaine – owing to a link with HIV transmission – has been subject to some serious scrutiny, other compounds that act on the transporters have received relatively scant attention with respect to a potential impact on immunity. Here, we argue that such imbalance should be redressed by: (1) elucidating the role of the transporters within the immune system; and (2) ascertaining whether immune function might be modified – for good or ill – by the drugs that target them.

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Evidence that lymphocytes carry transporters for serotonin and dopamine

Some relevant pharmacological characteristics of the transporters involved in active uptake of serotonin, dopamine, and noradrenaline are outlined in Table 1. Outside the brain, the most widely described of these is the serotonin transporter (SERT) in platelets.

SERT

Platelet SERT appears to be identical to that carried by neurons and stored serotonin can be rapidly released in response to a variety of inflammatory signals [1] (Box 1). Lymphocytes also carry SERT. High affinity uptake of serotonin by peripheral-blood lymphocytes has been described with corresponding inhibition by SSRIs [2]. Specific saturable binding of the labelled SSRI paroxetine (Seroxat®) with pharmacological characteristics consistent with SERT has also been demonstrated [3,4]. A capacity to accumulate 5-HT seems to have been evolutionarily conserved among lymphocytes; kinetics and pharmacology comparable to that in humans has been described for rainbow trout, indicating that lymphoid SERT has a fundamental role, if not essential function, within the periphery [5].

Lymphoblastoid lines have been used both for typing polymorphisms in the upstream regulatory region of the *sert* gene [6] and demonstrating modulation of SERT activity by brain-derived neurotrophic factor [7] and interleukin-4 (IL-4) [8]. Recently, we described an active uptake mechanism for 5-HT in Burkitt lymphoma lines, the consequence of which (apoptosis) could be reversed by SSRIs, although left untouched by 5-HT receptor antagonists. SERT protein carried by these neoplastic equivalents to germinal-centre B cells appears to be smaller (70 kDa) than that of its neuronal counterpart (90 kDa) [9].

Catecholamine transporters

Although unsuccessful in finding literature on lymphocytes carrying the noradrenaline transporter (NAT), evidence is available for them expressing the dopamine transporter (DAT). Faraj and co-workers [10] were among the first to indicate a dopamine uptake system in lymphocytes, describing drug-competable specific binding sites for dopamine, which were dependent on time, temperature and sodium, and following Michaelis–Menten kinetics. *In vivo*, significant uptake of labelled

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Table 1. Pharmacology of monoamine transporters

Compounds	Compound affinity (pKD)[b] SERT[a]	DAT	NAT
Monoamines			
5-HT	5.68	<4	< 4
DA	<4	5.62	4.49
NA	<4	4.79	5.66
SSRIs			
Citalopram	8.93	4.55	5.39
Fluoxetine	9.09	5.44	6.12
Paroxetine	9.88	6.31	7.40
TCAs			
Desipramine	7.75	5.50	9.08
Imipramine	8.86	5.07	7.43
Clomipramine	9.55	5.66	7.42
Psychotropics			
MDMA[c]	5.67	6.32	5.86
Cocaine	6.47	6.66	5.85
Fenfluramine[d]	6.57	<4.7	5.70

[a]Abbreviations: DA, dopamine; DAT, dopamine transporter; 5-HT, 5-hydroxytryptamine; MDMA, 3,4-Methylenedioxymethamphetamine; NA, noradrenaline; NAT, noradrenaline transporter; SERT, serotonin transporter; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.
[b]Affinity values for the neuronal transporters SERT, DAT and NAT given as $pK_D = -\log_{10}$ molar concentration of the equilibrium dissociation constant of the drug derived from Tatsumi *et al.* [46], unless otherwise indicated.
[c]$pIC_{50} = -\log_{10}$ molar concentration to inhibit radiolabelled monoamine uptake by 50%, [47].
[d]$pK_i = pIC_{50}$ adjusted according to the Cheng–Prusoff equation [48].

dopamine into lymphoid tissues is observed [11]. Bergquist and colleagues elegantly showed that in the presence of a dopamine-synthesis inhibitor (to block autocrine production), the monoamine continued to accumulate in cloned CD4 T cells through uptake from the external medium [12]. In a seminal study, Amenta and co-workers unequivocally demonstrated DAT in human lymphocytes by classical pharmacology, dopamine uptake, immunoblotting and immunocytochemistry [13]. Caronti *et al.* confirmed DAT immunoreactivity in lymphocytes and, interestingly, found it present at lower than normal levels in patients with Parkinson's disease [14]. Recently, Mill and colleagues demonstrated DAT mRNA in human lymphocytes by reverse transcriptase (RT)–PCR [15]. The proposed cellular pathways of transporter activity and function in lymphocytes – for both dopamine and serotonin – are given in Figure 1.

Impact on immune function of drugs that target biogenic monoamine transporters

Here, we concisely review some of the literature pertaining to how psychotropic drugs and antidepressants that target the monoamine transporters might perturb the proper functioning of the immune system.

Ecstasy and fenfluramine

3,4-Methylenedioxymethamphetamine (MDMA) or to give it its street name – Ecstasy – acts predominantly as a protein kinase C-dependent serotonin-releaser following its binding at SERT and also reduces inward transport of 5-HT. Both mechanisms lead to an increase in 5-HT available to postsynaptic serotonergic receptors in the brain. A multiparameter *in vitro* study of MDMA actions against murine lymphocytes concluded that although not effecting B-cell function it could selectively enhance T-cell IL-2 production, augment natural-killer (NK) activity and suppress cytotoxic T-lymphocyte (CTL) induction [16]. Connor and co-workers, investigating the effects of MDMA on the immune function of rats, have noted several changes, among which include: a rapid reduction in total leukocyte number with depressed *in vitro* mitogenic responses of lymphocytes to Con A (concanavilin A) [17]; suppressed IL-1β and tumour necrosis factor-α (TNF-α) production on lipopolysaccharide (LPS) challenge *in vivo* [18]; selective suppression of a keyhole limpet haemocyanin (KLH)-specific IgG2a antibody response with

Box 1. Where, when and why might lymphocytes 'see' serotonin and dopamine?

Serotonin

The major producers of 5-HT (5-hydroxytryptamine) are the enterochromaffin cells of the gut accounting for ~90% of serotonin in the body. Serotonin released by these cells can be taken up by platelets that then act as 'mobile storage units' for 5-HT. Triggers for the rapid release of platelet 5-HT include IgE-containing immune complexes, PAF (platelet activating factor) and C5a. Thus, at sites of inflammation, lymphocytes could be exposed to high amounts of platelet-derived 5-HT, estimated as reaching ≤1 μM in the local milieu. Another source of 5-HT for lymphocytes might be through the neuroimmune axis. Autonomic innervation of lymphoid organs can be extensive, with nerve fibres in close contact with lymphocytes. It has been suggested that noradrenergic nerve termini in lymphoid tissues might take up 5-HT to be released on later stimulation of the nerves [1].

Dopamine

In the spleen, the noradrenergic sympathetic fibres can take up circulating dopamine, particularly during (psychological) stress, which could then be released on sympathetic activation to be taken up in turn by neighbouring immunocytes [48]. The major source for lymphocytes, however, could lie within the immune system itself. Catecholamines, including dopamine, are present in lymphocytes, macrophages and neutrophils: the first two of which, at least, have been shown capable of actively synthesizing dopamine from amino acid precursor tyrosine through the intermediate, levodopa (L-DOPA). Lymphocytes can seemingly take up dopamine through active transport, whether its initial production and release is autocrine or paracrine [49–51].

Transporters and receptors

Appreciating that lymphocytes accumulate serotonin and dopamine then begs the question: 'why'? Part of the answer might relate to the fact that lymphocytes also express receptors for the neurotransmitters, the precise repertoire of which might dictate fine-tuning of an immune response: active uptake would be capable of regulating monoamine exposure within the locality of an immune reaction by limiting bioavailability where appropriate. Of the 14 receptor subtypes for serotonin identified so far, immune cells appear to express at least seven [52]. Dopamine receptors, of which there are five, are classified into D_1-like and D_2-like subfamilies, reflecting different biochemical and pharmacological properties: lymphocytes can seemingly express representative members of both groups [53]. A growing body of evidence indicates important roles for the monoamine receptors in regulating lymphocyte behaviour (reviewed in Ref. [36]). Alternatively, or in addition, and as we have argued for lymphoid SERT (serotonin transporter), the transporters could have direct signalling consequences in their own right [9]. Finally, loading a lymphocyte with monoamine at one locale (e.g. site of inflammation) might then enable its release at another (e.g. lymphoid tissue microenvironments) where it becomes available for paracrine signalling to neighbouring cells.

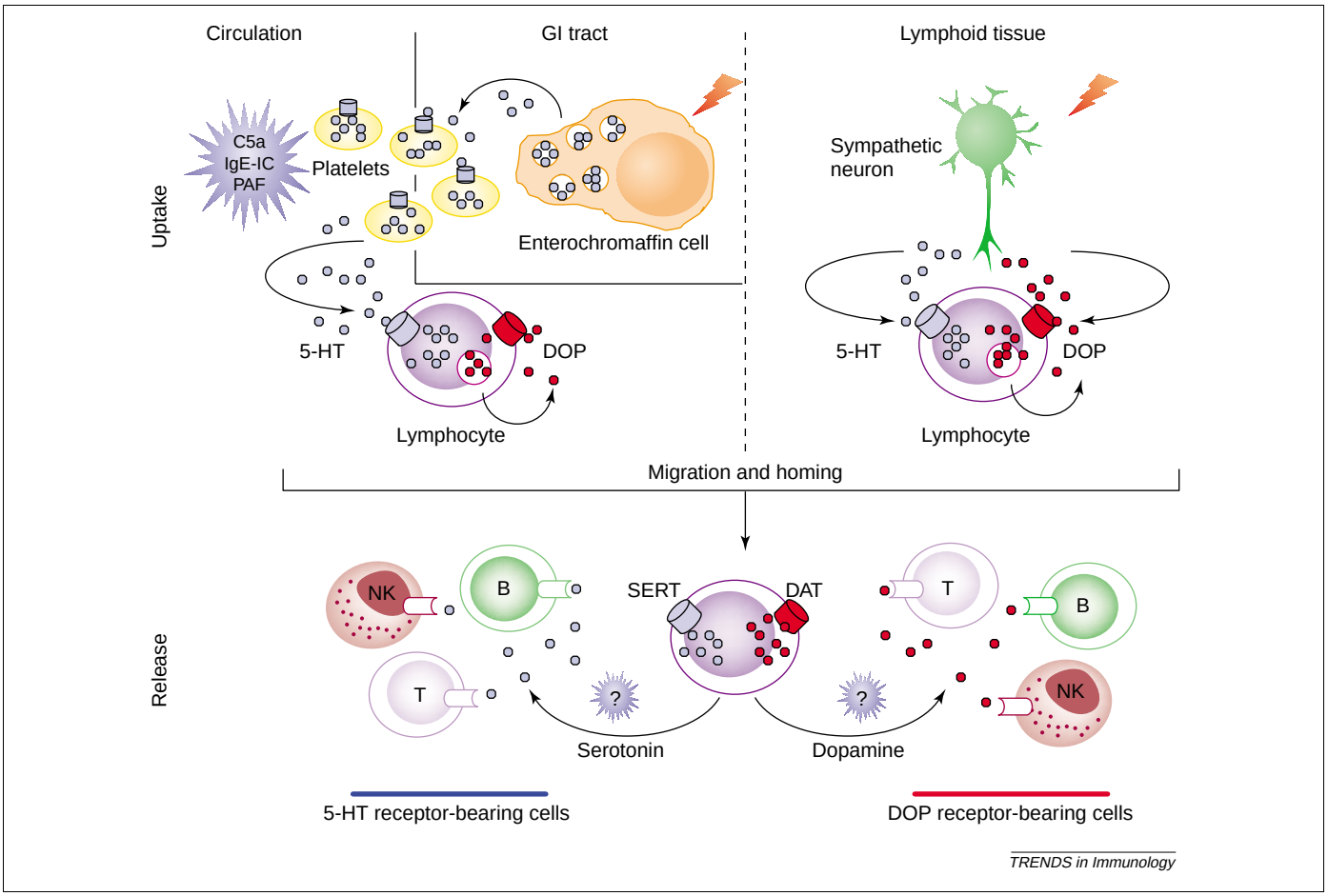

Fig. 1. Model for cellular pathways involved in the uptake, transport and release of serotonin and dopamine by lymphocytes. Stimulation of serotonin- and/or dopamine-loaded sympathetic neurons within lymphoid tissues triggers monoamine release with uptake by lymphocytes through corresponding transporters. Within the GI tract, 5-HT released from enterochromaffin cells in response to (mechanical) stress is taken up by platelets, then subsequently discharged on stimulation for uptake by SERT-carrying lymphocytes. Dopamine can be both synthesized and, on its release, taken up by DAT-carrying lymphocytes. Lymphocytes migrate from sites of uptake and then release (in response to as yet undefined signals: '?') monoamine(s) with resultant signalling of neighbouring receptor-bearing cells: here, illustrated by B, T and NK cells but could also include other immunocytes and even, potentially, non-immune cells. Abbreviations: B, B cell; DAT, dopamine transporter; DOP, dopamine; GI, gastro-intestinal; 5-HT, 5-hydroxtryptamine; IC, immune complexes; NK, natural killer cell; PAF, platelet activating factor; SERT, serotonin transporter; T, T cell.

corresponding decrease in KLH-specific interferon-γ (IFN-γ)-production in cultured splenocytes [19].

Particularly interesting are studies by Pacifici and colleagues on the immunomodulatory effects of MDMA in humans. They first assessed the influence of a single acute administration revealing that despite an unchanged total leukocyte count there were decreases in circulating CD4 T cells with diminished *in vitro* responses to mitogens but NK cells were significantly increased [20]. Repeated administration prolonged immunocompromise [21]. A 'double whammy' of Ecstasy and alcohol caused an even higher suppression of CD4 T cells and a greater NK augmentation [22].

Similar to Ecstasy, the amphetamine derivative d-fenfluramine (d-FEN) releases 5-HT following intracellular transport by SERT. Marketed widely as the appetite-suppressant Redux®, it was voluntarily withdrawn in 1998 owing to links with heart valve problems. Connor *et al.* compared FEN (racemic mix) directly with MDMA and found the two to be similar in their ability to suppress immune function in rats [23]. Clancy and colleagues, however, reported that at sub-anorexic doses, d-FEN could enhance a variety of immune parameters in mice [24], whereas chronic exposure corrected levels of NK-cell and T-cell activity otherwise disturbed in elderly animals [25]. They also found that d-FEN boosted a local immune response to the opportunistic microbial pathogen *Candida albicans*, which was accompanied by a CD8 lymphocytosis [26].

Cocaine

Cocaine is a non-selective competitive inhibitor of serotonin and catecholamine re-uptake, binding promiscuously at all three transporters (Table 1). Its intravenous use has been cited as an important risk factor in developing HIV. This appears not simply to relate to shared needle usage because increased risk remains even where cocaine is smoked [27]. The resultant implication – that the drug might directly impact on the pathobiology of HIV infection – has prompted numerous studies on its potential immunomodulatory role. However, conflicting data have emerged with a consensus difficult to discern. The confusion could relate to investigations alternating between *in vitro* and *in vivo* and ranging across species, cell types, functional outcomes, drug concentrations and chronicity of usage and administration. As with MDMA, cocaine administration leads to a rapid increase (2.7-fold) in NK-cell number and activity (3.8-fold) – both

parameters returning to baseline within two hours. A similar NK augmentation was noted in patients admitted to the emergency room after overdose. However, in no study were effects noted on NK cells *in vitro*. For T cells a single injection of cocaine produced no change in overall number, although overdosed patients in ER had a significantly lowered CD4 count. One *in vitro* study noted suppressive effects of cocaine on the phytohaemaglutinin (PHA)-stimulation of T cells together with a reduced Ca^{2+} response and IL-2 release; by marked contrast, where immobilised anti-CD3 was used as a stimulant, cocaine enhanced these read-outs (studies reviewed in Refs [27,28]).

Antidepressants

Here, we briefly note a few examples of studies addressing the impact on immune cells of two classes of widely used antidepressants that act relatively broadly or more selectively on one or more of the biogenic amine transporters: respectively, tricyclic antidepressants (TCAs) and SSRIs (Table 1).

For example, Xia *et al.*reported how, at relatively high concentration (50 μM), the TCA clomipramine substantially reduces *in vitro* lymphocyte survival [29]. Monocytes are similarly affected whereas growth of the Molt-4 T cell and U-937 monocytic lines are severely curtailed by TCA (and SSRI) antidepressants. The authors demonstrated features of apoptosis arising in both lymphocytes and monocytes as a consequence of TCA exposure, a finding confirmed and extended in later studies [30]. Edgar *et al.* showed, for both murine and human T cells, that the SSRI fluoxetine potently inhibits Con A-induced mitogenic responses, however, at sub-mitogenic concentrations of the lectin, low fluoxetine doses were stimulatory [31,32]. More recently, the same group described similar dual activity of fluoxetine on B-cell receptor (BCR)-driven murine B-cell proliferation [33].

NK-cell activity is significantly reduced in patients suffering from depression. Correspondingly, successful antidepressant therapy corrects this deficit. Although at least a component of this reversal might be indirect, Frank and colleagues demonstrated direct augmentation of NK-cell activity among mononuclear cells on *in vitro* exposure to fluoxetine and paroxetine [34]. Interestingly, the same group failed to recapitulate the SSRI effects with St. John's Wort, a popular herbal supplement used to treat depression and possessing serotonergic properties [35].

Is drug-induced immune disturbance mediated through lymphocyte monoamine transporters?

he existence of bi-directional communication among the immune, neuroendocrine and central nervous systems is beyond dispute, even if current insight probably represents just the tip of an iceberg [36,37]. Stress clearly perturbs immune function, as does mood and emotion. Main players mooted responsible for such crosstalk between mind and body are the sympathetic nervous system and the hyopthalmus–pituitary–adrenal (HPA) axis. Thus, with investigations *in vivo*, it becomes difficult to untangle effects of the drugs resultant on psychological change from any that might target lymphocytes directly. An overview of the *in vitro* studies indicates at least the possibility of direct targeting, especially for the antidepressants. However, caution must be adopted in interpretation with respect to: (1) whether concentrations used to register change are ever reached under normal dosing regimes; and (2) whether any observed effect is indeed through actions on the appropriate transporter(s).

Even SSRIs do not target SERT exclusively. Our own studies illustrate how SSRIs can influence lymphoid function through both serotonergic and non-serotonergic pathways. As mentioned earlier, we showed how the tendency of SERT-carrying Burkitt lymphoma cells to undergo apoptosis in response to serotonin could be reversed by SSRIs [9]. Later, we described how at slightly higher concentration, SSRIs initiated killing in their own right [38]. Thus, even on a single cell type, these antidepressants can exert either 5-HT-dependent or 5-HT-independent change. Importantly, however, our demonstration that SSRIs can reverse serotonin-driven modulation of lymphoid-cell function at concentrations close to those achieved therapeutically indicate the potential for these drugs directly impacting on immune function otherwise modified by 5-HT.

An argument in favour of, at least part of, the ability of SSRIs to augment (or restore in depressed patients) NK-cell activity being a direct effect on the serotonergic system in immune cells has been made by Helgason *et al.* [35]. This was based on the fact that exposure of NK cells to 5-HT *in vitro*invokes a similar change in activity to that seen on administering the antidepressants *in vivo*. Pellegrino and Bayer also reached the conclusion that, with respect to the suppression of lymphocyte responses resulting from fluoxetine administration to rats, the drug worked through its impact on the serotonergic axis. However, they favoured the notion that the resultant immunomodulation was indirect in-as-much as administration of the SSRI into the lateral ventricle of the brain recapitulated systemic infusion of the drug [39].

With regards the *in vivo* actions of Ecstasy on immune parameters, Connor and colleagues tend to discount a direct affect and favour the documented ability of the drug to impact on the HPA axis and the sympathetic nervous system. For example, they noted a concomitant dose-dependent rise in circulating corticosterone levels that mirrored the MDMA-induced suppression of immune function. They also concluded that the capacity of MDMA to suppress LPS-induced proinflammatory cytokine release was independent of its serotonin-releasing properties, postulating instead a role for dopamine in this outcome [40]. By contrast, when reviewing the literature as well as their own studies with regards to the influence of cocaine on immune function, Pellegrino and Bayer felt that the serotonergic system was primarily responsible for any suppression observed with this drug [28]. Although not dismissing direct actions on transporter-carrying immune cells, they argued – that in common with MDMA – cocaine might be exerting its influence primarily through the HPA axis, a conclusion reasserted in a study from Xu *et al.* [41]. Studies showing that cocaine perturbs *in vitro* T-cell function at concentrations corresponding to those found circulating in users do, however, indicate a possible direct action on lymphocytes.

Box 2. Questions arising from knowing that lymphocytes express the transporters for serotonin and dopamine

(1) How widely distributed are the transporters among immune cells; is their expression regulated and if so how? Here, analysis of distinct lymphoid microenvironments probed *in situ* and defined subsets isolated *ex vivo* would be studied using approaches, such as immunocytochemistry, pharmacology, immunoblotting and uptake studies, outlined by Amenta and colleagues for dopamine [13] and Serafeim *et al.* for serotonin [9]. A definitive conclusion as to whether NAT (noradrenaline transporter) is found in lymphocytes also needs to be reached. There is also the issue of whether and how transporter expression is regulated in lymphocytes. The two papers from Mossner and colleagues demonstrate the capacity for modulation of SERT (serotonin transporter) expression by growth factors in immortalised B-lymphoblastoid lines [7,8]: defining the signals that lead to altered expression of the transporters in normal subsets could help to provide insights into the role they have in lymphocyte biology.

(2) What is the physiological role for the biogenic monoamine transporters within the immune system? This is clearly the most pressing issue. Mice genetically deficient in each of the three transporters (i.e. those for dopamine, noradrenaline, serotonin) have been engineered and this might enable some insight into how their functioning might act to modify an immune response. However, any phenotype observed would need careful interpretation: untangling effects resulting as a disturbance of neural-immune cross-talk from those impacting on lymphocytes directly could be problematic. The key here would be to generate tissue-specific knockouts, exclusively targeting lymphoid cells for genetic ablation of the transporters. Given that there is some degree of promiscuity between the transporters for the monoamines, it might be necessary to investigate and generate double (or even triple) knockouts. One hypothesis to be tested is whether lymphocytes might have harnessed the transporters because, like platelets, they are a highly mobile cell type capable of delivering monoamines at sites distal from their production: this could extend to targets beyond the immune system itself.

(3) Do the antidepressants and psychostimulants discussed herein indeed impact on an immune response through transporter targets expressed in lymphocytes? Again, the above tools would enable a crucial approach to elucidating this question – at least for mice.

(4) Could transporter expression by lymphocytes provide surrogate markers for psychiatric disorders? Although this is among the most speculative of questions raised it is also one of the more intriguing. Platelet SERT expression has been used as a quantitative peripheral marker of numerous CNS (central nervous system)-related traits and disorders (most notably in relation to episodes of 'romantic love' [54]). At least with regards major depression, a recent study indicates that lymphoid SERT levels might also prove an indicator of CNS disturbances: or their reversal on medication [55].

Transports of delight?

In this Review we have posed the following questions: (1) do lymphocytes express functional transporters for serotonin and catecholamines; (2) do drugs in widespread use that target these structures disturb immune behaviour; and (3) are such effects dependent on direct modulation of lymphocyte-associated transporter activity? Although there appears to be sufficient (although still far from complete) evidence to answer affirmatively to the first question there remains confusion and/or simple lack of knowledge in our ability to offer firm conclusions to the other two. Moreover, an appreciation that immune cells carry receptors as well as transporters [36] for classical neurotransmitters raises even more questions, some of which are highlighted in Box 2.

Although being mindful of the potential to compromise optimal immune functioning through drugs that target the monoamine transporters (the 'agony'), we believe there are grounds for considering their exploitation to the benefit of individuals suffering immune disturbance in one guise or another (the 'ecstasy'). For example, an ability to enhance NK-cell number and function appears to be common to all the drugs under discussion. More specifically, Mathews *et al.* indicated the use of d-FEN in immunocompromised individuals, such as AIDS patients. Thus, the appetite suppressant augmented the capacity of CD8 lymphocytes to inhibit the growth of *C. albicans* and enhanced the ability of CD4 lymphocytes to proliferate in response to Con A while increasing the amount of IL-2 produced by both T subsets [42].

Perhaps most tantalising is the prospect of exploiting psychostimulants and antidepressants that target biogenic amine transporters to redirect an inappropriately polarised immune response. For example, cocaine can seemingly enhance IFN-γ while decreasing IL-10 production [43]. By contrast, Pacifici *et al.* showed that MDMA (and especially when co-administered with alcohol) promoted a switch from Th1 to Th2 cytokines together with increases in the T-regulatory cytokines, IL-10 and transforming growth factor-β (TGF-β) [22]. Similarly, on reviewing the literature with respect to antidepressants, Kenis and Maes noted a common theme of these drugs decreasing IFN-γ:IL-10 ratios and suppressing the production of proinflammatory cytokines, such as TNF-α, suggesting potential anti-inflammatory benefits [44]. Particularly exciting is the finding that both tricyclic and SSRI antidepressants seemingly suppress clinical symptoms in a rat model of experimental autoimmune neuritis, this being accompanied by a reduction in Th1 cells secreting IFN-γ in response to neuritogenic myelin proteins [45]. Could it be we are glimpsing the dawn of a new discipline: neuroimmunopharmacotherapy?

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