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REVIEW

ABOUT COVER

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REVIEW

Glutamate transporters, EAAT1 and EAAT2, are potentially important in the pathophysiology and treatment of schizophrenia and affective disorders

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Abstract

Glutamate is the predominant excitatory neurotransmitter in the human brain and it has been shown that prolonged activation of the glutamatergic system leads to nerve damage and cell death. Following release from the pre-synaptic neuron and synaptic transmission, glutamate is either taken up into the pre-synaptic neuron or neighbouring glia by transmembrane glutamate transporters. Excitatory amino acid transporter (EAAT) 1 and EAAT2 are Na^+-dependant glutamate transporters expressed predominantly in glia cells of the central nervous system. As the most abundant glutamate transporters, their primary role is to modulate levels of glutamatergic excitability and prevent spill over of glutamate beyond the synapse. This role is facilitated through the binding and transportation of glutamate into astrocytes and microglia. The function of EAAT1 and EAAT2 is heavily regulated at the levels of gene expression, post-transcriptional splicing, glycosylation states and cell-surface trafficking of the protein. Both glutamatergic dysfunction and glial dysfunction have been proposed to be involved in psychiatric disorder. This review will present an overview of the roles that EAAT1 and EAAT2 play in modulating glutamatergic activity in the human brain, and mount an argument that these two transporters could be involved in the aetiologies of schizophrenia and affective disorders as well as represent potential drug targets for novel therapies for those disorders.

Key words: Glia; Excitatory amino acid transporter;

Psychiatry; Affective disorders; Glutamate transporter; Glutamate; Schizophrenia

Core tip: Following release from the presynaptic neuron, the majority of glutamate within the human cortex is taken up into glia cells where it is converted into glutamine for recycling back into glutamate. Glutamate transporters excitatory amino acid transporter (EAAT) 1 and EAAT2 are predominantly localized in the glial plasma membrane, and are responsible for the majority of glutamate uptake within the human brain. Here we provide a comprehensive review of the unique regulation of EAAT1 and EAAT2 mRNA and protein in health and psychiatric disorder, and in response to medication use.

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GLUTAMATE AND EXCITOTOXICITY

Glutamate has long been recognized as the principal excitatory neurotransmitter of the mammalian brain[1]. It has been shown that glutamate concentration in the CNS extracellular space (approximately 0.6-2 μmol/L)[2-4] is comparatively lower than levels in cell cytoplasm (approximately 1-10 mmol/L)[5-7]. It is necessary for the level of extracellular glutamate to be kept low to allow for a high signal-to-noise ratio following the release of glutamate into the synapse and to prevent glutamate-mediated neuronal degeneration[8-10] as high levels of extracellular glutamate causes excitotoxicity and nerve damage[10]. The extracellular/intracellular glutamate gradient is at least partly maintained through the activity of Na^+-dependent excitatory amino acid transporters (EAATs) which are in the membrane of pre-synaptic neurons and glia. Whilst glutamate uptake into astrocytes is also mediated by Na^+-independent, chloride-dependent antiporters, this family of transporters appears to be responsible for less than 5% overall glutamate uptake[11]. Thus, this review will focus solely on the Na^+-dependent EAAT family.

THE EAAT FAMILY: OVERVIEW

The EAAT family of transporters consists of five Na^+-dependent high-affinity glutamate transporters termed EAAT1 [also known as solute carrier family 1 member 3 (SLC1A3)][12,13], EAAT2/SLC1A2[13,14], EAAT3/SLC1A1[13,15], EAAT4/SLC1A6[16] and EAAT5/SLC1A7[17]. In this review, the accepted nomenclature of "EAAT" will be used in discussing data from both humans and other mammalians. These subtypes are quite differentiated - EAAT1, EAAT2 and EAAT3 only share 51%-55% amino acid sequence homology[18].

THE EAAT FAMILY: FUNCTION

The functionality of the glutamate transporters reflects their coupling to the electrochemical potential gradients of Na^+, K^+ and H^+/OH^-. Specifically, glutamate is co-transported across the plasma membrane 1:2-3 with Na^+ and 1:1 H^+ (or counter-transport of OH^-) and counter-transported 1:1 with K^+[4,19,20]. This ionic association provides a net positive charge to glutamate transport[20] however a relatively slow turnover rate of approximately 70 ms makes it unlikely that this electrogenic attribute contributes significantly to the electrochemical gradient of the cell[21,22]. Furthermore, this slow turnover rate suggests that the transporters act first to buffer glutamate away from the synapse, and transport glutamate into glia at a slower rate. The quantity of charge transferred per molecule of glutamate is highly voltage dependant due to the existence of a thermodynamically uncoupled, transporter substrate-specific movement of chloride ions through the transporter[16,23]. The ion- and voltage- dependant uptake of glutamate makes this process highly susceptible to changes in the immediate cellular environment and plasma membrane potential.

THE EAAT FAMILY: LOCALISATION

Some of the functional properties of the EAATs can be attributed to their differential localisation. EAAT3 and EAAT5 are exclusively neuron-specific[17,24], with EAAT5 expression restricted to neurons and Müller cells of the retina[17]. By contrast, EAAT1 and EAAT2 are predominantly localised on astrocytes and are highly expressed in the cerebellum and hippocampus, respectively[25-27]. EAAT1, EAAT2 and EAAT3 have been reported to make up approximately 20%, 80% and 1% of all cell-surface glutamate transporters in the adult rat hippocampus, respectively[24]. EAAT4 is found in Purkinje neurons of the cerebellum[28].

THE EAAT FAMILY: ROLE IN GLIAL METABOLISM OF GLUTAMATE

Glial metabolism of glutamate is now recognised as a major factor in the control of glutamatergic neurotransmission[9], as, following the release of glutamate from the pre-synaptic neuron, the majority of the neurotransmitter diffuses out of the synaptic cleft where it is taken up into glial cells[29-31]. This effectively means that the astrocytic EAATs play a significant role in controlling the extent of glutamatergic activation by preventing neurotransmitter spill-over into neighbouring

synapses[20,22,32].

Once taken up by astrocytes, glutamate is either converted to glutamine by the glia-specific glutamine synthetase (GS)[33], or converted to α-ketoglutarate (α-KG) (also known as 2-oxoglutarate) through oxidation by glutamate dehydrogenase (GLDH) or transamination by aspartate aminotransferase[34,35]. α-KG may then be converted to succinate with a by-production of ATP (Figure 1) as part of the tricarboxylic acid cycle[34,35]. The formation of glutamine - the predominant metabolic pathway for glutamate - is followed by the transport of glutamine to neurons where it undergoes synthesis back into glutamate *via* the enzyme glutaminase[36] (Figure 1).

It is now acknowledged that glutamate excitotoxicity can potentially lead to problems in CNS functioning[8]. Significantly, it is now being speculated that glutamatergic excitotoxicity may in part be due to a reduction in glutamate uptake by astrocytic glutamate transporters and subsequent conversion of glutamate to glutamine by GS. Such an hypothesis is supported by research showing that inhibition or antisense oligonucleotide-knockdown of astrocytic glutamate transporters EAAT1 and EAAT2 results in excitotoxic oligodendrocyte death and nerve damage[37]. Moreover, whilst glutamate is usually taken into astrocytes under physiological conditions, it has been suggested that Ca^{2+}-dependent activation of select signalling molecules, intracellular increase in Na^{+} and glutamate, ATP depletion or cell swelling can lead to glutamate release through EAAT transporter reversal/reverse uptake[11,38]. Such an outcome represents another mechanism that can lead to glutamate excitotoxicity.

THE EAAT FAMILY: A FOCUS ON EAAT1 AND EAAT2

The focus of this review is the potential involvement of glutamate transporters in the aetiology of psychiatric disorders. At present, most evidence implicating glutamate transporters in the aetiologies of psychiatric disorders is from the study of EAAT1 and EAAT2. Hence the remainder of this review will focus on those two forms of glutamate transporters.

EAAT1

The *EAAT1* gene has been localised to human chromosome 5p11-12[39] and 5p13[40,41]. *EAAT1* is highly expressed in the glial cells of the cerebellar Purkinje cell layer[12,13,42] and generally is expressed at higher levels in astrocytes and oligodendrocytes compared to microglia[43].

The *EAAT1* gene encodes a 59 kDa protein that undergoes glycosylation to produce native 64 kDa and 70 kDa glycoproteins[44-46]. Post-translational modification of EAAT1 is developmentally regulated, with glycosylated EAAT1 increasing and non-glycosylated EAAT1 decreasing after birth[47]. Glycosylation does not affect EAAT1 transport activity, however research by Conradt *et al*[46] suggests that the addition of carbohydrates does impact EAAT1 homodimerisation. This is significant because it has been suggested that activation of astrocytes increases homodimerisation of EAAT1 and trafficking of the transporter to the plasma membrane[48]. In addition to homodimers, EAAT1 protein has shown potential to form homotrimers[49]. Moreover, it has been reported that cell-surface protein expression of EAAT1 is upregulated by exogenous glutamate[50-52], a process suggested to be mediated through transporter activity[51] and/or ionotropic glutamate receptor signalling[50,52]. EAAT1 protein levels have also been reported as being downregulated by the group Ⅰ metabotropic glutamate receptor (mGluR) agonist, DHPG, which selectively activates mGluR1 and 5, and upregulated by the group Ⅱ mGluR agonist, DCG-IV, which activates mGluR 2 and 3[53]. Further work is therefore required to fully elucidate which glutamate receptors are critical in controlling levels of EAAT1 at the cell surface.

At the level of protein localisation, high levels of EAAT1 protein can be observed on rat astrocytic membranes opposed to synaptically dense regions of the CNS and at higher levels on astrocytes facing capillaries, pia mater or stem dendrites[27]. Although predominantly a glial transporter, EAAT1 protein has also been detected in deep cerebellar rat neurons and transient protein expression of EAAT1 has been observed in cultured rat embryonic hippocampal neurons[26,54]. Rat neuronal EAAT1 protein expression appears to be restricted to perinuclear localizations, in particular the Golgi apparatus and associated vesicles[26]. Soluble factors secreted from astrocytes appear to induce the repression of neuronal protein expression of EAAT1[55], which supports the aforementioned lack of EAAT1 protein at the neuronal cell surface. It is possible that, at least in the adult brain, neuronal EAAT1 is redundant, and may be upregulated where there is an inadequate presence of EAAT1-expressing astroglia.

The *EAAT1* gene is expressed in its full length coding sequence of 10 exons[39] and as three splice variants which encode shorter protein isoforms known as EAAT1a, EAAT1b and EAAT1c.

EAAT1a lacks exon 3 - which ordinarily encodes the first and second transmembrane domain and the first extracellular loop[45] - and has been detected in rat brain and retina[45,56]. Significantly, it has been proposed that the splicing of exon 3 may reverse the orientation of EAAT1a within the membrane, and therefore the direction of glutamate transport[45]. EAAT1a has been shown to be predominantly expressed within internal vesicles, rather than on the cell membrane, in an osteocyte cell line[57]. Interestingly, an unglycosylated variant of EAAT1a protein has also been detected in rat brain[45]; as glycosylation has previously been associated with trafficking of EAAT1 to the cell surface, this may explain the localization of EAAT1a to internal vesicles.

mRNA for EAAT1b, which lacks exon 9[58], has been

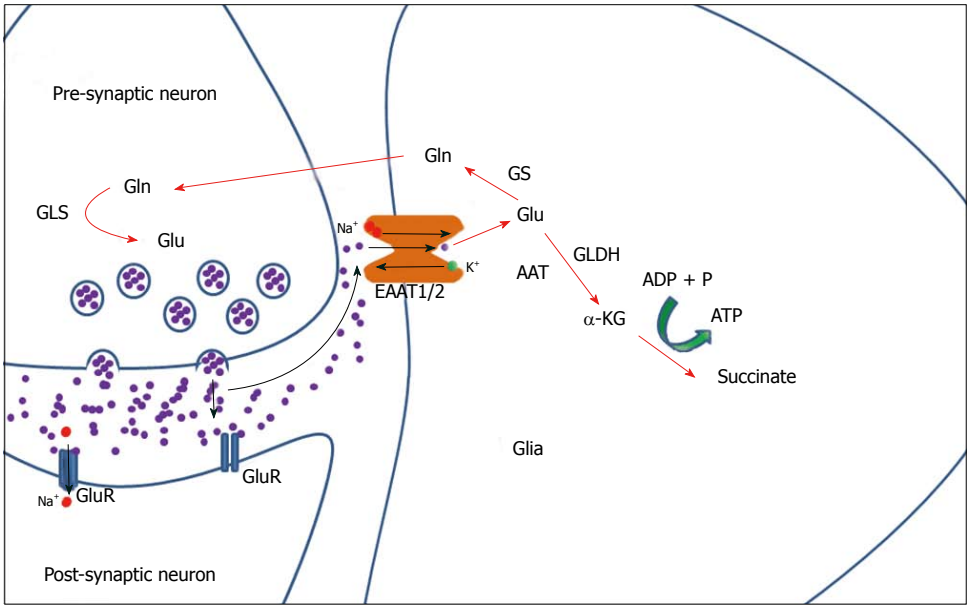

Figure 1 The metabolic fate of glutamate in glia cells. Glutamate is released from the pre-synaptic neuron to induce activation of the post-synaptic neuron *via* GluR. Spill-over of excess glutamate from the synaptic cleft is prevented through uptake by glial glutamate transporters EAAT1 and EAAT2, following which glutamate is converted to glutamine by GS for recycling back into glutamate, or α-KG for entrance into the tricarboxylic acid cycle. AAT: Aspartate Aminotransferase; α-KG: Alpha ketoglutarate; EAAT1/2: Excitatory amino acid transporter 1 and 2; GLDH: Glutamate dehydrogenase; Gln: Glutamine; GLS: Glutamate synthase; GS: Glutamine synthase; Glu: Glutamate; GluR: Glutamate receptors; Na+: Sodium; K+: Potassium.

detected in both human grey matter and axonal tracts at levels that are 10%-20% that of the full length EAAT1[58]. EAAT1b localization has been associated with the endoplasmic reticulum[58], intracellular inclusions and the plasma membrane[59], where it appears to have no functional glutamate uptake activity[58]. Rather, it has been suggested that EAAT1b negatively regulates levels of full-length EAAT1 at the cell surface by preventing the insertion of the latter into the plasma membrane[58], possibly by interacting directly with full length EAAT1. EAAT1b appears to be particularly localised to cortical neurons[59] where it is suggested to play a role in preventing ectopic neuronal expression of EAAT1. Immunoperoxidase-labelling of EAAT1b was associated with the plasma membrane for morphologically healthy neurons and in punctate intracellular inclusions of neurons that appeared degenerate[59]. Furthermore, EAAT1b expression was increased in neuronal populations - and to a lesser extent astrocytes - that were subject to hypoxia, in histologically degenerate brain regions which also displayed a downregulation of glial EAAT1[60].

EAAT1c mRNA, which lacks exon 5 and 6 of the full coding sequence, has been detected in astrocytes and oligodendrocytes of the human cortex, retina and optic nerve, as well as pig forebrain, midbrain, hindbrain and cerebellum[61]. The approximately 47 kDa glia-specific EAAT1c protein has been shown to have an intracellular perinuclear localization, with rapid redistribution to the cell surface following astrocytic stimulation[61]. Furthermore, EAAT1c does not undergo glycosylation[61]. Unfortunately the only study to examine EAAT1c was not able to determine its function, with the two most likely (default) candidates remaining as a regulator of full length EAAT1 cell surface expression, or as a *bona fide* transporter trafficked to the cell surface under appropriate conditions.

EAAT2

The EAAT2 gene is localized within human cytogenetic bands 11p12-13[62], and is responsible for the majority of glutamate uptake within the EAAT family (see[63] for review). EAAT2 expression appears to be restricted to the brain and placenta in humans[13] and has also been detected in cultured hippocampal neurons from rat embryo[64]. The mRNA expression of EAAT2 was found to be greater in cultured rat oligodendrocytes than astrocytes or microglia[43].

While EAAT2 protein expression has an overall homogenous brain distribution, it predominates across the forebrain with a particular focus in the hippocampus[26,65,66]. EAAT2 appears to be a predominantly glial glutamate transporter, with up to 80% of EAAT2 protein detected in glia plasma membrane, 6% localized to plasma membrane of pre-synaptic neurons and 8% to the axonal plasma membrane in the stratum radiatum of the rat hippocampal subregion CA1[67]. In line with the detection of EAAT2 mRNA[64], EAAT2 protein expression has similarly been recorded in cultured rat embryonic hippocampal neurons[54,64]. Like EAAT1, EAAT2 protein undergoes glycosylation, which produces a 5-15 kDa shift in molecular weight[48,68] and mediates its cell surface expression[68]. EAAT2 also exists in the plasma membrane as a multimer, with the potential to form dimers and trimers[49,69].

EAAT2 would seem to have a complex role in devel-

opment as it is transiently detected in neurons throughout ovine forebrain and cerebellum at 71 d gestation, and lost by 136 d gestation[70]. In addition, EAAT2 and glial fibrillary acidic protein (GFAP) have been reported to not be expressed by the same cells at 71 d ovine gestation but showed region-specific colocalization by 136 d gestation[70]. A similar transition from prenatal axonal pathways to astrocytic EAAT2 protein expression was also seen in rat[47]. Interestingly, it appears as if neuronal soluble factors may be required for proper expression of EAAT2 in neighbouring astrocytes[53,71] with EAAT2 protein levels quantitatively higher in the synaptically dense regions of glial cells[27].

The human *EAAT2* gene consists of 11 exons[72]. Two functional splice variants of EAAT2, termed EAAT2b and EAAT2c, contain unique C-terminal domains and have been detected in rat brain (EAAT2b) and retina (EAAT2c)[73,74]. Additional splice variants which share the C-terminals of EAAT2 and EAAT2b, but have a unique N-terminal domain, have also been detected in mouse and rat liver and referred to as mGLT-1A/rGLT-1A and mGLT-1B, respectively[75] (see[74] for a comparison of amino acid terminal sequences). While the unique N-terminals has been proposed to regulate tissue-specific expression, the function of the differing C-terminal of mGLT-1A and mGLT-1B is less clear - the authors propose that it may be related to the retention time of the transporter in the plasma membrane[75]. As mGLT-1A/rGLT-1A and mGLT-1B are localized to the liver[75], they will not be discussed further in this review. Rather, further evidence for the role of the C-terminus can be presented using data on rat EAAT2b, which will be discussed in the following paragraph.

Full length EAAT2 (commonly referred to as EAAT2a) expression is about 25-fold and 10-fold higher than EAAT2b in human and rat brain, respectively[76]. EAAT2b protein is similarly found in glia, localized close to or within the plasma membrane[77-79], however transcript and protein have also been detected in neurons[78]. While EAAT2 is constitutively trafficked to the cell surface membrane, localization of EAAT2b is mediated through its C-terminal, which is predicted to interact with the postsynaptic density-95/Discs large/zona occludens-1 (PDZ) domain-containing protein disks large homolog-1 (DLG1)[80]. The interaction between EAAT2b and DLG1 is itself regulated through AMPA-associated intracellular calcium levels, with exogenous glutamate resulting in dissociation of EAAT2b and DLG1 and subsequent internalization of EAAT2b[80]. EAAT2b also coimmunoprecipitates with the excitatory postsynaptic density scaffolding protein, PSD-95, as well as the ionotropic N-methyl-D-aspartate receptor (NMDAR), both found within the postsynaptic neuron[81]. Significantly, EAAT2 has also been detected in these protein complexes, through the indirect formation of a hetero-oligomer with EAAT2b[81]. This suggests that EAAT2b may assist in conditional neuronal cell-surface expression of EAAT2. EAAT2b represented 6% of total rat hippocampal EAAT2 variants at 8 wk of age, compared to EAAT2 at 90%, whereas the equally functional EAAT2c sits at just 1%[77].

EAAT2c is made up of exons 1-10 from the EAAT2 transcript plus a unique eleventh exon and C-terminus spliced from intron 10 - thereby losing the original eleventh exon from EAAT2 - similarly contains a PDZ-binding domain and is pre-synaptically expressed in the rat and human retina[74].

Aberrant EAAT2 splice variants which skip exons have also been discovered: In particular, EAAT2 exon7skipping and EAAT2 exon9skipping lack glutamate transport functionality and must form multimers with functional EAAT2 or EAAT2b (see[82] for review). These splice variants add another layer of complexity to what has been considered a predominantly astrocytic glutamate transporter.

EAAT1 vs EAAT2: A summary

Despite belonging to the same family of transporters, sharing 52% amino acid identity[18] and being localized within the same astrocytic plasma membrane[49], EAAT1 and EAAT2 display many differences in their functionality. EAAT1 protein levels have been shown to function approximately 6 times slower than EAAT2, and be expressed at a level approximately 6 times higher in the adult rat cerebellar molecular layer[21,65]. On the other hand, EAAT2 protein levels are upwards of 4 times higher than EAAT1 in the adult murine hippocampus[65]. Furthermore, unlike EAAT1, cell-surface protein expression of EAAT2 appears unaffected by exogenous glutamate levels but rather, is regulated by neuronal soluble factors[50-52]. Finally, within the EAAT family, only EAAT2 can be competitively inhibited by kainic acid (KA) and dihydrokainic acid (DHK)[13]. Conversely, KA has been shown to increase EAAT1 protein levels[50]. It is possible that this increase in EAAT1 protein in response to KA is a homeostatic mechanism, counterbalancing for the inhibition of EAAT2.

The fact that EAAT1 and EAAT2, and their functionally distinct splice variants, may be differentially regulated by internal and external factors presents us with two distinct transporters that are part of a system that is highly responsive to cellular physiology. In the following paragraphs, we will present the current knowledge surrounding EAAT1 and EAAT2 in psychiatric illness, as well as their responsiveness to medication and potential as drug targets (refer to Table 1 for a summary).

EAATS IN PSYCHIATRIC ILLNESS

Glial dysfunction has been implicated in a range of psychiatric illnesses, including major depressive disorders (MDD)[83,84], schizophrenia[85], bipolar disorders (BD)[86] and post-traumatic stress disorder (PTSD)[87]. Glia dysfunction has also been associated with suicide completion[88,89]. The association between the glutamatergic

Table 1 The role of EAAT 1 and 2 in psychiatric disorder and medication use

EAAT1	Genetic studies	
	BD	SNP rs2731880 T/T genotype associated with worse working memory and selective attention during a depressive episode[102]
		SNP rs2731880 T/T genotype increased negative fMRI BOLD coupling between the amygdala and AnCg[103]
	Scz	SNP rs2731880 T/T genotype associated with worse executive function, verbal fluency and verbal memory[104]
		No association between EAAT1 SNPs rs1428973, rs2033267, rs426040, rs4869684, rs1544795, rs3776585, rs962686, rs2303716, rs3776586, rs1049524, rs1529461 and Scz[112]
	mRNA studies	
	MDD	↓Lower levels in the DLPFC[83], AnCg[83], locus coeruleus[105] and hippocampus[106]
		↑Higher cortical levels in suicide completers with a MDD diagnosis compared to those without a diagnosis[118]
	Scz	↑Higher mRNA in the cerebellar vermis[113], AnCg[114], thalamus[115] and prefrontal cortex[116]
		→No change in the DLPFC or primary visual cortex[76,114]
		↓Lower levels in the prefrontal cortex of subjects who completed suicide compared to those who did not[117]
	Medication use	↑Haloperidol has been associated with an increase in EAAT1 mRNA in the thalamic medial dorsal nucleus[121]
		↑Chronic sodium valproate resulted in an upregulation of EAAT1 mRNA in chick cerebellar BGC culture[110]
	Protein studies	
	Scz	↓Decreased in the prefrontal cortex[114]
		↓N-glycosylation of EAAT1 monomer was decreased in the AnCg[114, 119]
	PTSD	↓Hippocampal EAAT1 protein was lower in a single prolonged stress (SPS) rat model of PTSD[108]
	Medication use	→Clozapine did not affect EAAT1 protein levels in rat[113,122]
		↑Chronic sodium valproate resulted in an upregulation of EAAT1 protein in rat hippocampus and chick cerebellar BGC culture[109,110]
EAAT2	Genetic studies	
	Scz	SNP rs4354668 G/G associated with poorer working memory performance[104,138] and a reduction in frontal grey matter[139]
	mRNA studies	
	MDD	↓Lower levels in DLPFC and AnCg[83]
		↑Higher levels in subjects who had completed suicide without a diagnosis of MDD compared to those with a diagnosis[118,127]
		↓Lower levels in the hippocampus, cerebral cortex and striatum of a rat model of depression[128, 129]
	Scz	↓Lower levels in the parahippocampal gyrus[140] and prefrontal cortex[141]
		↑Higher levels in the thalamus[115] and prefrontal cortex[142]
		→No change in EAAT2 or EAAT2b mRNA in the DLPFC or primary visual cortex[76]
	Medication use	↓Clozapine decreased levels in hippocampal CA1, parietal temporal, frontal and cingulate cortical[144], and striatal[145] brain regions of male Sprague-Dawley rats
		↓Haloperidol decreased frontal and cingulate cortical[144], as well as striatal[145], EAAT2 expression in rat
		↓Levels were higher in untreated subjects with Scz than in those prescribed typical or atypical antipsychotics[142]
		↓Increased levels caused by chronic stress were normalised by tianeptine treatment in rat[130]
		↓Increased hippocampal levels caused by stress were normalised by lithium administration in rat[137]
		↑Fluoxetine increased rat hippocampal and cortical levels[136]
		↑Tranylcypromine increased levels in rat amygdala[136]
	Protein studies	
	Scz	↓ N-glycosylation of EAAT2 multimer was lower in the DLPFC[119]
		↑ EAAT2b increased in extrasynaptic membrane/cytosol fractions from the DLPFC[143]
	PTSD	↓Hippocampal EAAT2 protein was lower in the single prolonged stress (SPS) rat model of PTSD[108]
	Medication use	↓Clozapine decreased protein levels in astrocyte culture[147]
		↓Clozapine reduced protein levels in the cerebral cortex of adult rats[146]
		↓Increased levels caused by chronic stress were normalised by tianeptine treatment in rat[130]
		→Increases in EAAT2b protein caused by chronic stress were unaffected by tianeptine treatment in rat[130]
		↑Chronic sodium valproate increased hippocampal EAAT2 protein in rat[109]

All research refers to human studies unless explicitly stated otherwise. References numbered as they are in the Reference section. ↑: Increase; ↓: Decrease; →: No change; AnCg: Anterior cingulate cortex; BGC: Bergmann glia cell; DLPFC: Dorsolateral prefrontal cortex; BD: Bipolar disorders; BOLD: Blood-oxygen dependent contrast imaging; fMRI: Functional magnetic resonance imaging; MDD: Major depressive disorders; PTSD: Post-traumatic stress disorder; Scz: Schizophrenia.

neurotransmitter system and psychiatric illness is not new[90,91]; originally based on the observation that phenylcyclidine (PCP), and later ketamine - both NMDA receptor antagonists - could induce schizophrenia-like positive and negative symptoms, as well as cognitive impairment[92-94]. While glutamatergic dysfunction is also hypothesized to be involved in other neurodevelopmental disorders such as autism[95] and attention deficit hyperactivity disorder[96,97], neurodegenerative disorders such as dementia[98,99], substance abuse/addiction[100] and chronic pain[101] (the latter two referenced reviews are written with a focus on EAATs), these topics are outside the scope of the current review.

EAAT1 in psychiatric illness

The rs2731880 (C/T) single nucleotide polymorphism (SNP) of *EAAT1* has been associated with deficits in working memory and selective attention in patients with

Type 1 bipolar disorder during a depressive episode, with T/T homozygotes displaying significantly worse performance[102]. Furthermore, bipolar disorder patients with the rs2731880 T/T genotype have displayed an overall negative correlation between amygdala and subgenual anterior cingulate cortex (AnCg) functional magnetic resonance imaging (fMRI) blood-oxygen-level dependent (BOLD) contrast imaging during a task which involved the processing of emotional or neutral faces, whereas in carriers of the C allele the coupling was absent[103]. SNP rs2731880 is a putative functional polymorphism within the promoter region of *EAAT1*, with the T/T genotype proposed to be associated with lower expression[104]. In support of the hypothesis that lower EAAT1 expression is associated with affective disorders, lower levels of EAAT1 mRNA have been reported in the human dorsolateral prefrontal cortex (DLPFC)[83], AnCg[83], locus coeruleus[105] and hippocampus[106] from subjects with MDD. Interestingly, Group II mGluR receptors - the agonists of which have been shown to upregulate EAAT1 protein levels[53] - are also decreased in MDD[107].

Furthermore, Feng *et al*[108] detected an increase in CSF glutamate levels and decrease in hippocampal EAAT1 protein levels in the single prolonged stress (SPS) rat model for PTSD. Interestingly, administration of fibroblast growth factor 2 (FGF2) alleviated the SPS-induced PTSD-like behaviour, promoted glutamate uptake and increased EAAT1 protein expression, thereby suggesting that astrocyte activation (and EAAT1 upregulation) may be advantageous in the treatment of PTSD[108].

Short-term sodium valproate treatment augmented EAAT1 translocation to the cell membrane, whereas prolonged or chronic sodium valproate treatment resulted in an upregulation of EAAT1 mRNA and protein levels, as well as glutamate transport and production of glutamine[109,110]. The ability of sodium valproate treatment to increase EAAT1 mRNA and protein levels, which are downregulated in affective disorders, contributes EAAT1 dynamics to an understanding of the medication's effectiveness[111]. This is not surprising, given that sodium valproate is used to treat both epilepsy - a disorder of excitotoxicity - and bipolar disorders.

Carriers of the rs2731880 SNP T/T genotype with a diagnosis of schizophrenia performed worse in tests of executive function, verbal fluency and verbal memory than the C carrier group[104]. This association has overlap with cognitive performance of subjects with bipolar disorder I[102]. Furthermore, Deng *et al*[112] analysed 11 *EAAT1* SNPS - exclusive of rs2731880 - in a Japanese population and found no association between *EAAT1* genotype and schizophrenia. These genotypic association studies suggest that while there may exist a relationship between *EAAT1* genotype and cognition, particularly within the context of psychiatric disorder, it is not a susceptibility locus specific to either schizophrenia or bipolar disorders.

Levels of EAAT1 mRNA have been reported as higher in the cerebellar vermis[113], AnCg[114], thalamus[115] and prefrontal cortex[116] of subjects with schizophrenia. In comparison, other studies have found no changes in EAAT1 expression in the DLPFC[76,114] or primary visual cortex[76] of subjects with schizophrenia. However, lower levels of EAAT1 mRNA were found in the prefrontal cortices of subjects with schizophrenia who completed suicide relative to those who did not[117], a confounding factor that many studies have not taken into consideration. Conversely, it has also been reported that EAAT1 mRNA levels were higher in the cortex of suicide completers without a prior diagnosis of MDD but not those with the diagnosis[118]. These data suggest complex expression x diagnoses x suicide factors that need to be considered when contemplating the role of EAAT1 in psychiatric disorders. Finally, monomeric EAAT1 protein expression was decreased in the DLPFC of elderly subjects with schizophrenia[114], while N-glycosylation of EAAT1 protein monomer was decreased in the AnCg[119]. Animal models have shown that *EAAT1* knock-out mice displayed locomotor hyperactivity in response to a novel environment which was exacerbated by NMDAR antagonists – two phenotypes considered to be relevant models for the positive symptoms of schizophrenia[120].

Interestingly, the locomotor hyperactivity in *EAAT1* knock-out mice could be normalised by treatment with haloperidol or the mGluR 2/3 agonist LY379268[120]. As EAAT1 protein levels have previously been shown to increase with administration of the mGluR 2/3 agonist DCG-IV[53], the data in *EAAT1* knockout mice suggests that activation of mGluR 2/3 may impact on a function downstream of glial glutamate uptake. Haloperidol has similarly been associated with an increase in EAAT1 RNA in the thalamic medial dorsal nucleus in subjects with schizophrenia[121]. On the other hand, administration of clozapine did not appear to affect EAAT1 protein levels in treated Sprague-Dawley rats[113,122], possibly due to the fact that clozapine, but not haloperidol, increases NMDAR-mediated neurotransmission through synaptobrevin-associated glial release of glutamate and D-serine[123]. If clozapine administration induces glutamate release from glia, it seems unlikely that glia would simultaneously increase a means to re-uptake glutamate. In conclusion, it appears that while the actions of haloperidol indirectly affect EAAT1 expression, clozapine seems to act through an alternative mechanism that is unrelated to EAAT1.

In summary, current data suggest an overall decrease in EAAT1 mRNA in affective disorders, while an increase in EAAT1 mRNA and decrease in EAAT1 protein is associated with schizophrenia; results which are further complicated by suicide completion and medication use. These factors must be taken into consideration when studying EAAT1, and the glutamatergic system as a whole, in terms of treatment for psychiatric illness.

EAAT2 in psychiatric illness

EAAT2 translation may be regulated by a large range

of molecules, including the stress-related glucocorticoids[124-126], creating a putative link between EAAT2 protein levels and stress-induced biological responses. To date, one study has recorded a lower levels of EAAT2 mRNA in the DLPFC and AnCg of subjects with MDD[83]. Interestingly, as with EAAT1, levels of EAAT2 mRNA were higher in the cortex of subjects who had completed suicide without a history of MDD, but not in those with a prior diagnosis[118,127]. EAAT2 mRNA was also lower in the hippocampus and cerebral cortex of learned helplessness rats – an established animal model of depression[128] and in the hippocampus, striatum, and frontal cortex of prenatally, restraint- stressed juvenile rats displaying increased behavioural despair[129].

Hippocampal EAAT2 protein levels were also observed to be lower in a SPS rat model of PTSD, which, like EAAT1 protein levels, could be alleviated by treatment with FGF2[108]. Interestingly, a rat model of chronic stress produced upregulated EAAT2 mRNA and protein levels in the hippocampus[130], suggesting that EAAT2 regulation may respond differently to the type, duration and severity of stress stimuli. Finally, amygdala specific DHK-inhibition of EAAT2 activity in rat resulted in reduced social interaction – a behavioural phenotype that could be blocked by the NMDA receptor antagonist, AP5[131].

Cerebrospinal fluid glutamate levels have been reported as higher in patients with obsessive compulsive disorder (OCD)[132,133]. To date however, an association between OCD and the glutamate transporters has only been proposed for the neuronal EAAT3, which is significantly less involved in glutamate uptake when compared to EAAT1 and EAAT2[134]. That said, astrocyte-specific inducible knockout of EAAT2 in adolescent - but not prenatal or adult - mice has been shown to result in glutamatergic hyperexcitability-related pathological repetitive self-grooming and tic-like head shakes[135]. Interestingly, these mice did not present with increased anxiety or social impairments[135].

It seems that the lower EAAT2 expression associated with depression and learned helplessness can be rescued by mood stabilizers. Chronic sodium valproate treatment increased EAAT2 protein levels in the rat hippocampus, but not other brain regions[109]. The antidepressant fluoxetine (class: SSRI) also produced rat hippocampal and cortical increases in EAAT2 expression, while tranylcypromine (class: monoamine oxidase inhibitor) resulted in an amygdala-specific increase[136]. In contrast to a depressive state, increases in EAAT2 mRNA and protein caused by chronic stress could be normalised by the antidepressant tianeptine in rat[130]. Interestingly, EAAT2b protein, but not mRNA, was also increased by chronic stress, however remained unaffected by tianeptine treatment[130]. A similar stress-induced increase in hippocampal EAAT2 mRNA expression was countered by food-based administration of lithium in rat[137]. This increase in EAAT2 expression in response to chronic stress lies in stark contrast to the previously mentioned decreases in EAAT2 expression associated with depression and learned helplessness, and suggests that EAAT2 is highly responsive to, or correlated with, different mood states.

EAAT2 SNP rs4354668 (T/G), located in the gene promoter region and associated with lower transport activity, has been correlated to cognitive dysfunction in schizophrenia, with the lower activity G allele linked to poorer working memory performance[104,138] and a reduction in frontal grey matter[139]. EAAT2 mRNA levels have been reported as lower in the parahippocampal gyrus -but not other hippocampal regions[140] and prefrontal cortex[141], and higher in the thalamus of subjects with schizophrenia[115]. In contrast, Matute *et al*[142] found an increase in EAAT2 expression in the prefrontal cortex. Finally, Lauriat *et al*[76] found no change in EAAT2 or EAAT2b mRNA in the DLPFC or primary visual cortex of subjects with schizophrenia, however the authors acknowledge the potential masking effect of antipsychotics on their results. N-glycosylation of the EAAT2 multimer was reduced in the DLPFC from subjects with schizophrenia[119], which may be associated with ER retention and reduced trafficking of EAAT2 to the plasma membrane[68]. The splice variant EAAT2b was increased in extra-synaptic membrane/cytosol post-mortem fractions from the DLPFC of subjects with schizophrenia[143]. As EAAT2b cell-surface expression is internalised in response to increases in intracellular calcium[80], it is possible that the elevated cytosolic localization of EAAT2b is a countermeasure to excitotoxicity.

Clozapine treatment has been reported to decrease EAAT2 expression in hippocampal CA1, parietal temporal, frontal and cingulate cortical[144], and striatal[145] brain regions of male Sprague-Dawley rats. EAAT2 protein levels and glutamate uptake were similarly reduced in the cerebral cortex of clozapine-treated adult rats with an accompanying increase in extracellular glutamate[146]. Clozapine also induced a decrease in EAAT2 protein in astrocyte culture, which was accompanied by a reduction in glutamate uptake[147]. This response to clozapine, which contrasts with the lack of effect that clozapine had on EAAT1 expression (discussed earlier in this review), suggests once again that the two EAAT subtypes are intrinsically different. Haloperidol similarly decreased frontal and cingulate cortical[144], as well as striatal[145], EAAT2 expression in rat. Matute and colleagues have provided support to the argument that antipsychotic drug treatment can affect EAAT2 expression by showing the higher levels of EAAT2 mRNA in the prefrontal cortex of untreated subjects with schizophrenia were not detectable in those with the disorder who had received typical or atypical antipsychotics[142].

CONCLUSION

Given the differential expression of EAAT1 and EAAT2 throughout brain development[47] and their importance in normal brain development[148], it is not surprisingly

that abnormal levels of these glutamate transporters have been found in the pathophysiology of psychiatric illness. It is imperative, however, that the subtype splice variants and glycosylation states be taken into consideration when researching the EAATs, as their unique attributes make them just as susceptible to disorder. The ability to analysis EAAT dynamics in a pre-mortem setting will assist in understanding the cause for their dysregulation and through that, the glutamatergic role in psychiatric disorder. Such information will allow for the prescription of medication with an understanding of how it may, or may not, affect the glutamatergic system. This review concludes with the contention that the EAAT family is dynamically regulated by a range of internal and external factors and offer a viable means to region-specific, subtype-specific therapeutic targets with the potential to respond to the immediate environment. However, a better understanding of the dynamic regulation of EAATs within the convoluted context of psychiatric disorder will be advantageous in advancing drug discovery.

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REVIEW

Melatonin in drug addiction and addiction management: Exploring an evolving multidimensional relationship

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Abstract

Melatonin is a pleiotropic signalling molecule that regulates several physiological functions, and synchronises biological rhythms. Recent evidences are beginning to reveal that a dysregulation of endogenous melatonin rhythm or action may play a larger role in the aetiology and behavioural expression of drug addiction, than was previously considered. Also, the findings from a number of animal studies suggest that exogenous melatonin supplementation and therapeutic manipulation of melatonin/melatonin receptor interactions may be beneficial in the management of behavioural manifestations of drug addiction. However, repeated exogenous melatonin administration may cause a disruption of its endogenous rhythm and be associated with potential drawbacks that might limit its usefulness. In this review, we examine the roles of melatonin and its receptors in addictive behaviours; discussing how our understanding of melatonin's modulatory effects on the brain rewards system and crucial neurotransmitters such as dopamine has evolved over the years. Possible indications(s) for melatonergic agents in addiction management, and how manipulations of the endogenous melatonin system may be of benefit are also discussed. Finally, the potential impediments to application of melatonin in the management of addictive behaviours are considered.

Key words: Dopamine; Drug dependence; Biological rhythms; Neuroplasticity; Brain reward

Core tip: Melatonin is a pleiotropic signalling molecule that regulates several physiological functions, and synchronises biological rhythms. Recent evidences are beginning to reveal that a dysregulation of endogenous melatonin rhythm or action may play a larger role in the aetiology and behavioural expression of drug addiction, than was previously considered. This review, using inf-

ormation garnered from extant literature, examines the roles played by melatonin and its receptors in addictive behaviours, addiction related changes in brain chemistry and brain plasticity; and its possible benefits in the management of drug associated withdrawal syndrome, relapse and behavioural sensitisation.

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INTRODUCTION

Drug addiction or substance use disorder has been defined as a chronic disease of the brain which is characterised by uncontrollable and compulsive drug-seeking and use; and which is associated with the development of a negative emotional state in the absence of drug access[1,2]. There have been suggestions that drug addiction is both a social and a medical problem dating as far back as recorded human history[3] and which continues to be a cause for global health concern[4]. Reports suggest that an estimated total of 246 million people, or approximately 1 in 20 people aged between 15 and 64 years were exposed to illicit drug in 2013; with surveys showing that approximately 1 in 10 of these have a drug-addiction problem[5]. Substance use disorder is arguably a serious public health issue, with a significant economic and health burden on affected individuals and their families[5]. There is also a significant societal burden measured in lost productivity, lawlessness, crime and increased health-care costs. Substance-use disorders have also been associated with worsening of co-morbid psychiatric and/or medical illness, risky behaviours and increasing mortality. While the global and economic burden of addiction continues to increase worldwide, current psychopharmacological therapies are falling short of the desired goals of therapy[6,7].

Over the past few centuries, several theories (social, biological or psychological) have been proposed to aid in understanding the aetiology of drug addiction[8]. Also, while the distinct aetiological bases for drug addiction are yet unclear, advances in neuroscience have continued to aid our understanding of the possible mechanisms that underlie the alterations in emotional balance and decision-making ability that occur with drug addiction[9]. Genetic, environmental, neurodevelopmental and sociocultural factors have been listed as important contributors to the development of drug addiction[10]. These factors have also been shown to increase the susceptibility of an individual to initiation or sustenance of drug use; and potentiate the development of structural brain changes that perpetuate drug use and are characteristic of drug addiction[9,11,12].

Presently, there is a growing body of evidence associating disruptions in circadian rhythms and circadian genes with the development and progression of drug addiction[13,14]. Studies in human subjects have demonstrated circadian rhythm disruptions in individuals with addiction, with suggestions that environmental and/or genetic alteration of the normal sleep wake cycle increases vulnerability to drug use[13,15]. Studies in rodents have also demonstrated that diurnal variations in the behavioural responses to different addiction paradigms exist[16-18]. In rodents, an increase in cocaine self-administration, and the intake of drugs of abuse have been observed at night[16,17,19,20]. There have also been suggestions that the continued craving for drugs of abuse is potentiated through the entrainment of the circadian clock[16,21,22].

Melatonin is a neurohormone that is important in the entrainment of circadian rhythms, as well as in the modulation of behaviour and physiological functioning in all mammals[23]. Some studies have observed a reduction in melatonin levels, and a delay in attaining its nocturnal peak concentration in alcohol-dependent humans and rodents[23]. Studies have also demonstrated melatonin's ability to modulate the reinforcing effects of a number of drugs of abuse with suggestions that it may play a crucial role in drug addiction[24]. In this review, we examine the roles of melatonin and its receptors in drug addiction, by discussing how our understanding of melatonin's modulatory effects on the brain reward system and crucial neurotransmitters such as dopamine has evolved over the years. Possible indications(s) for melatonergic agents in addiction management, and how manipulations of the endogenous melatonin system may be of benefit are also discussed. Finally, the potential impediments to application of melatonergic agents in the management of addictive behaviours are considered.

Neurobiological and neurochemical basis of drug addiction

Substance dependence can be described as a disorder which involves the motivational systems of the brain[25]. Repeated exposure to drugs of abuse has been linked to the development of long-lasting alterations in brain structure and neuronal circuitry. In the last decade or more, studies have demonstrated that repeated use of addictive drugs can alter the neural circuitries that are involved in reward/ motivation, learning/memory, affect, stress response and decision-making[26]. These regions which include the ventral tegmental area (VTA), nucleus accumbens (NAc) and amygdala form a part of the mesolimbic dopaminergic system and are important in reward-related processes[27]. Adaptations in cortical regions, including the prefrontal cortex, orbitofrontal cortex and the anterior cingulate gyrus, which form the mesocortical pathway, have also been implicated in addiction[28]. Increase in dopamine release in the mesolimbic or mesocortical brain regions have been sug-

gested to occur in parallel, appearing to mediate different phases or aspects of drug addiction. The mesolimbic regions (amygdala and hippocampus) have been linked to mediating conditioned learning in addiction; while the prefrontal cortex, orbitofrontal cortex and the anterior cingulate gyrus mediate executive control and emotional response to drugs[26].

Chronic drug use has also been associated with alterations in the "anti-reward" pathway which include the hypothalamic-pituitary-adrenal axis[29,30]. Adaptations in stress response, involving levels of corticosterone cortisol releasing factor and the adrenocorticotrophic hormone have also been reported to occur with drug addiction[31,32].

Brain neurotransmitter/neuromodulator changes also occupy a central role in the establishment, management and extinction (or otherwise) of addictive behaviours. In the brain, the neurochemical targets for a number of drugs of addiction have been identified[33]. Also, while the pharmacological profiles of the drugs of addiction are diverse, drug-receptor interactions can largely explain the wide range of physiological and behavioural changes that occur with drug use[26]. Also, there have been reports that suggest that despite the diversity of behavioural responses, drugs of addiction may share a common reward neural circuitry. Studies have shown that most of the addictive drugs appear to activate the reward system, directly or indirectly stimulating dopamine release[34,35].

Research has shown that dopaminergic (DA) neurons that project from the VTA to the NAc play a crucial role in the processing of stimuli associated with substance-related reward[36]. As a part of their pharmacological effects, substances with abuse potentials stimulate the brain reward system by increasing DA release from the NAc[37]. Also, there are reports that drugs of abuse induce their initial reinforcing effect by stimulating supraphysiologic levels of DA in the NAc. These DA surges (acting *via* D1 receptors) activate the striatal pathway (direct), while inhibiting the striato-cortical pathway (indirect) through D2 receptors[10]. Repeated drug use has also been associated with triggering neuroplastic changes that involve the glutamatergic inputs to the striatum and midbrain DA neurons, these alterations enhance the ways the brain reacts to drug cues, weakening self-regulation, reducing sensitivity to non-drug rewards and increasing sensitivity to stress[10].

There is also ample scientific evidence to suggest that there are DA-independent reinforcement pathways in the acute rewarding or pleasurable effects of addictive drugs. A number of studies in animals have shown that alcohol, opioids, nicotine and amphetamines may produce reinforcing effects *via* DA-independent mechanisms[38-40].

The involvement of some other neurochemicals and neuromodulators such as opioids, gamma-aminobutyric acid, glutamate, noradrenaline, cannabinoids and serotonin in drug addiction have also been suggested[41]. Reports from brain imaging studies have demonstrated an increase in opioid receptors density in persons experiencing withdrawal from alcohol[42], opioids[43] and cocaine[44]. Studies have also shown that the corticostriatal glutamate pathway may be important in the initiation and/or expression of a number of addictive behaviours; examples include conditioned place preference, drug seeking behaviour and locomotor sensitisation[45]. The overall conclusion is that although a number of neurotransmitters and neuromodulators are involved in the short-term reinforcing effects of addictive drugs; the dopaminergic reward pathway is central to the reinforcing properties of drugs and the initiation of the cycle of addiction. However, other mediators are believed to exert their influence *via* dopamine modulation[26,41].

The roles played by neuropeptides in addiction-related behaviours have also been examined; and for the most part, neuropeptides including signalling molecules like substance P, endogenous opioids, and neuropeptide Y have been studied extensively as possible therapeutic targets for addiction management[26].

Drug addiction and circadian rhythm/gene abnormalities

There is ample scientific evidence to suggest the importance of chronological events like the biological rhythms in determining response to drugs of abuse. Earlier studies have argued that chronobiological variables including time of day, sleep-wake patterns and light-dark cycles may modulate the development and maintenance of drug addiction[46]. More recent evidences derived from animal models suggest and support the existence of strong links between appetitive processes and various circadian genes[47]. Also, while details of the exact mechanisms are still being studied; it is becoming more obvious that a strong relationship exists between disturbance of circadian rhythms (as a result of factors like alteration of normal light-dark cycle) and the development of addiction[48].

It also appears that circadian phase-shifting activities such as repeated travels across time zones may influence the pattern of consumption of certain substances with addictive potentials; and this has also been demonstrated in experimental animals[49]. Using male Sprague-Dawley rats, Doyle *et al*[49] studied the effects of experimentally-induced chronic jet lag on methamphetamine consumption; and concluded that pre-exposure to methamphetamine (*via* 2 wk of forced consumption through drinking water) was associated with a significantly higher consumption of methamphetamine in phase-shifted rats (four consecutive 6-h advancing phase shifts of the light-dark cycle) during the second week following abstinence, when compared to those with undisturbed rhythms[49].

Earlier studies in humans had observed that drug-seeking behaviours are probably linked to mutations in certain key genes that are related to circadian rhythm maintenance; suggesting a link between abnormalities of circadian rhythm maintenance and addiction[47]. However, the associations between these genetic alterations and addiction have also been demonstrated in animals by using specific experimental paradigms. In male

Wistar rats, a month of constant light exposure exerts a significant effect on voluntary consumption of morphine, exhibition of withdrawal symptoms, plasma concentration of melatonin [evaluated by enzyme-linked immunosorbent assay (ELISA)] and the mRNA expression of period homolog genes (Per1, Per2) and dopamine (D1) receptors in the striatum and prefrontal cortex[48]. One month exposure to constant light caused a significant decrease in melatonin concentration, an upregulation of mRNA levels of Per2 and D1 receptor in the striatum and prefrontal cortex, up-regulation of Per1 gene in the striatum of rats under constant light (in comparison to those under standard light cycle), increased morphine consumption and preference ratio, and also a significant increase in severity of naloxone-induced withdrawal syndrome[48]. In humans, more studies are beginning to demonstrate that core genes that are involved in circadian rhythm maintenance are also important regulators of reward-related behaviours which occur in response to common substances of abuse[50]. On the other hand, substance use has been known to cause disruptions in circadian rhythms and affect functions such as the sleep/wake cycle; hence, the relationship that exists between substance abuse/addiction and circadian rhythm abnormalities is bidirectional, such that one could lead to the other, and vice versa. Also, abnormalities of sleep and circadian rhythms appear intimately linked to substance abuse, and they could appear as either predictors or consequences of substance abuse[47]. Documented effects of substance use on sleep is not only dependent on the class of agents, but also on the phase of usage, with acute sleep effects, chronic sleep effects, and sleep effects due to withdrawal or abstinence being described. Acute ingestion of drugs such as cocaine and amphetamine which have stimulant effects have been associated with a light, restless and disrupted sleep[51]; while ingestion of drugs with depressant effects such as benzodiazepines, alcohol and opiates can have an initial sleep-promoting effect (increased daytime sleepiness and reduced sleep latency) but sleep disruptions (increased night awakenings) later in the night, as a result of acute withdrawal effects[51,52].

There are reports that chronic use of substances may alter sleep quality and quantity in ways that are similar across different substances[53]. Extended sleep onset latency (SOL), a reduction in total sleep time (TST), increased frequency of night-time awakenings, reduced slow-wave sleep (SWS) and rapid eye movement (REM) sleep have all been described[47]. However, withdrawal from alcohol or stimulants may be associated with distinct time-related changes in pattern of TST and REM sleep[51,54]. Acute withdrawal from substance use may also be associated with sleep disturbances such as extended SOL, reduced TST, and reduced SWS[51]. Also, sleep disturbances such as REM sleep disturbances may continue weeks into abstinence; and polysomnographic evidences in cocaine-dependent participants still show increased SOL, and decreased TST, SWS, and REM sleep[55]. In heavy marijuana users, polysomnography had shown that over two weeks of abstinence, increases in wake time after sleep onset (WASO) and decreases in TST, sleep efficiency and REM sleep had been observed[56]. A persistence of sleep disturbances is believed to be a risk factor for relapse[55,56]. Overall, the relationship between circadian rhythm abnormalities/sleep disorders and addiction/substance abuse appears to be a complex one; with one pair increasing the predisposition to the other pair, and vice-versa.

MELATONIN

Melatonin is an endogenously-produced indolamine that is predominantly secreted by the pineal gland, and widely recognised as a regulator of several physiological functions. Melatonin production is controlled by the photoperiod through the suprachiasmatic nucleus (SCN), with production peaking at night and being at its lowest in daytime. In mammals, melatonin is a master synchroniser of biological rhythms, a regulator of physiological processes such as cardiac function; and an important modulator of behaviours, body posture and balance[57-59]. Fluctuations in melatonin levels (in a 24 h period) tune the body's cellular activities to the actual time-of-day; and while high levels of melatonin potentiate behaviours and physiological functions associated with darkness, low levels attenuate such behaviours and functions[23].

In biological systems, melatonin's effects are exerted *via* interactions with melatonin receptors (MT_1 and MT_2), orphan nuclear receptors, and intracellular proteins like calmodulin[60-62]. As an ampiphillic molecule, melatonin is capable of autocrine, paracrine and endocrine signalling; and it permeates several body compartments to exert effects on a variety of functions such as diurnal/seasonal rhythms, reproduction, neurobehaviour, antioxidant defense and general immunity.

Over the years, exogenous melatonin and melatonin analogues have been known to have an established role in the management of a range of sleep disorders. However, melatonin's therapeutic application is not limited to the central nervous system; and research has continued to shed light on the potential use of melatonergic drugs for the management of an increasing number of disorders/diseases including respiratory ailments such as asthma, pneumonias, chronic obstructive airway diseases, pleural cavity diseases, vascular pulmonary disease, and even lung cancer[63] Melatonin administration had also been shown to be protective against intestinal ischaemic-reperfusion injury in young male Sprague-Dawley rats[64] and Wistar albino rats[65].

From the foregoing, it is obvious that due to its unique chemical characteristics and diverse effects, melatonin may be useful in the management of several human diseases/disorders including those of the central nervous system such as drug addiction. Therefore, a better understanding of melatonin's role in addiction might open a new door in addiction management.

Melatonin and drug addiction

The roles played by circadian rhythm/gene abnormalities in the development or entrainment of addiction–related behaviours, or in potentiating changes in neurohormone, neuromodulator or neurotransmitter levels which result in the development of addiction are well-documented[24]. Melatonin's role in the entrainment of circadian rhythms is also well-documented. Observations that alcohol consumption altered the circadian profile of melatonin production in alcohol-dependent humans and alcohol drinking rodents[66,67] have also increased interests in the importance of melatonin in addiction[23].

Studies by Uz *et al*[68], and Kurtuncu *et al*[69] demonstrated loss of diurnal variation in cocaine-induced locomotor sensitisation and cocaine-induced place-preference respectively, in melatonin-deficient pinealectomised mice, suggesting that the cocaine-induced diurnal variations were mediated by melatonin[68,69]. There have also been reports suggesting that (drug-induced) hypothermic responses to injections of morphine, nicotine or ethanol varied with the light-dark cycle[70]. There have been suggestions that disturbances in sleep observed after months of abstinence in humans with alcohol-dependence could be linked to delayed peak of melatonin's nocturnal rise and lower melatonin levels[68,71]. Studies in rodents have also demonstrated similar alterations[67], further buttressing the role of the melatonergic system in drug addiction.

The effects of exogenous melatonin in modulating behavioural responses to specific drugs of abuse have also been studied. Vengeliene *et al*[23] demonstrated that administration of melatonin modulates alcohol-seeking or wanting and/or relapse-like drinking behaviours[23]. Results of *in vitro* electrophysiological studies have also shown that in cerebellar neurons, nicotine-stimulated currents decreased with application of increasing concentrations of melatonin[72]. Markus *et al*[73] also reported nocturnal elevations of melatonin-mediated nicotine-induced glutamate release by cerebellar neurons[73]. Finally, studies have demonstrated that melatonin is able to modulate the reinforcing or relapsing effects of certain drugs of abuse[23,24].

Melatonin receptors and drug addiction

Melatonin exerts its effects on behaviours and physiological functions largely *via* the melatonin (MT) receptors 1 and 2[74,75]. Also, while research has continued to demonstrate the possible roles that melatonin may play in drug addiction, including modulation of the development of dopaminergic behaviours, like drug-seeking behaviours or psychostimulant-induced diurnal locomotor sensitisation; the contributions of melatonin receptors, especially as it relates to specific drugs, are still being evaluated[23]. Research has demonstrated the presence of the MT1 receptor subtype in a number of brain regions, including areas like the prefrontal cortex, hippocampus, nucleus accumbens and amygdala which have been associated with regulating the effects of addictive drugs or behaviours[76,77]. Uz *et al*[76] studied the expression pattern of MT1 receptors in the dopaminergic system of the human and rodent brain, and observed the presence of MT1 receptor in these regions of the post-mortem human brain; while in the mouse brain, they observed a diurnal variation (high protein levels and low mRNA at night) in the expression of the mouse MT1 receptor in the dopaminergic system[76]. A few studies have also observed an increase in melatonin receptor-related cyclic AMP in the mesolimbic dopaminergic system[62]. In another study, prolonged treatment with antidepressants and cocaine was associated with alteration in the content of melatonin receptor mRNA, with the effects of these drugs on MT1/MT2 mRNAs being brain region-specific[78]; however, prolonged cocaine use did not alter MT2 receptor expression[78,79]. There have been reports suggesting that genetic deletion of MT1 and MT2 receptors abolished the development and expression of methamphetamine-induced locomotor sensitisation[79], and methamphetamine-induced reward[80] in melatonin-expressing C3H/HeN mice. Uz *et al*[68] however reported that MT1, and not MT2 receptor was required for cocaine-induced locomotor sensitisation in rodents. In another study by Hutchinson *et al*[81], this time comparing the differences in locomotor sensitisation observed following a single dose of methamphetamine in low melatonin-expressing C57BL/6 wild-type and MT1 knockout mice, to melatonin-proficient C3H/HeN mice; it was reported that methamphetamine pre-treatment induced locomotor sensitisation during the light period in C3H and C57 wild-type mice. A diminution in magnitude of sensitisation in C57 mice in the dark period, and a complete abrogation in the MT1 receptor knockout (MT1KO) mice was observed; buttressing the role of MT1 receptors in the possible management of drug addiction[81]. On the other hand, MT2 receptors have been linked to the modulation of hippocampal-dependent long-term potentiation; with a few studies demonstrating loss of long-term potentiation in transgenic mice deficient of MT2 receptors[82]. There were also reports of loss of experience–dependent short term latency to enter the closed arm on the second day of elevated plus maze exposure; a feature which suggests that MT2 receptors may play an important role in modulating memory processes and hippocampal synaptic plasticity[82]. These properties may prove useful in the management of addiction-related neuroplasticity.

MELATONIN AND THE PHARMACOLOGIC MANAGEMENT OF DRUG ADDICTION

Information garnered from years of research into the aetiopathogenesis of addiction point to the conclusion that drug-dependence is a multifactorial behavioural and biological disorder, which is amenable to medical treatment. The current treatment protocol for drug

use disorders involves the use of psychosocial and pharmacological interventions[5]. The main goals of management include: (1) reduction of drug use and drug craving; (2) improvement of general wellbeing and functioning of the individual; and (3) decreasing the risk of the development of complications and/or recurrence[5]. However, currently-available treatment options remain inadequate, with varying addiction relapse rates, depending on the drugs involved[83,84]. Thankfully, advances in science and research are opening new vistas for possible therapeutic interventions, and as such, current research interests are directed at developing or discovering new treatments options like the use of melatonin (a regulator of the circadian rhythm and potent antioxidant) that could be beneficial in reducing craving/withdrawal period and preventing relapse.

The ability of melatonin to mitigate different aspects of addiction neurobiology has been examined extensively. Studies have reported the efficacy of melatonin supplementation in the control of drug-seeking behaviour, opiate withdrawal/ relapse[24], behavioural sensitisation[84,85], regulation of the sleep and or circadian rhythm disorders[86], neuroplasticity, and prevention of: Mitochondrial-induced autophagy, apoptosis, oxidative stress and neurotoxic injury[84] in brain areas linked to reward and emotionality.

Melatonin, withdrawal syndrome and relapse

Prolonged use or abuse of drugs (such as opioids) by humans have been linked to the development of physical dependence and/or addiction, which is usually associated with alterations in brain biochemistry and hormone levels; and disruption of the sleep/wake cycle[87-89]. Also, sudden clearance or reduction in the plasma concentration of opioids of abuse results in withdrawal symptoms, including circadian rhythm disturbances like insomnia, jitteriness and restlessness[90,91]. Studies in animals have reported that chronic morphine administration resulted in a reduction in total activity within a 24 h period, and a dampening of the circadian amplitude in locomotor activity rhythm[92,93]. Abrupt withdrawal of morphine administration in rats has also been associated with sustained disruption of the circadian rhythms in locomotor activity, and alterations in plasma melatonin, β-endorphin, corticosterone, adenocorticothrophic hormone, and orexin concentrations[93-95]. Studies have also reported evidence of anxiety-related behaviour following cocaine withdrawal[96].

The possible effects of melatonin on withdrawal symptoms have also been examined; and while there is a dearth of clinical trials, studies in rodents have demonstrated its effectiveness. Zhdanova and Giorgetti[96] assessed the effects of melatonin supplementation on cocaine-induced anxiety-like behaviour and nucleus accumbens cyclic adenine monophosphate (AMP) levels in rats. In their study, melatonin (200 ng/mL) was administered in drinking water (at night) to groups of rats that had been exposed to repeated cocaine administration (15 mg/kg *i.p.*), or during its withdrawal. Results showed that melatonin caused a reduction in anxiety-like behaviour in a defensive withdrawal paradigm, 48 h after the last injection of cocaine[96]. Melatonin pretreatment also attenuated the augmentation of cAMP levels in the nucleus accumbens following acute administration of cocaine. These results suggest that a low-dose night-time melatonin treatment was effective in militating against symptoms of cocaine-withdrawal in rats[96]. Bondi *et al*[97] conducted a single-centre, randomised, double-blind, placebo-controlled, parallel-group trial to assess the effect of melatonin (5 mg) compared to placebo as adjuvant treatment (alongside behavioural and pharmacotherapy) on weekly self-reported severity of depression, anxiety, stress, and insomnia complaints in recovering substance use disorder subjects males (aged 18 years or older) who were at a residential program. Results showed no significant differences were observed for baseline characteristics; although the frequency of reported adverse events was higher in the melatonin group[97]. The authors were of the opinion although the diversity of medication regimens and behavioural interventions provided increase the complexity of assessing melatonin's efficacy with regards to the measured outcome, there is insufficient evidence to demonstrate melatonin's benefits as an adjuvant in addiction recovery[97].

The use of melatonin for its antioxidant effects during recovery from drug abuse has also been studied. The naloxone-induced heroine withdrawal syndrome has been associated with derangement in antioxidant enzymes and bio-elements which are essential for the maintenance of life[98]. Cemek *et al*[98] examined the effect of melatonin supplementation on the levels of antioxidant enzymes and bio elements in naloxone induced heroine withdrawal syndrome and reported a reversal in heroine withdrawal related alteration in glutathione, catalase levels, and the levels of bio elements (iron, manganese, magnesium, aluminium, calcium and copper). The researchers concluded that exogenous melatonin could be effective in militating bio element and antioxidant enzyme derangements in heroine withdrawal syndrome[98].

A very powerful challenge to drug addiction treatment is the high incidence of drug- use relapse during abstinence[99]. However, years of extensive clinical and preclinical research on drug-use relapse[100,101] have done little to reduce relapse rates[83,102]. Reports from a number of studies have reported that drug-use relapse is usually triggered by acute exposure to the self-administered drug[103], stress[102], the presence of drug-related cues and contexts[104], and protracted periods of withdrawal or exposure to cure that have been previously associated with withdrawal[105]. Extensive research work has led to the identification of possible cellular, neurotransmitter, and/or receptor mediated mechanisms that increase the risk of relapse to drug-use with the intent of identifying novel pharmacological treatment options[106-108]. Takahashi *et al*[24] assessed

the effects of melatonin supplementation (administered at either 25 or 50 mg/kg body weight) on cocaine self-administration and relapse-like behaviours in male Sprague-Dawley rats (which had been exposed to long-term cocaine self-administration training). Behavioural parameters measured included the motivation for cocaine self-administration in the break point test, relapse-like behaviour in the cue-induced reinstatement test, sucrose preference and distance travelled in the open field. Results showed a reduction in the cocaine-seeking behaviour and the desire to self-administer cocaine. The researchers concluded that melatonin supplementation could be beneficial in reducing relapse[24].

Melatonin, addiction-related behavioural sensitisation, neuroplasticity and neurotoxicity

Chronic intermittent use of cocaine and a number of other psychostimulants have been associated with the development of a progressive, long-lasting enhancement of psychomotor effects which have been referred to as cocaine or psychostimulant sensitisation. Studies have demonstrated that behavioural sensitisation to psychostimulants is associated with an increase in nitric oxide synthase[109]. While examining the effect of melatonin on cocaine-induced behavioural sensitisation in rats, Sircar[85] reported that: (1) acute or repeated melatonin injections on its own did not affect locomotor behaviour in rats; (2) acute melatonin pre-exposure augmented the acute locomotor effects of cocaine; and (3) repeated melatonin pre-exposure prevented the development of cocaine induced behavioural sensitisation, while a single injection of melatonin did not halt behavioural sensitisation in rats already sensitised to cocaine. Sircar[85] concluded that while melatonin supplementation increased cocaine's acute behavioural effects and prevented the development of cocaine's behavioural sensitisation, it had no effect in militating fully-developed cocaine behavioural sensitisation[85]. Itzhak *et al*[110] studied the effects of melatonin supplementation on the development of methamphetamine (METH)-induced behavioural sensitisation and reported that pre-treatment with melatonin at 10 mg/kg body weight prevented the development of METH-induced depletion of dopamine and/or its metabolites and depletion of dopamine transporter binding sites. It also attenuated METH-induced behaviours and diminished METH-induced hyperthermia, although it did not reverse fully-developed METH–induced behavioural sensitisation[110]. Feng *et al*[84] also examined the effects of melatonin on morphine-induced behavioural sensitisation and reported that pre-treatment with melatonin prevented the development of morphine-induced behavioural sensitisation and analgesic tolerance; effects which were dose-dependent[84].

The development of long-lasting addiction-related behavioural dysfunction and structural deficits in the brain have been linked to alterations in the methylation processes for purine metabolism/serotonin pathways[111], oxidative stress-induced autophagy[84], mitochondrial mediated apoptosis[112,113], alteration in mitochondrial DNA copy number in distinct brain regions[81], and neurotoxicity[84,114]. Li *et al*[95] also reported that protracted opiate withdrawal in rats was associated with the disruption of the circadian rhythm of hormones (adrenocorticotropin, orexin and corticosterone), leading to the induction of neurobiological changes which may worsen the risk of relapse[95].

Feng *et al*[84] examined the ability of melatonin to militate against the deleterious effects of opiate addiction and reported that melatonin was able to reverse morphine induced mitochondrial dysfunction and oxidative stress, in cultured cells. They also demonstrated that melatonin reversed morphine-induced autophagy and changes in mitochondrial DNA copy number in cultured cells and neurons[84]. *In vivo* studies using a mouse model of morphine addiction demonstrated that melatonin also counteracted morphine–induced autophagic effects and decrease in mitochondrial DNA copy number in the hippocampus[84].

Melatonin in the management of drug-addiction related sleep and circadian rhythm disorders

Sleep and circadian rhythm disorders have been well-defined in a number of substances use disorders, including those of marijuana[115], alcohol[52,116,117], nicotine[118], benzodiazepines[86,119] and cocaine[51]. Also, results from a number of rodent studies have reported interactions between alcohol and homeostatic mechanisms[120] and/or circadian systems[121-123]. Treatment options for insomnias in drug addiction are limited, largely because traditional hypnotics that target benzodiazepine receptors are associated with abuse potential, withdrawal effects, and the potential for overdose. Melatonin supplement has been found particularly valuable in the management of circadian rhythm disorders[124], in the treatment of insomnias in subjects with chronic schizophrenia[125], in the elderly[126,127], and among children with sleep onset insomnia[128]. However, its benefits in addiction-related sleep and/or circadian rhythm disorders are still being evaluated. A double-blind cross-over control study that examined melatonin's ability in militating sleep difficulties associated with benzodiazepine (BDZ) withdrawal reported that while melatonin did not increase the likelihood of BDZ discontinuation, it improved sleep quality, especially in subjects who continued to use BDZ[86].

Its use in alcohol addicts have been supported by studies that have reported low plasma melatonin levels in this group of substance users[66,129]. Other studies have examined the efficacy of melatonin analogs in militating addiction-related sleep disorders. Brower *et al*[116] examined the ability of the melatonin receptor agonist ramelteon to attenuate insomnia in recovering alcoholics, and reported an improvement in sleep quality and quantity. Another study using ag-

omelatine (a melatonergic agonist at MT1 and MT2 receptors, and a 5-HT2C antagonist approved for use as an antidepressant) reported improved sleep in alcohol-dependent subjects with insomnia; with participants reporting improved subjective sleep quality after 6 wk of administration[130].

CONCLUSION

To date, melatonin and its analogs have continued to show promise in the management of drug addiction. However, the use of melatonin may be limited by its short half-life and an additive sedative effect when used alongside BDZs and other drugs such as morphine; also, its safety in the younger age groups are still being debated. Despite these, evidences from both animal and human studies continue to show the potentials of melatonin and its analogs in the management of drug addiction. Therefore, research must continue to focus on the applications of melatonergic agents in drug addiction management, especially, beyond their established use for associated sleep disorders.

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