



REVIEW ARTICLE

Connexin 43: insights into candidate pathological mechanisms of depression and its implications in antidepressant therapy

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Major depressive disorder (MDD), a chronic and recurrent disease characterized by anhedonia, pessimism or even suicidal thought, remains a major chronic mental concern worldwide. Connexin 43 (Cx43) is the most abundant connexin expressed in astrocytes and forms the gap junction channels (GJCs) between astrocytes, the most abundant and functional glial cells in the brain. Astrocytes regulate neurons' synaptic strength and function by expressing receptors and regulating various neurotransmitters. Astrocyte dysfunction causes synaptic abnormalities, which are related to various mood disorders, e.g., depression. Increasing evidence suggests a crucial role of Cx43 in the pathogenesis of depression. Depression down-regulates Cx43 expression in humans and rats, and dysfunction of Cx43 also induces depressive behaviors in rats and mice. Recently Cx43 has received considerable critical attention and is highly implicated in the onset of depression. However, the pathological mechanisms of depression-like behavior associated with Cx43 still remain ambiguous. In this review we summarize the recent progress regarding the underlying mechanisms of Cx43 in the etiology of depression-like behaviors including gliotransmission, metabolic disorders, and neuroinflammation. We also discuss the effects of antidepressants (monoamine antidepressants and ketamine) on Cx43. The clarity of the candidate pathological mechanisms of depression-like behaviors associated with Cx43 and its potential pharmacological roles for antidepressants will benefit the exploration of a novel antidepressant target.

Keywords: depression; Connexin 43; astrocyte; prefrontal cortex; hippocampus; Ca²⁺ wave; ATP; antidepressant target

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INTRODUCTION

Major depression disorder (MDD), one of the most severe mental disorders troubling over 350 million people worldwide, is also the main reason for disability [1]. Besides, MDD imposes a considerable economic cost and brings a significant burden to society [2]. The current consensus view is that cellular and molecular abnormalities caused by genetic and environmental interactions contribute a lot to depression [3]. It is traditionally believed that depression is related to the monoaminergic neurotransmitter system in the brain [3]. However, the pathological phenomena of depression are complicated, and the monoaminergic hypothesis has many limitations, which cannot fully explain these phenomena. Thorough cognition of depression pathophysiology and pathogenesis is still lacking. Thus, it is essential to consider more pharmacological mechanisms of MDD to explore new therapeutic targets. The GJC is currently a hot research content, and it may also be a potential target for antidepressant therapy.

Gap junction dysfunction in the prefrontal cortex (PFC) can induce depressive-like behaviors [4]. GJCs are formed by connexins [5]. Connexin is synthesized by the endoplasmic reticulum (ER) and transported to the membrane surface [6]. The six penetrating gap junction proteins constitute homomeric or heteromeric connexons [7]. A pair of connexons on the corresponding surfaces of adjacent cell membranes form a GJC which allows small molecules whose molecular weight is <1 kDa and diameter is <1.5 nm. These small

molecules include ions, metabolic molecules, and second messengers to pass through mediating information exchange between cells [8]. Uncoupled connexon acts as a hemichannel (HC) to promote the chemical connection between the intracellular and extracellular spaces [7]. GJCs act as a critical role in regulating nerve cell growth, differentiation, and physiological functions by participating in the metabolic coupling of material exchange between cells, the electrical coupling of electrical signal transmission, and the transmission of information between cells [9]. Under physiological conditions, HCs on the cell membrane keep closed. Only under special circumstances can the HCs be activated, including stress and acute injury, causing some molecules to enter and exit the cell through the channel [10]. A short-term opening is beneficial to increase the adaptability of cells, but long-term activation may cause damage to the cell, which further induces many diseases, e.g., depression [11]. There are two families of gap junction proteins in mammals: connexins (21 members in humans) [12] and pannexins (three members) [13]. However, several pieces of evidence indicates that HCs formed by pannexins cannot be assembled into GJCs [14]. Thus, this article only discusses connexin. Connexin is widely expressed in all tissues except differentiated skeletal muscle, circulating erythrocytes, and mature sperm cells [15]. More than half of the connexin is expressed in the nervous system and 1/3 in the central nervous system (CNS), mainly in the glial. Cx43 is the main subtype of connexin of astrocytes [7, 16],

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and other subtypes including Cx30 [17], Cx26, Cx45, Cx40, and Cx46 [18] are also slightly expressed. Astrocyte dysfunction is an important pathological feature and pathogenesis of depression, of which Cx43 dysfunction plays an important role [4]. Inactivation of the Cx43 gene in astrocytes increased the acute antidepressant effect of fluoxetine [19]. Notably, Cx43 is widely distributed [20]. The abnormal function of Cx43 in other tissues and cells such as liver tissue [21] and microglia [22] also contributes directly or indirectly to the induction of depression-like behaviors, which will be elaborated in this article. In short words, the relationship between Cx43 dysfunction and depression-like behaviors is far more complicated than expected. Therefore, in the present review, the authors summarize the latest views on the role of Cx43 in depression-like behaviors.

CX43 ABNORMALITIES AND DYSFUNCTION IN DEPRESSION

At the earliest, postmortem studies found that Cx43 was down-regulated in the locus coeruleus [23], frontal cortex [24], mediodorsal thalamic nucleus [25], and caudate nucleus [26] in patients with MDD compared to healthy individuals. It suggests that Cx43 expression in the above areas may act a vital role in the pathophysiology of depression. Later, studies on Cx43 expression and function changes were carried out (Table 1). Cx43 gene levels are reduced in the orbitofrontal cortex [26] and neocortex [25] in patients with depression. However, there was no difference in the DNA methylation of the Cx43 gene in the PFC between patients with clinically well-defined depressed patients and healthy people. It suggests that the contribution of Cx43 to depression may be derived from downstream protein levels and functions. This section will discuss Cx43 abnormalities of several related brain areas in the depression model *in vivo* and *in vitro*.

Prefrontal cortex (PFC)

Rats subjected to chronic unpredictable stress (CUS) showed a significant decrease in Cx43 protein [4] and mRNA expression [27], accompanied by GJC dysfunction in the PFC [4]. Loss of Cx43 delayed the growth rate of astrocytes [28]. The gap between the two neighboring astrocytes was around 1.5-fold wider than the control group [4]. CUS suppressed gap junction permeability and decreased gap junction density [4]. In another study, the Cx43 content of the orbitofrontal cortex of rats subjected to CUS decreased, and the myelin basic protein area fraction was positively correlated with the density of Cx43-positive puncta in the orbitofrontal cortex, suggesting that the change of Cx43 may be related to the myelin morphology disorder in depression [29]. Furthermore, rats subjected to CUS have higher levels of endogenous corticosterone (CORT) [30]. Rats treated with chronic CORT administration consistently showed decreased Cx43 protein and GJC dysfunction in the PFC [31]. The observations suggested that the increase of endogenous CORT caused by CUS might cause reduced Cx43 expression and dysfunction of Cx43 [32]. Rats exposed to chronic restraint stress also showed decreased Cx43 protein and GJC dysfunction in the PFC [33]. Another study found that Cx43 expression decreases in internal prefrontal cortex (mPFC) astrocytes of chronic social defeated stress (CSDS) mice was associated with neuronal activity decreases [34]. Both lipopolysaccharide (LPS) and CORT have been proposed as an inducer of depressive-like context [35]. In rat cultured astrocytes, chronic administration of exogenous CORT reduced Cx43 expression via enhancing the degradation and suppressing the synthesis of Cx43 [36, 37]. The passways of degradation affected include the ubiquitin-proteasomal and autophagy-lysosomal pathways of Cx43 [38]. CORT damaged GJC function by reducing the distribution of Cx43 and enhancing the phosphorylation of Cx43 at Ser368 [37]. LPS activated Cx43 HCs in primary cultured cortical astrocytes of the mouse but had no effects on Cx43 protein [39].

Hippocampus

CUS reduced the expression of Cx43 and the ratio of Cx43/glia fibrillary acidic protein (GFAP) in rat hippocampal CA1 area and impaired the function of GJCs in the rats. However, there are no differences in GFAP expression. It suggests that CUS treatment influenced the expression of Cx43 in astrocytes [40]. In addition, the expression of Cx43 was significantly decreased in the hippocampus of CSDS mice and was strongly associated with decreases in neuronal activity [34]. Acute restraint-induced activation of Cx43 HCs in astrocytes of the mouse was further enhanced by chronic restraint stress [11]. Interestingly, the enhancement of neuronal HC activities caused by chronic restraint stress was inhibited by Cx43 HC blockers, e.g., Gap26, Gap27, and Cx43E₂. It indicated that Cx43 HCs was involved the enhancement of neuronal HC activities. However, neurons were confirmed to express Panx1 HCs [41] and Cx36 HCs [42], but not Cx43 HCs. The author speculated that Cx43 HC activities of astrocytes were a prerequisite condition of enhancing neuronal HC activities induced by chronic stress [11]. It suggests that the enhancement of Cx43 HC opening in astrocytes caused by acute stress is an adaptation to stress in the short term. In contrast, the enhancement of neuronal HC activities induced by long-term stress can damage the crucial functions of brain physiology [43] involved in the mechanism of depression-like behavior. However, the level of Cx43 protein did not change under both acute and chronic resistant stress [11]. It can be speculated that dysfunction of Cx43 in the depression model is more important than changes of Cx43 protein levels. Consistently, CORT exposure increased the level of phosphorylated Cx43 at Ser368 in mice hippocampus [44], but not affect total Cx43 expression [37, 44].

In summary, reduced Cx43 protein levels and dysfunction, including GJC dysfunction and HC activation, were shown in the PFC and hippocampus in various models of depression.

CX43 AFFECTS BEHAVIORS IN DEPRESSION

Prefrontal cortex (PFC)

Mitterauer et al. considered that decrease of connexins was related to the pathogenesis of depression firstly [45]. After that, various drugs that could regulate the function of GJCs and HCs were used to study the role of connexins in depression-like behavior [46, 47]. Cx43 overexpression in the mPFC increased neuronal activity and improved depressive-like behaviors of CSDS mice, while Cx43 suppression in normal mice reduced neuronal activity and induced depressive-like behaviors [34]. Furthermore, infusion of the non-selective GJC inhibitor carbenoxolone (CBX) into the PFC of healthy rats induced anhedonia in the sucrose preference test [4]. Cx43 specific GJC inhibitor Gap26 and Gap27 also displayed similar effects reversed by fluoxetine [4]. Reduced Cx43 protein levels and GJC dysfunction caused by CBX infusion in the PFC could be reversed by classic antidepressant fluoxetine [33]. Notably, CBX also inhibits HCs [48]. Gap26 and Gap27 specifically block Cx43 GJCs and HCs [49, 50]. It suggested that inhibiting the expression and function of Cx43 in the PFC contributed to the pathological mechanism of depression-like behaviors.

Hippocampus

Overexpression of Cx43 in the hippocampus astrocytes increased neuronal activity and inhibited depressive-like behaviors of CSDS mice, while suppression of Cx43 in normal mice was sufficient to reduce neuronal activity and induced depressive-like behaviors [34]. Bilateral infusion of gap junction blocker CBX into hippocampal CA1 area of healthy rats induces depression-like behavior [40]. This may be caused by GJC dysfunction in astrocytes. It was found that the Cx43 content in astrocytes increased [40], which may be a compensatory increase. Bilateral

Table 1. Abnormalities of Cx43 in experimental models.

Experimental objects	Type of cells	Species	In vivo or in vitro	Models	Dose and duration	Effect on Cx43 expression	Effect on the ratio of Cx43 to GFAP	Effect on Cx43 phosphorylation	Research method	Effect on Cx43 function	Ref
Hippocampus	Astrocytes	Mouse	In vivo	Acute restraint stress	2 h × 1 time	No effect (protein)	-	-	Acute slices, Dye (Etd) uptake experiment	Augmentation (HCs)	[11]
Prefrontal cortex	Unknown	Rat	In vivo	Chronic restraint stress	2 h × 10 times	No effect (protein)	-	-	Dye (LY) transfer, Electron microscopy	Further augmentation (HCs) Inhibition (GJCs)	[4, 27, 29]
Hippocampus	Unknown	Rat	In vivo	CUS	28 days	Decrease (mRNA and protein)	Decrease	-	Dye (LY) transfer	Inhibition (GJCs)	[40]
Hippocampus	Unknown	Rat	In vivo	CBX	1, 10, 100 mM, PLC (bilateral)	Decrease (protein)	Decrease	-	Dye (LY) transfer	Inhibition (GJCs)	[40]
Hippocampus	Unknown	Mouse	In vivo	CORT (in water)	45 days	Decrease (protein)	Decrease	-	Dye (LY) transfer, Electron microscopy	Inhibition (GJCs)	[44]
Cortical astrocytes	Astrocytes	Rat	In vitro	CBX	100 mM, Hippocampal CA1 (bilateral)	Increase (protein)	Increase	-	Dye (LY) transfer	-	[36]
Cortical astrocytes	Astrocytes	Rat	In vitro	CORT	5 mg · kg ⁻¹ · d ⁻¹ , 28 days	No effect (protein)	-	Increase	-	-	[44]
Hippocampal astrocytes	Astrocytes	Rat	In vitro	CORT	5–50 μM, 16 days	Decrease (protein)	Decrease	-	-	-	[36]
Hippocampal astrocytes	Astrocytes	Rat	In vitro	CORT	50 μM, 24 h	Decrease (protein)	-	Increase (Ser368)	Dye (LY) transfer	Inhibition (GJCs)	[37]
Hippocampal astrocytes	Astrocytes	Rat	In vitro	CORT	50 μM, 24 h	Decrease (protein)	-	-	-	-	[37]

CBX carbenoxolone, CORT corticosterone, CUS chronic unpredictable stress, Etd ethidium bromide, GJCs gap junction channels, GFAP glial fibrillary acidic protein, HCs hemichannels, LY lucifer yellow, PLC prefrontal cortex, Ref reference.

Table 2. Summary of the effects of antidepressants for classes: TCA, SSRI, SNRI, NRI, and NMDAR antagonist.

Classes	Anti-depressants	Experimental objects	Type of cells	Species	In vivo or in vitro	Model	Treatment (dose and duration)	Effect on Cx43 expression	Effect on Cx43 function	Possible mechanisms of antidepressant effect	Ref
TCA	Amitriptyline	Cortical astrocyte	Astrocyte	Rat	In vitro	NO	25 μM, 24 h	Increase (mRNA, protein)	Increase (GJCs)	Activation of p38 MAPK and the subsequent increase in AP-1 activity by translocation of c-Fos from the cytosol to the nucleus	[62]
	Clomipramine	Cortical astrocyte	Astrocyte	Rat	In vitro	NO	10 μM, 48 h	Increase (protein)	-	-	-
	Amitriptyline	Cortical astrocyte	Astrocyte	Mouse	In vitro	NO	20 μM, 24 h	No effect	Inhibition (GJCs)	Control Cx43 HC activity through the production of TNF-α and/or IL-1β as the result of a reduction of microglial activation	[39]
	Imipramine	Cortical astrocyte	Astrocyte	Mouse	In vitro	NO	20 μM, 24 h	No effect	Mild inhibition (HCs) No effect (GJCs)	-	-
	Fluoxetine	Cortical astrocyte	Astrocyte	Mouse	In vitro	NO	10 μM, 24 h	No effect	Mild inhibition (HCs) Inhibition (GJCs) Total inhibition (HCs)	-	-
SSRI	Fluoxetine	PFC	Unknown	Rat	In vivo	NO	20 mg/kg, 21 days	Increase (protein)	-	The expression of GFAP was slightly reduced	[61]
	Fluoxetine	PFC	Unknown	Rat	In vivo	NO	10 mg/kg, 21 days	Increase (protein)	No effect (GJCs)	-	[4]
	-	-	-	-	-	CUS	-	Increase (mRNA, protein)	Augmentation (GJCs)	-	-
	Fluoxetine	HIP	Unknown	Mouse	In vivo	CORT (water)	18 mg/kg, 28 days	Decrease (p-Cx43)	-	The degree of Cx43 phosphorylation may be an important process to regulate the selectivity of neurotransmitters released by HCs	[44]
	Fluoxetine	Astrocytoma cell line	Astrocytoma cell line	Human	In vitro	NO	10 μg/mL, 20 μg/mL, 24 h	Increase (mRNA, protein)	-	Partial effect of cAMP in total effect of fluoxetine on Cx43 expression	[58]
SNRI	Fluvoxamine	Cortical astrocyte	Astrocyte	Rat	In vitro	NO	25 μM, 48 h	Increase (protein)	-	-	[62]
	Paroxetine	Cortical astrocyte	Astrocyte	Mouse	In vitro	NO	5 μM, 24 h	No effect	Increase (GJCs)	The antidepressant inhibitory effects on HCs are acting downstream to the microglial step and/or more directly on the Cx43 HC function	[39]
	Duloxetine	Cortical astrocyte	Astrocyte	Mouse	In vitro	NO	5 μM, 24 h	No effect	Total inhibition (HCs) Mild augmentation (GJCs) No effect (GJCs)	-	-
	Duloxetine	PFC	Unknown	Rat	In vivo	NO	10 mg/kg, 21 days	Increase (mRNA, protein)	Total inhibition (HCs) No effect (GJCs)	-	[4]
	Duloxetine	Cortical astrocyte	Unknown	Rat	In vivo	CUS	25 μM, 48 h	No effect (protein)	Augmentation (GJCs)	-	[62]
NRI	Milnacipran	Cortical astrocyte	Astrocyte	Rat	In vitro	NO	25 μM, 48 h	No effect (protein)	-	-	[62]
	Venlafaxine	Cortical astrocyte	Astrocyte	Mouse	In vitro	NO	5 μM, 24 h	No effect	Inhibition (GJCs)	Control Cx43 HC activity through the production of TNF-α and/or IL-1β as the result of a reduction of microglial activation	[39]
	Reboxetine	Cortical astrocyte	Astrocyte	Mouse	In vitro	NO	10 μM, 24 h	No effect	Mild inhibition (HCs) No effect (GJCs) Mild inhibition (HCs)	-	-

Table 2. continued

Classes	Anti-depressants	Experimental objects	Type of cells	Species	In vivo or in vitro	Model	Treatment (dose and duration)	Effect on Cx43 expression	Effect on Cx43 function	Possible mechanisms of antidepressant effect	Ref
NMDAR antagonist	Ketamine	Cortical astrocyte	Astrocyte	Mouse	In vitro	NO	300 μ M, 30 min	-	Inhibition	Act directly on NMDAR and GABA _A R receptors in astrocytes	[73]
						LPS, 200 ng/mL	20 μ M, 30 min	-	-	Inhibition	
						TNF- α , IL-1 β , 20 ng/mL	50 μ M, 30 min	-	-	Inhibition	

AP-1 activator protein-1, *cAMP* cyclic adenosine monophosphate, *CORT* corticosterone, *CUS* chronic unpredictable stress, *GFAP* glial fibrillary acidic protein, *GABA_AR* γ -aminobutyric acid receptor, *GJCs* gap junction channels, *HCs* hemichannels, *HIP* hippocampus, *IL-1 β* interleukin-1 β , *LPS* lipopolysaccharide, *NMDAR* N-methyl-D-aspartic acid receptor, *NRI* selective noradrenaline reuptake inhibitor, *p38* MAPK p-38 mitogen-activated protein kinase, *p-Cx43* phosphorylated connexin 43, *PFC* prefrontal cortex, *Ref* reference, *SNRI* serotonin noradrenaline reuptake inhibitor, *SSRI* selective serotonin reuptake inhibitor, *TCA* tricyclic, *TNF- α* tumor necrosis factor- α .

infusion of CBX into the ventral hippocampus (vHIP) decreased anxiety-like behavior in the elevated plus maze test and the open field test (OFT) [51]. It may be due to the electrical signaling, which generates synchronized activities between vHIP and mPFC, drives anxiety-like behaviors [51], and there is a unidirectional ipsilateral nerve projection from the CA1 area of the hippocampus to mPFC in rat brain [52]. In fact, unilateral injection of CBX into vHIP combined with contralateral injection into mPFC produced similar anxiolytic effects [51]. However, no change in anxiety behaviors were observed with CBX in single unilateral vHIP [51]. Moreover, the dorsal hippocampus (dHIP) is more related to the memory function of the hippocampus [53]. Consistently, infusion of CBX into bilateral dHIP did not induce changes in anxiety-like behavior [51]. CBX is a non-selective blocker that can block the gap junctions of both neurons and astrocytes [54]. It influenced electrical signaling due to disturbing the theta rhythm in the vHIP and mPFC [51]. Although Cx43 is not expressed on neurons, studies have found that inhibiting Cx43 in astrocytes reduced the activities of neurons [34], and Cx43 GJCs could regulate the synaptic plasticity of neurons. However, mice with constitutive deficiency of Cx43 in hippocampal astrocytes showed less despair behaviors in the TST and more exploratory behaviors in the OFT [44]. These animals lack Cx43 throughout development, leaving open the possibility of long-term compensatory mechanisms [51]. Furthermore, Cx43 levels decreased in the hypothalamus of these mice, so the incompletely consistent function of Cx43 in different brain regions may be an important factor [44].

Generally, the evidence we have collected suggests that Cx43 dysfunction of PFC and hippocampus may play a role in the onset of depression-like behaviors. Improving GJC dysfunction and inhibiting the activity of HCs may be new directions for improving depression-like behaviors. Moreover, it is more important to consider the function of Cx43 (HCs and GJCs) associated with depression but not Cx43 individual kinetics. Also, mice with the conditional knockout of Cx43, as achieved by crossing Cx43^{fl/fl} mice with GFAP-cre mice, may not be the best tool for studying Cx43 function in a particular brain region. Injecting pseudotyped lentivirus containing the Cre-recombinase locally to drive the inactivation of Cx43 can be a better strategy. Furthermore, since gap junction blockers using currently can block both GJCs and HCs, there is no definite research and evidence for the isolated role of GJCs or HCs in the development of depression-like behaviors, and more relevant studies are needed in the future. Roles of Cx43 in other areas in the brain related to depression need to explore as well further because Cx43 in different brain regions have different functions [55]. Also, the Cx43 gap junction has a regulatory effect on the activities and function of neurons [34]. The neural circuit of vHIP-mPFC has been shown to play a role in depression-like behaviors. Therefore, further research on the effect of Cx43 on neural circuits is also a potential new direction.

EFFECTS OF ANTIDEPRESSANTS ON CX43

The antidepressants currently in clinical use mainly include tricyclic and tetracyclic antidepressants (TCAs), selective 5-HT reuptake inhibitors (SSRIs), 5-HT, and NE reuptake inhibitors (SNRIs) [56]. Rapid antidepressants, e.g., ketamine, are also a hot spot of current research. Many studies reported that the treatment with these antidepressants caused alterations in the expression of Cx43 in astrocytes.

Monoamine antidepressants

Ten monoamine antidepressants from four therapeutic classes were tested (Table 2). The results showed that 24 and 48 h treatment induced an increase in the Cx43 expression at the mRNA and protein level. The effect of fluoxetine on Cx43 was pronounced, which has been verified in several models in vivo and in vitro [4, 57, 58]. Thus, it can be inferred that the expression

of Cx43 in the brain of patients with MDD decreases, and antidepressant treatment is beneficial to the upregulation of Cx43 [59]. Jeanson et al. systematically tested the gap junction communication and HC activities in primary cultured astrocytes with seven antidepressants from four categories (TCA, SSRI, NRI, SNRI) [39]. It is known that these cells only express Cx43 [60]. The results showed that these antidepressant drugs had different effects on both Cx43 GJC and HC functions of astrocyte, although the level of Cx43 did not change significantly (Table 2) [39]. These reports have contradictions and inconsistencies. Treatment with fluoxetine in vivo [61] and in vitro showed an increase in Cx43 expression level [4, 57, 58]. Given treatment with amitriptyline to primary cultured rat astrocytes, it showed an increase in Cx43 expression level [62] and inhibition of GJC function. However, both fluoxetine and amitriptyline did not change the expression level of Cx43, and only treatment with amitriptyline showed an inhibitory effect on GJCs [39]. Moreover, in the preliminary research of our laboratory, fluoxetine and duloxetine were administered to rats chronically in the control group and CUS model group. Through dye tracer experiment and electron microscopy analysis, fluoxetine and duloxetine had reversal effects on the GJC dysfunction of astrocytes in the PFC caused by CUS, but no impact on the GJC function without CUS in rats [4]. However, fluoxetine had an inhibitory effect on the GJC function of mouse frontal astrocytes cultured in vitro, while duloxetine had no noticeable impact [39].

As a whole result, the effect of antidepressants on Cx43 function is far more complicated than the current literature reports. That opposed effects are observed within the same therapeutic class. There may be several reasons for these contradictions: First, the models used in these studies are different. The preliminary research in our laboratory worked on rats in vivo [4], whereas Jeanson et al. used cultured mouse astrocytes [39]. Furthermore, other cell culture models, e.g., human astrocytoma [58] and rat astrocytes [62], were also used. Secondly, the dosage and period of treatment differed. Cell culture models from different species and cell types may have different sensitivity and tolerance to antidepressants. Moreover, in the experiments that were operated in vivo models, animals are given chronic treatment indeed involved more integrated and complex mechanisms.

In addition, the effects of antidepressants on the Cx43 HCs of astrocytes have been less studied. LPS is considered an inducer of a depression-like context [35, 63]. It can activate Cx43 HCs in astrocytes by releasing pro-inflammatory factors tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), and astrocytes stimulated by LPS can be used to study the mechanism of antidepressant drugs [64, 65]. Seven monoamine antidepressants tested (fluoxetine, amitriptyline, paroxetine, imipramine, reboxetine, duloxetine, and venlafaxine) all had an inhibitory effect on LPS-induced HC activities in astrocytes [39]. At the same time, fluoxetine [66, 67], amitriptyline [66, 68], paroxetine [69], and imipramine [70] have all been found to inhibit the production of TNF- α and IL-1 β . In contrast, venlafaxine has been found to increase the level of TNF- α [67]. Meanwhile, the inhibitory effect of venlafaxine on LPS-induced HC activities was the lowest [67]. Therefore, it can be preliminarily speculated that antidepressants may inhibit the activation of Cx43 HCs to decrease the activity of astrocytes by reducing the generation of TNF- α and/or IL-1 β levels [39]. Besides, Cx43 HCs in astrocytes have also been found to mediate glutamate release [48, 71]. Combined with the hypothesis that glutamate cycle disorders cause depression [72], the antidepressant inhibits actions on HC activities can also support the current hypothesis [39]. However, more studies are needed to confirm and further explore the effects of antidepressants on the function of Cx43, Cx43 GJCs, and HCs.

Ketamine

In addition to mainstream monoamine antidepressants, other antidepressants, e.g., *N*-methyl-*D*-aspartic acid receptor (NMDAR)

antagonist ketamine, also received attention. There are also a small number of studies on the effects of ketamine on Cx43 (Table 2). Acute administration of ketamine (20 μ M, 30 min) had a significant inhibitory effect on Cx43 HCs of mouse cortical astrocytes, whereas ketamine (300 μ M, 30 min) had an inhibitory effect on the GJCs [73]. It is generally believed that the plasma concentrations of ketamine exerting antidepressant effects in humans and rats are 10 and 20 μ M, separately [74, 75]. Therefore, the acute treatment with ketamine at a therapeutic-relevant concentration had an inhibitory effect on the activity of the Cx43 HCs but not GJCs [32]. The mechanism may be inhibiting the release of inflammatory factors TNF- α and IL-1 β [73]. The mechanism of high concentration ketamine to inhibit the function of GJCs may be directly acting on NMDARs and gamma-aminobutyric acid receptors (GABARs) on astrocytes [76, 77].

CANDIDATE PATHOLOGICAL MECHANISM OF DEPRESSION-LIKE BEHAVIORS ASSOCIATED WITH CX43

Cx43 and gliotransmission

Synaptic plasticity is the specific structural and functional change of synapses caused by the continuous activity of neurons, and it is closely related to the pathophysiological process of a variety of neuropsychiatric diseases [78]. Astrocytes are the most abundant and functional glial cells in the CNS [79]. They can affect neurons' synaptic strength and function by expressing receptors and regulating neurotransmitters, e.g., adenosine triphosphate (ATP), glutamate, γ -aminobutyric acid [80, 81]. Its dysfunction can cause synaptic abnormalities, which are related to various mood disorders, e.g., depression [82]. Therefore, the concept of "tripartite synapse" was first proposed over 20 years ago to describe the intimate relationship between neurons and glutamatergic synaptic astrocytes [83]. In addition, microglia in resting states can also interact with astrocytes and neurons, so the hypothesis of "quad-partite synapse" has been proposed [84]. Due to the significant contribution of astrocyte dysfunction to depression [85], this section only discusses the contribution of Cx43 expression and function in the "tripartite synapse" to gliotransmission and synaptic plasticity.

Cx43 and glutamate-glutamine cycle. Presynaptic neurons release glutamate through vesicles [86]. Glutamate in the synaptic cleft binds to the glutamate receptors on postsynaptic neurons. Then, glutamate is quickly cleared up from the synaptic cleft, and more than 90% [87] is absorbed by astrocytes via the astrocyte-specific glutamate transporter GLT-1 [88], which is homologous to the excitatory amino acid transporter (EAAT2) in the brain of human [89]. In astrocytes, glutamate is transformed to glutamine by glial-specific glutamine synthetase. Then glutamine shuttles back to the presynaptic neurons and is converted into glutamate by neuron-specific phosphoric acid-activated glutaminase to supplement and maintain the glutamate storage of presynaptic neurons [87] (Fig. 1).

In the PFC and hippocampus of rats exposed to CUS, the function of Cx43 GJCs was impaired [4] (Fig. 2). Moreover, after blocking the Cx43 GJCs in astrocytes, GLT-1 expression decreased [90]. Recent studies show that pharmacological inhibition of central astrocytic glutamate uptake with the GLT-1 inhibitor dihydrokainic acid (DHK) can induce anhedonia-/depressive-like behaviors. Brain region-specific inhibition of GLT-1 is sufficient to induce depression-like behaviors. The possible mechanism is that glutamate intake through GLT-1 is reduced, resulting in a decrease of the glutamate pool and excess glutamate in the synaptic cleft [91, 92]. Glutamate binds to over-activated NMDARs outside the synapse (particularly in GluN2B-containing NMDARs), increasing cell death and neuron loss [91, 93]. Also, dysfunction of GLT-1 damages glutamate release and uptake which further inhibits α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) and NMDAR action to affect the cortex, resulting in reduced synapse density and diameter and dendritic length and

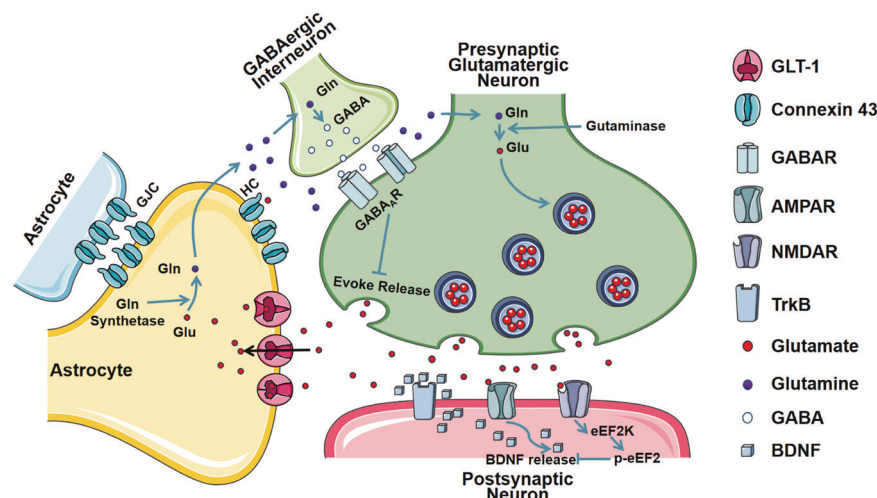


Fig. 1 Cx43 and glutamate-glutamine cycle under physiological conditions. Under physiological conditions, the function of Cx43 GJCs is normal, and most of the HCs remain closed. Presynaptic neurons release glutamate through vesicles. Glutamate in the synaptic cleft binds to the glutamate receptors on postsynaptic neurons, including NMDAR and AMPAR. Then, glutamate is quickly removed from the synaptic cleft, and more than 90% is taken up by astrocytes via the astrocyte-specific glutamate transporter GLT-1. In astrocytes, glutamate is converted to glutamine by glial-specific glutamine synthetase. A part of glutamine shuttles back to the presynaptic neurons. It is converted into glutamate by neuron-specific phosphoric acid-activated glutaminase to supplement and maintain the glutamate storage of the presynaptic neurons. Also, a part of glutamine enters GABAergic interneurons to synthesize GABA, binding to GABA_R on presynaptic neurons and inhibiting glutamate release. Through the above two aspects of regulation, the concentration of glutamate in the synaptic cleft keeps low. AMPAR α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, GABA γ-aminobutyric acid, GJC gap junction channel, GLT-1 glutamate transporter 1, HC hemichannels, NMDAR N-methyl-D-aspartate-receptor.

arborization [93] (Fig. 2). In addition, stress can also activate Cx43 HCs of astrocytes, and the outflow of a large amount of glutamate through HCs leads to a further increase of glutamate in the synaptic cleft [94]. Interestingly, postmortem studies found that glutamate decreased in the frontal limbic of patients with MDD [95]. Consistently, neuroimaging studies using proton magnetic resonance spectroscopy revealed reduced levels of glutamate in the brain areas, including PFC of patients with MDD [96]. However, ketamine might produce rapid antidepressant-like effects, at least in part by transiently increasing glutamate cycling in the PFC. It has been demonstrated in rodent and human studies [97, 98]. Therefore, we suspect that the effects of GJC and HC dysfunction on the glutamate cycle may be time-dependent. In the short term, glutamate release from activated HCs can be beneficial to adaptation to the stress [11]. However, continuous activation of HCs and dysfunction of GJCs induce the accumulation of glutamate in the synaptic cleft. The functional degradation of astrocytes caused by prolonged stress may cause the decreased glutamate levels in depressed individuals [85]. Thus, inhibiting the activated HC may be helpful to delay the progression of depression-like behavior, while activating HCs to release glutamate may produce a rapid antidepressant effect in individuals with severe depression.

Furthermore, glutamate-glutamine cycling is considered the primary source of glutamine in the brain [86]. The reduction of GLT-1 expression caused by GJC dysfunction also reduces glutamine synthesis. Glutamine is the precursor for synthesizing the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) [99]. Therefore, GJC dysfunction also leads to GABAergic system dysfunction. Gamma-aminobutyric acid (GABA) is responsible for fine-tuning and controlling excitatory transmission [100, 101], which is also a potential factor in the pathophysiology of MDD [102] (Fig. 2).

However, the specific mechanism by which Cx43 GJC dysfunction in astrocytes leads to reducing GLT-1 expression is still unclear, and a possible intermediate target is GFAP. The decrease in GFAP expression following stress exposure led to a strong glutamate transporter GLT-1 on the hippocampus and cortical astrocytes [85], which may be through a cAMP-protein kinase A-dependent pathway

[103]. GFAP seems to act as a scaffolding protein to involve the expression of GLT-1 on the membrane surface [85]. Furthermore, in previous studies of our laboratory, rats were given a single bilateral infusion of CBX in the hippocampal CA1 area and sacrificed 2 days later. Interestingly, the expression of GFAP in the hippocampus increased [40]. A cellular self-protection mechanism may induce a compensatory increase in GFAP expression, and long-term gap junction dysfunction may decrease GFAP expression.

Cx43 and ATP. ATP is generally considered the primary energy currency of cells [85]. It widely mediates astrocyte-neuronal signal communication [104] and participates in the regulation of synaptic plasticity [105] (Fig. 3). Glutamatergic signaling triggered Ca^{2+} influx into neurons through AMPAR or NMDAR, resulting in a localized decrease in the extracellular Ca^{2+} concentration [106]. Cx43 HCs in astrocytes open in response to low extracellular Ca^{2+} conditions and mediate the efflux of ATP. ATP binds to astrocytic P_2Y_1R and P_2Y_2R , and then activates the G-protein coupled with P_2YRs [107]. Activated G-protein further activates phospholipase C (PLC), leading to the release of intracellular inositol triphosphate (IP_3) [108]. Then, IP_3 binds to IP_3 receptors on the ER membrane, and subsequent calcium releases from the ES calcium stores into the cytoplasm [85]. The localized increase in cytosolic Ca^{2+} is named a calcium wave [109]. The intracellular Ca^{2+} signal in astrocytes triggers the vesicular or non-vesicular release of gliotransmitters, e.g., glutamate, GABA, ATP, into the synaptic cleft [110, 111]. These gliotransmitters, especially ATP and glutamate, bind to synaptic receptors, providing a feedback signal on synaptic transmission and neuronal activities [85, 112].

Furthermore, astrocytes can transmit the calcium signals to neighboring non-stimulated astrocytes, forming intercellular Ca^{2+} waves (ICWs) [113]. There are two possible pathways by which ICWs can be transmitted (Fig. 3). One is mediated by Cx43 GJCs [114]. Both Ca^{2+} [109] and IP_3 [115] can enter the cytoplasm of adjacent astrocytes through GJCs and induce the calcium waves in adjacent astrocytes. The other pathway is through ATP in the synaptic cleft. ATP activates P_2YRs on the membrane of neighboring cells and then induces the release of IP_3 and subsequent calcium release from the ES calcium stores [109]. These two pathways are not mutually

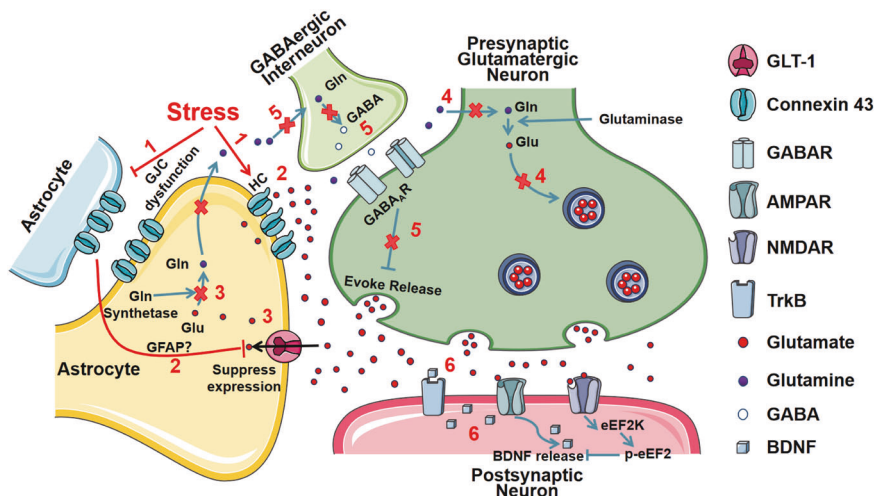


Fig. 2 Cx43 and glutamate-glutamine cycle under stress conditions. (1) Stress causes dysfunction of Cx43 GJCs and activation of Cx43 HCs. (2) Dysfunction of GJCs reduces the expression of GLT-1. A possible intermediate target is GFAP. The activated HCs release large amounts of glutamate into the synaptic cleft. (3) The clearance of glutamate through GLT-1 decreases, and glutamine synthesis in astrocytes decreases. (4) Glutamine shuttled back to the presynaptic neurons decreases, and glutamate storage of presynaptic neurons decreases. (5) Glutamine entering into GABAergic interneurons decreases, and the synthesis of GABA reduces. The inhibitory effect of GABAergic neurons on the release of glutamate from presynaptic neurons is weakened. (6) Reduced glutamate clearance by GLT-1, increased release of glutamate by HCs, and dysfunction of GABAergic system together leads to the accumulation of glutamate in the synaptic cleft. Accumulated glutamate in the synaptic cleft leads to excessive and continuous activation of NMDAR and AMPAR, resulting in a decrease of BDNF release, thereby contributing to the onset of depression. AMPAR α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, BDNF brain-derived neurotrophic factor, GABA γ -aminobutyric acid, GFAP glial fibrillary acidic protein, GJC gap junction channel, GLT-1 glutamate transporter 1, HC hemichannels, NMDAR *N*-methyl-*D*-aspartate-receptor.

exclusive but work together to promote the transmission of ICWs [109]. However, based on current research about ICWs in astrocytes, it seems to act crucial roles under pathological conditions but not physiological conditions [109, 116]. Spontaneous ICWs are sparsely seen in the mature CNS, even after physiological stimulation [109, 117]. This may be related to the low activities of the HCs under physiological conditions [118]. The low concentration of ATP is not enough to support the long-distance propagation of ICWs. In addition, the P_2X_7 receptor may also be involved in the propagation of ICWs [119]. Several GJC/HC blockers, e.g., heptanol, octanol, CBX, and mefloquine, prevented ATP-dependent spread of ICWs under low divalent cation solutions by affecting P_2X_7R [119]. But the specific mechanism needs to be further explored.

Moreover, ATP is degraded to ADP, activating P_2YR_1 on the interneuron [107, 120]. The interneuron is stimulated to depolarize and increase firing [107]. This pattern of neuron-glia signaling may be a negative feedback mechanism that increases inhibitory transmission while glutamatergic activities are excessive [107]. Also, blocking ATP release from astrocytes can induce a decrease in the number of hippocampal nerve spines in mice, leading to neurological disorders and depression-like behaviors [121]. Exogenous ATP or endogenous activation of astrocytes to promote ATP release can quickly reverse depression-like behaviors within a week [122]. This may be related to the ATP release of astrocytes, which can enhance inhibitory transmission relying on local excitation and serve as a brake on network excitatory output [107].

Astrocytes rapidly take up glutamate in the synaptic cleft to provide primary metabolic substrates glutamine to neurons and control synaptic activities by releasing ATP and generating calcium waves [109]. Cx43 HCs mediate ATP efflux [123]. Thus, Cx43 may be a promising antidepressant target worth developing.

Cx43 and metabolic disorders

As we all know, depression is a disease of environmental and genetic factors [3]. Its pathogenesis is very complicated, and it is not limited to brain cells and molecular abnormalities. Therefore, considering other related factors in the body is essential for

understanding depression. This section will explain the relationship among Cx43, metabolic disorders, and depression from the hypothalamus-pituitary-adrenal axis (HPA axis) dysfunction, insulin resistance, and thyroid hormone dysfunction (Fig. 4).

Cx43 and HPA axis dysfunction. The HPA axis is the primary link in coping with stress [124]. Acute or chronic stress can cause changes in the function of the HPA axis [125]. HPA axis dysfunction is one of the recognized biochemical changes in depression [126]. The stress activates the HPA axis and then induces the paraventricular nucleus to release corticotropin-releasing hormone (CRH). CRH triggers the hormone cascade release, eventually triggering the release of glucocorticoid (GC). GC inhibits the activity of the HPA axis through negative feedback [127]. Cortisol in humans and CORT in rodents are the primary GC that acts on central GC receptors and affects the function of the metabolic regulation hormone [127]. Reduced expression of Cx43 in the hippocampus and frontal cortex was found in several stress-related depression animal models, including CUS [4, 29, 128], acute/chronic restraint stress [11], and exogenous CORT models [44]. In vitro, CORT inhibits gap junctional intercellular communication (GJIC) in prefrontal and hippocampal astrocytes [37]. Furthermore, the expression of Cx43 was significantly reduced [129], while phosphorylated Cx43 significantly increased [37]. The possible mechanism of Cx43 reduction induced by CORT includes decreasing Cx43 biosynthesis and membrane distribution, increasing Cx43 degradation, and regulating Cx43 stability [37]. In addition, the phosphorylation of Cx43 at Ser368 might cause the decreased expression of Cx43 and GJC dysfunction [130, 131]. There are relatively few studies on Cx43 HCs. Studies have initially found that CORT activated HCs [11]. Thus, Cx43 may be involved in the pathophysiological changes of depression under the HPA axis hypothesis by weakening GJC functions and enhancing HC functions [32]. More experiments are needed to confirm the effect of CORT on Cx43 HCs and explore possible mechanisms. In addition, the effect of changes in Cx43 expression and functions on endogenous CORT secretion is also needed to explore.

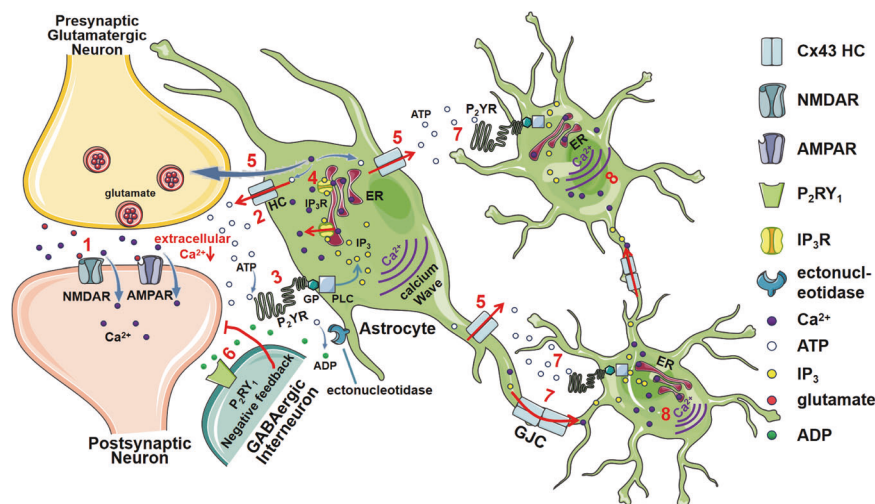


Fig. 3 Under stress conditions, ATP mediates the formation of ICWs through Cx43 GJCs and HCs. (1) Glutamatergic signaling triggers Ca^{2+} influx into neurons through AMPAR or NMDAR, resulting in a localized decrease in the extracellular Ca^{2+} concentration. (2) Cx43 HCs in astrocytes open in response to low extracellular Ca^{2+} conditions and mediate the efflux of ATP. (3) ATP binds to P_2YRs on astrocytes and then activates G protein and PLC, promoting the release of intracellular IP_3 . (4) The combination of IP_3 and IP_3R on the ER membrane causes calcium release from ER, forming calcium waves. (5) Calcium waves regulate the release of neurotransmitters from presynaptic neurons and further promote the release of ATP from HCs. (6) ATP is degraded to ADP. ADP activates interneuronal $\text{P}_2\text{Y1}$ receptors, stimulating depolarization and firing, thereby enhancing inhibitory transmission. (7) ICWs transmit through Cx43 GJCs and HCs. Both Ca^{2+} and IP_3 can enter the cytoplasm of adjacent astrocytes through GJCs and then induce the calcium waves in adjacent astrocytes. ATP released from HCs activates P_2YRs on the membrane of neighboring cells and then induces the release of IP_3 and subsequent calcium release from the ER calcium stores. (8) A large-scale calcium wave is formed in the astrocyte network to regulate neuronal activities under stress conditions. ADP adenosine diphosphate, AMPAR α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, ATP adenosine triphosphate, ER endoplasmic reticulum, GJC gap junction channel, HC hemichannels, ICW intercellular calcium waves, IP_3 inositol triphosphate, NMDAR N-methyl-D-aspartate-receptor, PLC phospholipase C.

Cx43 and insulin resistance. The results of multiple studies have shown that insulin resistance may be the cause of depression induced by obesity [132]. Insulin administration had antidepressant effects [133]. Alterations of Cx43 are related to insulin resistance. High-dose insulin increased the phosphorylation of Cx43 at Ser368 in vascular smooth muscle cell GJCs and decreased the expression of Cx43, causing GJC dysfunction. High-dose insulin treatment largely enhanced the H_2O_2 level in cells. Furthermore, pretreatment with catalase increased the expression of Cx43, decreased the phosphorylation level of Cx43 at Ser368, and recovered cellular GJC function compared with cells only treated with high-dose insulin [134]. The results suggest that high-dose insulin impairs intercellular GJC through the oxidative stress-activated signaling pathway [134]. It may be a possible mechanism by which excessive insulin secretion in the body leads to insulin resistance [135, 136]. However, the increased expression of Cx43 and cell-cell coupling in hepatocytes might be a factor that promoted the development of insulin resistance. Endoplasmic reticulum stress (ERS) is an essential character of obesity and type 2 diabetes [137]. It is also a crucial factor in the network of stress signals regulating insulin resistance and diabetes [138]. In hepatocytes, ERS increases Cx43 expression and cell-cell coupling. The liver Cx43 knockout mice can avoid the effects of highly nutritious diet-induced ERS and insulin resistance. In obese mice, Cx43-mediated cell-cell coupling promotes the transmission of ERS between cells [21]. It suggests that Cx43 and GJCs provide a pathway for the transmission of chronic stress signals [21], and may play a role in promoting the onset of insulin resistance and depression. As a way of cell communication, Cx43 GJC mediates the necessary communication between cells, however, it also facilitates the spread of harmful substances [139, 140]. Thus, the contribution of Cx43 to insulin resistance is also bidirectional, which may be related to the role of Cx43 in this tissue.

Cx43 and thyroid hormone dysfunction. The thyroid hormone plays a vital role in developing a healthy brain [141] because it

relates to neuron precursors' proliferation, migration, differentiation, and synapse formation [142]. The effect is mediated mainly by glial cells, particularly astrocytes [143]. Clinical studies have found that the levels of thyroxine (T4), free triiodothyronine (FT3), and free thyroxine (FT4) in female patients with depression were significantly lower than those in healthy groups, suggesting that patients with depression might be accompanied by hypothyroidism [144]. Also, the hyperfunction of the hypothalamus-pituitary-thyroid axis (HPT axis) affects brain function [145]. Various studies suggest that the HPT axis is involved in the pathophysiology and prognosis of depression [146]. Notably, some patients with depression have normal thyroid functions [147, 148]. The specific relationship between depression and thyroid hormone dysfunction has been demonstrated in cross-sectional studies, and findings have been inconsistent [149]. The existing literature suggests a link between subtle thyroid dysfunction and depression, but more data are needed [150]. In fact, screening for thyroid dysfunction is part of the routine assessment of depressed patient [144].

Like most tissues, thyrocytes communicate with each other through various types of intercellular junctions, including GJCs. There are two connexins expressed in thyrocytes: Cx43 and Cx32 [151]. Thyroid-stimulating hormone (TSH), produced in the brain's hypothalamus, promotes thyroid hormone production by increasing the synthesis of these two connexins and inducing the opening of GJCs in thyrocytes [20]. Cx43 GJC-mediated cell-to-cell communication is likely involved in controlling thyrocyte proliferation [151]. In patients with Hashimoto disease, the expression of Cx43 in thyroid epithelial cells decreased [152].

Furthermore, some studies showed that thyroid hormone had a regulatory function on the expression of connexin [153]. Thyroid hormone inhibits the growth and proliferation of Sertoli cells by inducing the expression of Cx43 [20]. Gap junction coupling inhibitors have been shown to significantly reverse the inhibitory effects of thyroid hormones on the growth and proliferation of

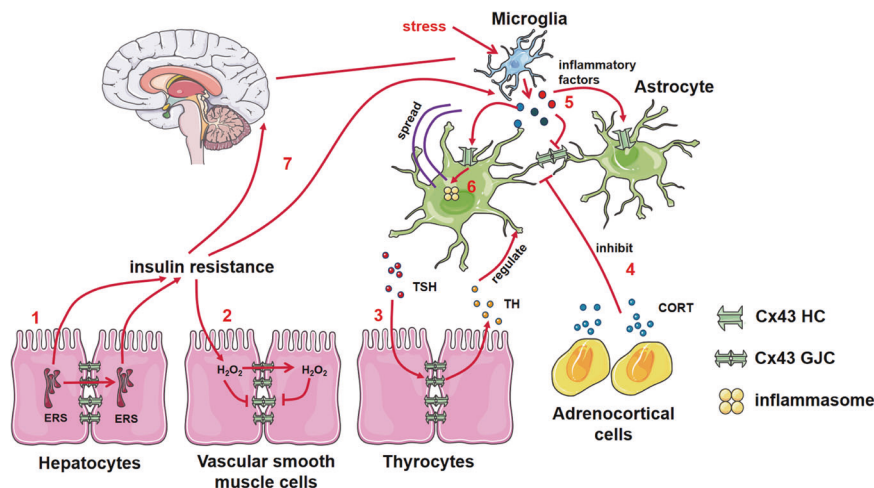


Fig. 4 Cx43 dysfunction contributes to depression through peripheral mechanisms. (1) ERS in hepatocytes spreads through Cx43 GJCs and promotes insulin resistance. (2) Insulin resistance causes Cx43 GJC dysfunction by increasing the level of H_2O_2 in vascular smooth muscle cells. (3) TSH promotes TH production by increasing the synthesis of Cx43 and inducing the opening of Cx43 GJCs in thyrocytes. (4) Excessive CORT produced by adrenocortical cells causes Cx43 GJC dysfunction of astrocytes in the brain. (5) Under conditions of stress, activated microglia releases inflammatory factors, promoting Cx43 HC opening and Cx43 GJC dysfunction of astrocytes. (6) Opened HCs further contribute to the activation and spread of the inflammasome pathway. (7) Insulin resistance can promote neuroinflammation and affect the brain function. CORT corticosterone, ERS endoplasmic reticulum stress, GJCs gap junction channels, HC hemichannel, TH thyroid hormone, TSH thyroid stimulating hormone.

Sertoli cells [20]. Antidepressant fluoxetine has been reported to cause male sexual dysfunction [154], but the target that induces this side effect is still unknown. Sertoli cells have nutritional and protective effects on developing sperm [155]. Fluoxetine may impair the integrity of the peritubular myoid cell-Sertoli cell and disturb spermatogenesis, which may be one reason why fluoxetine induces male sexual dysfunction [156]. As the critical target that regulates the growth and proliferation of Sertoli cells [157], Cx43 may be a potential target to improve this side effect of fluoxetine. In addition, cellular therapy is an emerging treatment strategy for depression [158]. As ‘nurse cells’ within the testis, Sertoli cells are considered an ideal substitute for neurotransmitter antagonists and inhibitors due to their anti-inflammatory, neuroprotective, and nutritional effects [157]. As the target of regulating the growth and proliferation of Sertoli cells, Cx43 also has great potential value for developing a new type of antidepressant therapy and understanding the cellular and molecular processes that underlie depression.

GJCs and HCs formed by aggregated connexin are essential for direct communication between cells [159]. Adjacent cells share cytoplasmic contents through GJCs, including ions, second messengers, and small metabolites [160, 161]. Under physiological conditions, hormones can regulate the expression of Cx43 through endocrine, autocrine, and paracrine methods. Under stress conditions, on the one hand, functional changes of Cx43 GJCs and HCs Cx43 can control the dramatic changes in the internal and external environment of cells caused by stress within a certain range. On the other hand, GJC can provide a way for the rapid spread of toxic substances, intracellular redox components, or signal molecules in neighboring cells, contributing to metabolic disorders [162]. Therefore, in a short time, changes of Cx43 may be beneficial to the adaptation of cells and the body to external factors. However, long-term stress will damage the function of Cx43 [4], inducing metabolic disorders. In turn, metabolic disorders affect the secretion of biologically active molecules, e.g., hormones, chemokines, cytokines, and lipids [162]. Changes in the secretion of these substances dynamically alter the communication among cells, neighboring and distant cells [162], thereby affecting the function of connexins [153]. In short, the function of connexin and metabolic disorders affect each other, and metabolic disorders are inseparable from depression. It suggests that improving metabolic disorders by

regulating the function of connexin may be a potential target for depression treatment.

Cx43 and neuroinflammation

The critical role of neuroinflammation in the occurrence and development of MDD has been testified by many experimental studies [163]. Neuroinflammation and Cx43 also have a complex relationship (Fig. 4).

On the one hand, neuroinflammation affects the expression and function of Cx43. In patients with MDD, the expression of pro-inflammatory factors, e.g., $TNF-\alpha$ [164] and interleukin-6 (IL-6) [165], increased, which was related to the severity of MDD. Some antidepressants can inhibit the production of these inflammatory factors [166]. Under conditions of neuroinflammation, microglia are activated and release these inflammatory factors to promote the opening of Cx43 HCs in astrocytes [48]. Opened HCs further contribute to the activation and spread of the inflammasome pathway [167]. Other inflammation and stress factors include fibroblast growth factor-2 (FGF-2) [168], transforming growth factor- β (TGF- β) [168], and arachidonic acid (AA) [169] have also been found to reduce the expression of Cx43 and inhibit the opening of GJCs. Another study found that NO and NO-derived compounds could enhance Cx43 HCs and reduce GJ communication [170, 171]. The Cx43 GJC dysfunction in astrocytes has been an essential condition for the onset of depression. Some studies suggest that the activation of Cx43 HCs may be a factor that induces depression [11]. Therefore, Cx43 may be a critical intermediate factor in inflammation-induced depression; however, the underlying complex mechanism is still unclear.

On the other hand, changes in the expression and function of Cx43 may promote the production of inflammatory responses. A recent study has found an encouraging result that inhibitors of Cx43 HCs (tonabersat) could inhibit inflammasome activation and damage in the CNS [172]. However, research on the mechanism is relatively scarce. Astrocytes cultured *in vitro* were found to release NAD^+ through HCs in the resting state, and then NAD^+ was transported from the cytosol to the active site of CD38 [173]. CD38 is a multifunctional enzyme that can enhance Ca^{2+} levels in cells and Ca^{2+} -dependent functions accordingly by autocrine and paracrine mechanisms [174]. Enhancing Ca^{2+} levels upmodulates various intercellular signaling pathways, e.g., inflammation

pathways [175]. In addition to astrocytes, the function of Cx43 in other cells may also be related to inflammation-induced depression. Depression and Alzheimer's disease (AD) always co-occur, and depression increases the risk of AD [176]. High Cx43 HC activities of mastocytes in the brain may play an essential role in the occurrence and development of AD [177]. Moreover, mastocytosis, rare activation and aggregation of mast cells in different kinds of tissues, may be involved in the process of inflammation-induced depression [178]. Thus, although the global study is sparse, as in the case of AD, high HC activities in mastocytes probably were early factors in the inflammation pathways linked to depression [177]. However, the cascade of events taking place among activation in mastocytes, involvement of Cx43 GJCs/HCs in astrocytes, and the damage of neurons need further studies [177].

CONCLUSION AND PERSPECTIVES

In this review, we summarized recent advances in Cx43 related to depression. It mainly includes the abnormalities of Cx43 in depression, the effects of Cx43 changes on depression, and the effects of antidepressants on Cx43. In addition, we emphatically describe the candidate pathological mechanisms of depression-like behaviors associated with Cx43. Cx43 dysfunction contributes to depression by the following aspects: affecting the gliotransmission, e.g., glutamate-glutamine cycle and ATP release; inducing dysfunction of HAP axis, insulin, and thyroid hormone; inducing neuroinflammation. However, these mechanism needs further studies to confirm.

The following outstanding questions need to be studied: (1) The current research on Cx43 changes under depression mainly focuses on astrocytes in the brain, but how does Cx43 change in other tissues and cells? (2) How do Cx43 GJCs and HCs independently participate in the pathogenesis of depression?

In addition, the glutamate cycle, ATP release, neuroinflammation, and metabolic disorders are all targets that can be used for the treatment of depression. Cx43 may be an upstream target that caused these changes, but the sequence of these changes needs further study. The etiology of depression is complex, and further research on Cx43 would contribute to shedding new light on the pathogenesis of depression. Cx43 also can develop into a novel, root cause-based antidepressant treatment target for depression.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

REFERENCES

- Milanovic SM, Erjavec K, Poljicanin T, Vrabec B, Brecic P. Prevalence of depression symptoms and associated socio-demographic factors in primary health care patients. *Psychiatr Danub*. 2015;27:31–7.
- Smith K. Mental health: a world of depression. *Nature*. 2014;515:181.
- Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature*. 2008;455:894–902.
- Sun JD, Liu Y, Yuan YH, Li J, Chen NH. Gap junction dysfunction in the prefrontal cortex induces depressive-like behaviors in rats. *Neuropsychopharmacology*. 2012;37:1305–20.
- John SA, Saner D, Pitts JD, Holzenburg A, Finbow ME, Lal R. Atomic force microscopy of arthropod gap junctions. *J Struct Biol*. 1997;120:22–31.
- Duffy HS, Delmar M, Spray DC. Formation of the gap junction nexus: binding partners for connexins. *J Physiol-Paris*. 2002;96:243–9.
- Okada M, Fukuyama K, Shiroyama T, Murata M. A working hypothesis regarding identical pathomechanisms between clinical efficacy and adverse reaction of clozapine via the activation of connexin43. *Int J Mol Sci*. 2020;21:7019.

- Loewenstein WR. Junctional intercellular communication: the cell-to-cell membrane channel. *Physiological Rev*. 1981;61:829–913.
- Orellana JA, Martinez AD, Retamal MA. Gap junction channels and hemichannels in the CNS: regulation by signaling molecules. *Neuropharmacology*. 2013;75:567–82.
- Orellana JA, Figueroa XF, Sanchez HA, Contreras-Duarte S, Velarde V, Saez JC. Hemichannels in the neurovascular unit and white matter under normal and inflamed conditions. *CNS Neurol Disord Drug Targets*. 2011;10:404–14.
- Orellana JA, Moraga-Amaro R, Diaz-Galarce R, Rojas S, Maturana CJ, Stehberg J, et al. Restraint stress increases hemichannel activity in hippocampal glial cells and neurons. *Front Cell Neurosci*. 2015;9:102.
- Willecke K, Eiberger J, Degen J, Eckardt D, Romualdi A, Guldenagel M, et al. Structural and functional diversity of connexin genes in the mouse and human genome. *Biol Chem*. 2002;383:725–37.
- Panchina Y, Kelmanson I, Matz M, Lukyanov K, Usman N, Lukyanov S. A ubiquitous family of putative gap junction molecules. *Curr Biol*. 2000;10:R473–R4.
- Bruzzone R, Barbe MT, Jakob NJ, Monyer H. Pharmacological properties of homomeric and heteromeric pannexin hemichannels expressed in *Xenopus* oocytes. *J Neurochem*. 2005;92:1033–43.
- Scemes E, Suadicani SO, Dahl G, Spray DC. Connexin and pannexin mediated cell-cell communication. *Neuron Glia Biol*. 2007;3:199–208.
- Naus CCG, Bechberger JF, Zhang Y, Venance L, Yamasaki H, Juneja SC, et al. Altered gap junctional communication, intercellular signaling, and growth in cultured astrocytes deficient in connexin43. *J Neurosci Res*. 1997;49:528–40.
- Nagy JI, Ionescu AV, Lynn BD, Rash JE. Coupling of astrocyte connexins Cx26, Cx30, Cx43 to oligodendrocyte Cx29, Cx32, Cx47: implications from normal and connexin32 knockout mice. *Glia*. 2003;44:205–18.
- Dermietzel R, Gao Y, Scemes E, Vieira D, Urban M, Kremer M, et al. Connexin43 null mice reveal that astrocytes express multiple connexins. *Brain Res Rev*. 2000;32:45–56.
- Portal B, Delcourte S, Rovera R, Lejards C, Bullich S, Malnou CE, et al. Genetic and pharmacological inactivation of astroglial connexin 43 differentially influences the acute response of antidepressant and anxiolytic drugs. *Acta Physiol (Oxf)*. 2020;229:e13440.
- Hussain MU. Connexins: the gap junction proteins. India:Springer India; 2014.
- Tirosh A, Tuncman G, Calay ES, Rathaus M, Ron I, Tirosh A, et al. Intercellular transmission of hepatic ER stress in obesity disrupts systemic metabolism. *Cell Metab*. 2021;33:319–33. e6.
- Georgin-Lavialle S, Moura DS, Salvador A, Chauvet-Gelinier JC, Launay JM, Damaj G, et al. Mast cells' involvement in inflammation pathways linked to depression: evidence in mastocytosis. *Mol Psychiatry*. 2016;21:1511–6.
- Bernard R, Kerman IA, Thompson RC, Jones EG, Bunney WE, Barchas JD, et al. Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. *Mol Psychiatry*. 2011;16:634–46.
- Ernst C, Nagy C, Kim S, Yang JP, Deng X, Hellstrom IC, et al. Dysfunction of astrocyte connexins 30 and 43 in dorsal lateral prefrontal cortex of suicide completers. *Biol Psychiatry*. 2011;70:312–9.
- Nagy C, Torres-Platas SG, Mechawar N, Turecki G. Repression of astrocytic connexins in cortical and subcortical brain regions and prefrontal enrichment of H3K9me3 in depression and suicide. *Int J Neuropsychopharmacol*. 2017;20:50–7.
- Miguel-Hidalgo JJ, Wilson BA, Hussain S, Meshram A, Rajkowska G, Stockmeier CA. Reduced connexin 43 immunolabeling in the orbitofrontal cortex in alcohol dependence and depression. *J Psychiatr Res*. 2014;55:101–9.
- Jin C, Wang ZZ, Zhou H, Lou YX, Chen J, Zuo W, et al. Ginsenoside Rg1-induced antidepressant effects involve the protection of astrocyte gap junctions within the prefrontal cortex. *Prog Neuropsychopharmacol Biol Psychiatry*. 2017;75:183–91.
- Nadarajah B, Jones AM, Evans WH, Parnavelas JG. Differential expression of connexins during neocortical development and neuronal circuit formation. *J Neurosci*. 1997;17:3096–111.
- Miguel-Hidalgo JJ, Moulana M, Deloach PH, Rajkowska G. Chronic unpredictable stress reduces immunostaining for connexins 43 and 30 and myelin basic protein in the rat prelimbic and orbitofrontal cortices. *Chronic Stress (Thousand Oaks)*. 2018;2:2470547018814186.
- Hurtubise JL, Howland JG. Effects of stress on behavioral flexibility in rodents. *Neuroscience*. 2017;345:176–92.
- Lou YX, Li J, Xia CY, Wang ZZ, Chen NH. Elevated glucocorticoid damages gap junction structure and function in rat prefrontal cortex. *Acta Pharm Sin B*. 2018;53:1645–52. Chinese.
- Okada M, Oka T, Nakamoto M, Fukuyama K, Shiroyama T. Astroglial Connexin43 as a potential target for a mood stabiliser. *Int J Mol Sci*. 2021;22:339.
- Zheng QL, Zhu HY, Xu X, Chu SF, Cui LY, Dong YX, et al. Korean red ginseng alleviate depressive disorder by improving astrocyte gap junction function. *J Ethnopharmacol*. 2021;281:114466.

34. Huang D, Li C, Zhang W, Qin J, Jiang W, Hu C. Dysfunction of astrocytic connexins 30 and 43 in the medial prefrontal cortex and hippocampus mediates depressive-like behaviours. *Behav Brain Res.* 2019;372:111950.
35. Mello BS, Monte AS, McIntyre RS, Soczynska JK, Custodio CS, Cordeiro RC, et al. Effects of doxycycline on depressive-like behavior in mice after lipopolysaccharide (LPS) administration. *J Psychiatr Res.* 2013;47:1521–9.
36. Miguel-Hidalgo JJ, Carter K, Deloach PH, Sanders L, Pang Y. Glucocorticoid-induced reductions of myelination and connexin 43 in mixed central nervous system cell cultures are prevented by mifepristone. *Neuroscience.* 2019;411:255–69.
37. Xia CY, Wang ZZ, Zhang Z, Chen J, Wang YY, Lou YX, et al. Corticosterone impairs gap junctions in the prefrontal cortical and hippocampal astrocytes via different mechanisms. *Neuropharmacology.* 2018;131:20–30.
38. Wang HQ, Yang SW, Gao Y, Liu YJ, Li X, Ai QD, et al. Novel antidepressant mechanism of ginsenoside Rg1: regulating biosynthesis and degradation of connexin43. *J Ethnopharmacol.* 2021;278:114212.
39. Jeanson T, Pondaven A, Ezan P, Mouthon F, Charveriat M, Giaume C. Antidepressants impact connexin 43 channel functions in astrocytes. *Front Cell Neurosci.* 2015;9:495.
40. Lou YX, Wang ZZ, Xia CY, Mou Z, Ren Q, Liu DD, et al. The protective effect of ginsenoside Rg1 on depression may benefit from the gap junction function in hippocampal astrocytes. *Eur J Pharmacol.* 2020;882:173309.
41. Thompson RJ, Zhou N, MacVicar BA. Ischemia opens neuronal gap junction hemichannels. *Science.* 2006;312:924–7.
42. Schock SC, Leblanc D, Hakim AM, Thompson CS. ATP release by way of connexin 36 hemichannels mediates ischemic tolerance in vitro. *Biochem Biophys Res Commun.* 2008;368:138–44.
43. Dhabhar FS, McEwen BS. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. *Brain Behav Immun.* 1997;11:286–306.
44. Quesseveur G, Portal B, Basile JA, Ezan P, Mathou A, Halley H, et al. Attenuated levels of hippocampal connexin 43 and its phosphorylation correlate with antidepressant- and anxiolytic-like activities in mice. *Front Cell Neurosci.* 2015;9:490.
45. Mitterauer BJ. Downregulation and upregulation of glial connexins may cause synaptic imbalances responsible for the pathophysiology of bipolar disorder. *CNS Neurosci Ther.* 2011;17:281–93.
46. Riquelme MA, Kar R, Gu S, Jiang JX. Antibodies targeting extracellular domain of connexins for studies of hemichannels. *Neuropharmacology.* 2013;75:525–32.
47. Schulz R, Gorge PM, Gorbe A, Ferdinandy P, Lampe PD, Leybaert L. Connexin 43 is an emerging therapeutic target in ischemia/reperfusion injury, cardioprotection and neuroprotection. *Pharmacol Ther.* 2015;153:90–106.
48. Abudara V, Roux L, Dallerac G, Matias I, Dulong J, Mothet JP, et al. Activated microglia impairs neuroglial interaction by opening Cx43 hemichannels in hippocampal astrocytes. *Glia.* 2015;63:795–811.
49. Desplantez T, Verma V, Leybaert L, Evans WH, Weingart R. Gap26, a connexin mimetic peptide, inhibits currents carried by connexin43 hemichannels and gap junction channels. *Pharmacol Res.* 2012;65:546–52.
50. Siller-Jackson AJ, Burra S, Gu S, Xia X, Bonevald LF, Sprague E, et al. Adaptation of connexin 43-hemichannel prostaglandin release to mechanical loading. *J Biol Chem.* 2008;283:26374–82.
51. Schoenfeld TJ, Kloth AD, Hsueh B, Runkle MB, Kane GA, Wang SS, et al. Gap junctions in the ventral hippocampal-medial prefrontal pathway are involved in anxiety regulation. *J Neurosci.* 2014;34:15679–88.
52. Laroche S, Davis S, Jay TM. Plasticity at hippocampal to prefrontal cortex synapses: dual roles in working memory and consolidation. *Hippocampus.* 2000;10:438–46.
53. McEown K, Treit D. Alpha2 GABAA receptor sub-units in the ventral hippocampus and alpha5 GABAA receptor sub-units in the dorsal hippocampus mediate anxiety and fear memory. *Neuroscience.* 2013;252:169–77.
54. Juszczak GR, Swiergiel AH. Properties of gap junction blockers and their behavioural, cognitive and electrophysiological effects: animal and human studies. *Prog Neuropharmacol Biol Psychiatry.* 2009;33:181–98.
55. Nadarajah B, Parnavelas JG. Gap junction-mediated communication in the developing and adult cerebral cortex. *Novartis Found Symp.* 1999;219:157–70. discussion 70–4
56. Lopez-Munoz F, Alamo C. Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. *Curr Pharm Des.* 2009;15:1563–86.
57. Fatemi SH, Folsom TD, Reutiman TJ, Lee S. Expression of Astrocytic markers aquaporin 4 and connexin 43 is altered in brains of subjects with autism. *Synapse.* 2008;62:501–7.
58. Mostafavi H, Khaksarian M, Joghataei MT, Hassanzadeh G, Soleimani M, Eftekhari S, et al. Fluoxetine upregulates connexin 43 expression in astrocyte. *Basic Clin Neurosci.* 2014;5:74–9.
59. Rajkowska G, Stockmeier CA. Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. *Curr Drug Targets.* 2013;14:1225–36.
60. Dermietzel R, Hertzberg EL, Kessler JA, Spray DC. Gap-junctions between cultured astrocytes - immunocytochemical, molecular, and electrophysiological analysis. *J Neurosci.* 1991;11:1421–32.
61. Fatemi SH, Folsom TD, Reutiman TJ, Pandian T, Braun NN, Haug K. Chronic psychotropic drug treatment causes differential expression of connexin 43 and GFAP in frontal cortex of rats. *Schizophr Res.* 2008;104:127–34.
62. Morioka N, Suekama K, Zhang FF, Kajitani N, Hisaoka-Nakashima K, Takebayashi M, et al. Amitriptyline up-regulates connexin43-gap junction in rat cultured cortical astrocytes via activation of the p38 and c-Fos/AP-1 signalling pathway. *Br J Pharmacol.* 2014;171:2854–67.
63. Ohgi Y, Futamura T, Kikuchi T, Hashimoto K. Effects of antidepressants on alterations in serum cytokines and depressive-like behavior in mice after lipopolysaccharide administration. *Pharmacol Biochem Behav.* 2013;103:853–9.
64. Custodio CS, Mello BS, Cordeiro RC, de Araujo FY, Chaves JH, Vasconcelos SM, et al. Time course of the effects of lipopolysaccharide on prepulse inhibition and brain nitrite content in mice. *Eur J Pharmacol.* 2013;713:31–8.
65. Painsipp E, Kofer MJ, Sinner F, Holzer P. Prolonged depression-like behavior caused by immune challenge: influence of mouse strain and social environment. *PLoS One.* 2011;6:e20719.
66. Tai YH, Tsai RY, Lin SL, Yeh CC, Wang JJ, Tao PL, et al. Amitriptyline suppresses neuroinflammation-dependent interleukin-10-p38 mitogen-activated protein kinase-heme oxygenase-1 signaling pathway in chronic morphine-infused rats. *Anesthesiology.* 2009;110:1379–89.
67. Valera E, Ubhi K, Mante M, Rockenstein E, Masliah E. Antidepressants reduce neuroinflammatory responses and astroglial alpha-synuclein accumulation in a transgenic mouse model of multiple system atrophy. *Glia.* 2014;62:317–37.
68. Obuchowicz E, Kowalski J, Labuzek K, Krysiak R, Pendzich J, Herman ZS. Amitriptyline and nortriptyline inhibit interleukin-1 release by rat mixed glial and microglial cell cultures. *Int J Neuropsychopharmacol.* 2006;9:27–35.
69. Liu RP, Zou M, Wang JY, Zhu JJ, Lai JM, Zhou LL, et al. Paroxetine ameliorates lipopolysaccharide-induced microglia activation via differential regulation of MAPK signaling. *J Neuroinflammation.* 2014;11:47.
70. Lee YH, Kim SH, Kim Y, Lim Y, Ha K, Shin SY. Inhibitory effect of the antidepressant imipramine on NF-kappaB-dependent CXCL1 expression in TNFalpha-exposed astrocytes. *Int Immunopharmacol.* 2012;12:547–55.
71. Ye ZC, Wyeth MS, Baltan-Tekkok S, Ransom BR. Functional hemichannels in astrocytes: a novel mechanism of glutamate release. *J Neurosci.* 2003;23:3588–96.
72. Gorman JM, Docherty JP. A hypothesized role for dendritic remodeling in the etiology of mood and anxiety disorders. *J Neuropsychiatry Clin Neurosci.* 2010;22:256–64.
73. Liu XH, Gangoso E, Yi CJ, Jeanson T, Kandelman S, Mantz J, et al. General anesthetics have differential inhibitory effects on gap junction channels and hemichannels in astrocytes and neurons. *Glia.* 2016;64:524–36.
74. Idvall J, Ahlgren I, Aronsen KR, Stenberg P. Ketamine infusions: pharmacokinetics and clinical effects. *Br J Anaesth.* 1979;51:1167–73.
75. Cohen ML, Chan SL, Way WL, Trevor AJ. Distribution in the brain and metabolism of ketamine in the rat after intravenous administration. *Anesthesiology.* 1973;39:370–6.
76. Lalo U, Pankratov Y, Kirchoff F, North RA, Verkhratsky A. NMDA receptors mediate neuron-to-glia signaling in mouse cortical astrocytes. *J Neurosci.* 2006;26:2673–83.
77. Velez-Fort M, Audinat E, Angulo MC. Central role of GABA in neuron-glia interactions. *Neuroscientist.* 2012;18:237–50.
78. Citri A, Malenka RC. Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropharmacology.* 2008;33:18–41.
79. Herculano-Houzel S. The glia/neuron ratio: how it varies uniformly across brain structures and species and what that means for brain physiology and evolution. *Glia.* 2014;62:1377–91.
80. Singh SK, Stogsdill JA, Pulimood NS, Dingsdale H, Kim YH, Pilaz LJ, et al. Astrocytes assemble thalamocortical synapses by bridging NRX1alpha and NL1 via hevin. *Cell.* 2016;164:183–96.
81. Allen NJ, Bennett ML, Foo LC, Wang GX, Chakraborty C, Smith SJ, et al. Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. *Nature.* 2012;486:410–4.
82. Perea G, Navarrete M, Araque A. Tripartite synapses: astrocytes process and control synaptic information. *Trends Neurosci.* 2009;32:421–31.
83. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* 1999;22:208–15.
84. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR 3rd, Lafaille JJ, et al. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. *Cell.* 2013;155:1596–609.

85. Wang Q, Jie W, Liu JH, Yang JM, Gao TM. An astroglial basis of major depressive disorder? an overview. *Glia*. 2017;65:1227–50.
86. Ransom BR, Ransom CB. Astrocytes: multitasked stars of the central nervous system. *Methods Mol Biol*. 2012;814:3–7.
87. Danbolt NC. Glutamate uptake. *Prog Neurobiol*. 2001;65:1–105.
88. Bak LK, Schousboe A, Waagepetersen HS. The glutamate/GABA-glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. *J Neurochem*. 2006;98:641–53.
89. Jimenez E, Nunez E, Ibanez I, Draffin JE, Zafra F, Gimenez C. Differential regulation of the glutamate transporters GLT-1 and GLAST by GSK3beta. *Neurochem Int*. 2014;79:33–43.
90. Figiel M, Allritz C, Lehmann C, Engle J. Gap junctional control of glial glutamate transporter expression. *Mol Cell Neurosci*. 2007;35:130–7.
91. Hertz L, Zielke HR. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. *Trends Neurosci*. 2004;27:735–43.
92. Popoli M, Yan Z, McEwen BS, Sanacora G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat Rev Neurosci*. 2011;13:22–37.
93. Valentine GW, Sanacora G. Targeting glial physiology and glutamate cycling in the treatment of depression. *Biochem Pharmacol*. 2009;78:431–9.
94. Stehberg J, Moraga-Amaro R, Salazar C, Becerra A, Echeverria C, Orellana JA, et al. Release of gliotransmitters through astroglial connexin 43 hemichannels is necessary for fear memory consolidation in the basolateral amygdala. *FASEB J*. 2012;26:3649–57.
95. Bernstein HG, Meyer-Lotz G, Dobrowolny H, Bannier J, Steiner J, Walter M, et al. Reduced density of glutamine synthetase immunoreactive astrocytes in different cortical areas in major depression but not in bipolar I disorder. *Front Cell Neurosci*. 2015;9:273.
96. Merkl A, Schubert F, Quante A, Luborzewski A, Brakemeier EL, Grimm S, et al. Abnormal cingulate and prefrontal cortical neurochemistry in major depression after electroconvulsive therapy. *Biol Psychiatry*. 2011;69:772–9.
97. Chowdhury GM, Behar KL, Cho W, Thomas MA, Rothman DL, Sanacora G. (1)H-[(1)(3)C]-nuclear magnetic resonance spectroscopy measures of ketamine's effect on amino acid neurotransmitter metabolism. *Biol Psychiatry*. 2012;71:1022–5.
98. Milak MS, Proper CJ, Mulhern ST, Parter AL, Kegeles LS, Ogden RT, et al. A pilot in vivo proton magnetic resonance spectroscopy study of amino acid neurotransmitter response to ketamine treatment of major depressive disorder. *Mol Psychiatry*. 2016;21:320–7.
99. Rae C, Hare N, Bubb WA, McEwan SR, Broer A, McQuillan JA, et al. Inhibition of glutamine transport depletes glutamate and GABA neurotransmitter pools: further evidence for metabolic compartmentation. *J Neurochem*. 2003;85:503–14.
100. Ghosal S, Hare B, Duman RS. Prefrontal cortex GABAergic deficits and circuit dysfunction in the pathophysiology and treatment of chronic stress and depression. *Curr Opin Behav Sci*. 2017;14:1–8.
101. Krystal JH, Sanacora G, Blumberg H, Anand A, Charney DS, Marek G, et al. Glutamate and GABA systems as targets for novel antidepressant and mood-stabilizing treatments. *Mol Psychiatry*. 2002;7:571–80.
102. Pehrson AL, Sanchez C. Altered gamma-aminobutyric acid neurotransmission in major depressive disorder: a critical review of the supporting evidence and the influence of serotonergic antidepressants. *Drug Des Devel Ther*. 2015;9:603–24.
103. Hughes EG, Maguire JL, McMinn MT, Scholz RE, Sutherland ML. Loss of glial fibrillary acidic protein results in decreased glutamate transport and inhibition of PKA-induced EAAT2 cell surface trafficking. *Brain Res Mol Brain Res*. 2004;124:114–23.
104. Cao X, Li LP, Wang Q, Wu Q, Hu HH, Zhang M, et al. Astrocyte-derived ATP modulates depressive-like behaviors. *Nat Med*. 2013;19:773–7.
105. Yang J, Yang H, Liu Y, Li X, Qin L, Lou H, et al. Astrocytes contribute to synapse elimination via type 2 inositol 1,4,5-trisphosphate receptor-dependent release of ATP. *Elife*. 2016;5:e15043.
106. Berridge MJ. Neuronal calcium signaling. *Neuron*. 1998;21:13–26.
107. Torres A, Wang F, Xu Q, Fujita T, Dobrowolski R, Willecke K, et al. Extracellular Ca²⁺ acts as a mediator of communication from neurons to glia. *Sci Signal*. 2012;5:ra8.
108. Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science*. 1990;247:470–3.
109. Scemes E, Giaume C. Astrocyte calcium waves: what they are and what they do. *Glia*. 2006;54:716–25.
110. Agulhon C, Petracvic J, McMullen AB, Sweger EJ, Minton SK, Taves SR, et al. What is the role of astrocyte calcium in neurophysiology? *Neuron*. 2008;59:932–46.
111. Parpura V, Basarsky TA, Liu F, Jęftinija K, Jęftinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature*. 1994;369:744–7.
112. Nedergaard M. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science*. 1994;263:1768–71.
113. Kuga N, Sasaki T, Takahara Y, Matsuki N, Ikegaya Y. Large-scale calcium waves traveling through astrocytic networks in vivo. *J Neurosci*. 2011;31:2607–14.
114. Finkbeiner S. Calcium waves in astrocytes-filling in the gaps. *Neuron*. 1992;8:1101–8.
115. Majumder P, Crispino G, Rodriguez L, Ciubotaru CD, Anselmi F, Piazza V, et al. ATP-mediated cell-cell signaling in the organ of Corti: the role of connexin channels. *Purinergic Signal*. 2010;6:167–87.
116. Weissman TA, Riquelme PA, Ivic L, Flint AC, Kriegstein AR. Calcium waves propagate through radial glial cells and modulate proliferation in the developing neocortex. *Neuron*. 2004;43:647–61.
117. Kumada T, Komuro H. Completion of neuronal migration regulated by loss of Ca²⁺ transients. *Proc Natl Acad Sci USA*. 2004;101:8479–84.
118. Montero TD, Orellana JA. Hemichannels: new pathways for gliotransmitter release. *Neuroscience*. 2015;286:45–59.
119. Suadicani SO, Brosnan CF, Scemes E. P2X7 receptors mediate ATP release and amplification of astrocytic intercellular Ca²⁺ signaling. *J Neurosci*. 2006;26:1378–85.
120. Wink MR, Braganhol E, Tamajusuku AS, Lenz G, Zerbini LF, Libermann TA, et al. Nucleoside triphosphate diphosphohydrolase-2 (NTPDase2/CD39L1) is the dominant ectonucleotidase expressed by rat astrocytes. *Neuroscience*. 2006;138:421–32.
121. Cao X, Li LP, Qin XH, Li SJ, Zhang M, Wang Q, et al. Astrocytic adenosine 5'-triphosphate release regulates the proliferation of neural stem cells in the adult hippocampus. *Stem Cells*. 2013;31:1633–43.
122. Jun M, Xiaolong Q, Chaojuan Y, Ruiyuan P, Shukun W, Junbing W, et al. Calhm2 governs astrocytic ATP releasing in the development of depression-like behaviors. *Mol Psychiatry*. 2018;23:1091.
123. Kang J, Kang N, Lovatt D, Torres A, Zhao Z, Lin J, et al. Connexin 43 hemichannels are permeable to ATP. *J Neurosci*. 2008;28:4702–11.
124. Joseph DN, Whirlidge S. Stress and the HPA axis: balancing homeostasis and fertility. *Int J Mol Sci*. 2017;18:2224.
125. Juruena MF, Erer F, Cleare AJ, Young AH. The role of early life stress in HPA axis and anxiety. *Adv Exp Med Biol*. 2020;1191:141–53.
126. Keller J, Gomez R, Williams G, Lembke A, Lazzaroni L, Murphy GM Jr, et al. HPA axis in major depression: cortisol, clinical symptomatology and genetic variation predict cognition. *Mol Psychiatry*. 2017;22:527–36.
127. Nicolaidis NC, Kyratzi E, Lamprokostopoulou A, Chrousos GP, Charmandari E. Stress, the stress system and the role of glucocorticoids. *Neuroimmunomodulation*. 2015;22:6–19.
128. Jin C, Wang ZZ, Zhou H, Lou YX, Chen J, Zuo W, et al. Ginsenoside Rg1-induced antidepressant effects involve the protection of astrocyte gap junctions within the prefrontal cortex. *Prog Neuro-Psychoph*. 2017;75:183–91.
129. Xia CY, Chu SF, Zhang S, Gao Y, Ren Q, Lou YX, et al. Ginsenoside Rg1 alleviates corticosterone-induced dysfunction of gap junctions in astrocytes. *J Ethnopharmacol*. 2017;208:207–13.
130. Lampe PD, TenBroek EM, Burt JM, Kurata WE, Johnson RG, Lau AF. Phosphorylation of connexin43 on serine368 by protein kinase C regulates gap junctional communication. *J Cell Biol*. 2000;149:1503–12.
131. Cone AC, Cavin G, Ambrosi G, Hakoziaki H, Wu-Zhang AX, Kunkel MT, et al. Protein kinase Cdelta-mediated phosphorylation of Connexin43 gap junction channels causes movement within gap junctions followed by vesicle internalization and protein degradation. *J Biol Chem*. 2014;289:8781–98.
132. Goldstein JM, Holsen L, Huang G, Hammond BD, Todd TJ, Cherkizian S. Prenatal stress-immune programming of sex differences in comorbidity of depression and obesity/metabolic syndrome. *Dialogues Clin Neurosci*. 2016;18:425–36.
133. Hryhorczuk C, Sharma S, Fulton SE. Metabolic disturbances connecting obesity and depression. *Front Neurosci*. 2013;7:177.
134. Bian O, Zhang H, Guan Q, Sun Y, Zeng D. High-dose insulin inhibits gap junction intercellular communication in vascular smooth muscle cells. *Mol Med Rep*. 2015;12:331–6.
135. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab*. 2001;86:1930–5.
136. Lewis GF, Uffelman KD, Szeto LW, Steiner G. Effect of acute hyperinsulinemia on VLDL triglyceride and VLDL apoB production in normal weight and obese individuals. *Diabetes*. 1993;42:833–42.
137. Ozcan U, Yilmaz E, Ozcan L, Furuhashi M, Vaillancourt E, Smith RO, et al. Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. *Science*. 2006;313:1137–40.
138. Hotamisligil GS. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. *Cell*. 2010;140:900–17.

139. Lin JH, Weigel H, Cotrina ML, Liu S, Bueno E, Hansen AJ, et al. Gap-junction-mediated propagation and amplification of cell injury. *Nat Neurosci*. 1998;1:494–500.
140. Spray DC, Hanstein R, Lopez-Quintero SV, Stout RF Jr., Suadicani SO, Thi MM. Gap junctions and bystander effects: good samaritans and executioners. *Wiley Interdiscip Rev Membr Transp Signal*. 2013;2:1–15.
141. Liu YY, Brent GA. Thyroid hormone and the brain: Mechanisms of action in development and role in protection and promotion of recovery after brain injury. *Pharmacol Ther*. 2018;186:176–85.
142. Bernal J. Thyroid hormone receptors in brain development and function. *Nat Clin Pr Endocrinol Metab*. 2007;3:249–59.
143. Dezonne RS, Lima FR, Trentin AG, Gomes FC. Thyroid hormone and astroglia: endocrine control of the neural environment. *J Neuroendocrinol*. 2015;27:435–45.
144. Joffe RT, Sullivan TB. The significance of an isolated elevated TSH level in a depressed patient: a clinical commentary. *Int J Psychiatry Med*. 2014;48:167–73.
145. Zhang ZJ, Li Q, Kang WH, Tan QR, Gao CG, Zhang FG, et al. Differences in hypothyroidism between lithium-free and -treated patients with bipolar disorders. *Life Sci*. 2006;78:771–6.
146. Duval F, Mokrani MC, Erb A, Gonzalez Lopera F, Alexa C, Proudnikova X, et al. Chronobiological hypothalamic-pituitary-thyroid axis status and antidepressant outcome in major depression. *Psychoneuroendocrinology*. 2015;59:71–80.
147. Hage MP, Azar ST. The link between thyroid function and depression. *J Thyroid Res*. 2012;2012:590648.
148. Tossell L, Rusby E. Palliative care in the undergraduate curriculum: a medical student's perspective. *Palliat Med*. 2010;24:839–40.
149. Almeida OP, Alfonso H, Flicker L, Hankey G, Chubb SA, Yeap BB. Thyroid hormones and depression: the health in men study. *Am J Geriatr Psychiatry*. 2011;19:763–70.
150. Dwyer JB, Aftab A, Radhakrishnan R, Widge A, Rodriguez CI, Carpenter LL, et al. Hormonal treatments for major depressive disorder: state of the art. *Am J Psychiatry*. 2020;177:686–705.
151. Munari-Silem Y. Gap junctions in the thyroid gland: distribution regulation, function. *Ann Endocrinol-Paris*. 1996;57:484–6.
152. Li GY, Jiang XY. Expression of Cx43 in thyroid epithelial cells from the patients with Hashimoto disease. *J Harbin Med Univ*. 2009;43:584–9. Chinese.
153. Munari-Silem Y. Gap junctions in the thyroid gland: distribution, regulation, function. *Ann Endocrinol (Paris)*. 1996;57:484–6.
154. Serretti A, Chiesa A. Treatment-emergent sexual dysfunction related to antidepressants: a meta-analysis. *J Clin Psychopharmacol*. 2009;29:259–66.
155. Griswold MD. The central role of Sertoli cells in spermatogenesis. *Semin Cell Dev Biol*. 1998;9:411–6.
156. Camara ML, Almeida TB, de Santi F, Rodrigues BM, Cerri PS, Beltrame FL, et al. Fluoxetine-induced androgenic failure impairs the seminiferous tubules integrity and increases ubiquitin carboxyl-terminal hydrolase L1 (UCHL1): possible androgenic control of UCHL1 in germ cell death? *Biomed Pharmacother*. 2019;109:1126–39.
157. Loftis JM. Sertoli cell therapy: a novel possible treatment strategy for treatment-resistant major depressive disorder. *Med Hypotheses*. 2011;77:35–42.
158. Tfilin M, Sudai E, Merenlender A, Gispán I, Yadid G, Turgeman G. Mesenchymal stem cells increase hippocampal neurogenesis and counteract depressive-like behavior. *Mol Psychiatry*. 2010;15:1164–75.
159. Balasubramanian V, Dhar DK, Warner AE, Vivien Li WY, Amiri AF, Bright B, et al. Importance of connexin-43 based gap junction in cirrhosis and acute-on-chronic liver failure. *J Hepatol*. 2013;58:1194–200.
160. Rouach N, Koulakoff A, Abudara V, Willecke K, Giaume C. Astroglial metabolic networks sustain hippocampal synaptic transmission. *Science*. 2008;322:1551–5.
161. SÁEz JC, Berthoud VM, BraÑEs MC, MartÍnez AD, Beyer EC. Plasma membrane channels formed by connexins: their regulation and functions. *Physiological Rev*. 2003;83:1359–400.
162. Yang YM, Seki E. Global spread of a local fire: transmission of endoplasmic reticulum stress via connexin 43. *Cell Metab*. 2021;33:229–30.
163. Capuron L, Miller AH. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacol Ther*. 2011;130:226–38.
164. Ma K, Zhang H, Baloch Z. Pathogenetic and therapeutic applications of tumor necrosis factor-alpha (TNF-alpha) in major depressive disorder: a systematic review. *Int J Mol Sci*. 2016;17:733.
165. Ting EY, Yang AC, Tsai SJ. Role of interleukin-6 in depressive disorder. *Int J Mol Sci*. 2020;21:2194.
166. Lang UE, Borgwardt S. Molecular mechanisms of depression: perspectives on new treatment strategies. *Cell Physiol Biochem*. 2013;31:761–77.
167. Kim Y, Davidson JO, Gunn KC, Phillips AR, Green CR, Gunn AJ. Role of hemichannels in CNS inflammation and the inflammasome pathway. *Adv Protein Chem Struct Biol*. 2016;104:1–37.
168. Reuss B, Dermietzel R, Unsicker K. Fibroblast growth factor 2 (FGF-2) differentially regulates connexin (cx) 43 expression and function in astroglial cells from distinct brain regions. *Glia*. 1998;22:19–30.
169. Martinez AD, Saez JC. Arachidonic acid-induced dye uncoupling in rat cortical astrocytes is mediated by arachidonic acid byproducts. *Brain Res*. 1999;816:411–23.
170. Belousov AB, Fontes JD, Freitas-Andrade M, Naus CC. Gap junctions and hemichannels: communicating cell death in neurodevelopment and disease. *BMC Cell Biol*. 2017;18:4.
171. Makarenkova HP, Shestopalov VI. The role of pannexin hemichannels in inflammation and regeneration. *Front Physiol*. 2014;5:63.
172. Kim Y, Griffin JM, Nor MNM, Zhang J, Freestone PS, Danesh-Meyer HV, et al. Tonabersat prevents inflammatory damage in the central nervous system by blocking connexin43 hemichannels. *Neurotherapeutics*. 2017;14:1148–65.
173. De Flora A, Zocchi E, Guida L, Franco L, Bruzzone S. Autocrine and paracrine calcium signaling by the CD38/NAD⁺/cyclic ADP-ribose system. *Ann N Y Acad Sci*. 2004;1028:176–91.
174. Beyer K. Age at onset: an essential variable for the definition of genetic risk factors for sporadic Alzheimer's disease. *Ann N Y Acad Sci*. 2005;1057:260–78.
175. Giepmans BN. Gap junctions and connexin-interacting proteins. *Cardiovasc Res*. 2004;62:233–45.
176. Caraci F, Spampinato SF, Morgese MG, Tascadda F, Salluzzo MG, Giambirtone MC, et al. Neurobiological links between depression and AD: the role of TGF-beta1 signaling as a new pharmacological target. *Pharmacol Res*. 2018;130:374–84.
177. Sarrouilhe D, Dejean C, Mesnil M. Connexin43- and pannexin-based channels in neuroinflammation and cerebral neuropathies. *Front Mol Neurosci*. 2017;10:320.
178. Georjin-Lavialle S, Moura DS, Salvador A, Chauvet-Gelinier JC, Launay JM, Damaj G, et al. Mast cells' involvement in inflammation pathways linked to depression: evidence in mastocytosis. *Mol Psychiatry*. 2016;21:1511–6.