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Emerging role of glutamate in the pathophysiology and therapeutics of Gulf War illness

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Emerging role of glutamate in the pathophysiology and therapeutics of Gulf War illness

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4. Abstract

Gulf War illness (GWI) is a chronic and multi-symptomatic disorder affecting veterans who served in the Gulf War. The commonly reported symptoms in GWI veterans include mood problems, cognitive impairment, muscle and joint pain, migraine/headache, chronic fatigue, gastrointestinal complaints, skin rashes, and respiratory problems. Neuroimaging studies have revealed significant brain structure alterations in GWI veterans, including subcortical atrophy, decreased volume of the hippocampus, reduced total grey and white matter, and increased brain white matter axial diffusivity. These brain changes may contribute to or increase the severities of the GWI-related symptoms. Epidemiological studies have revealed that neurotoxic exposures and stress may be significant contributors to the development of GWI. However, the mechanism underlying how the exposure and stress could contribute to the multi-symptomatic disorder of GWI remains unclear. We and others have demonstrated that rodent models exposed to GW-related agents and stress exhibited higher extracellular glutamate levels, as well as impaired structure and function of glutamatergic synapses. Restoration of the glutamatergic synapses ameliorated the GWI-related pathological and behavioral deficits. Moreover, recent studies showed that a low-glutamate diet reduced multiple symptoms in GWI veterans, suggesting an important role of the glutamatergic system in GWI. Currently, growing evidence has indicated that abnormal glutamate neurotransmission may contribute to the GWI symptoms. This review summarizes the potential roles of glutamate dyshomeostasis and dysfunction of the glutamatergic system in linking the initial cause to the multi-symptomatic outcomes in GWI and suggests the glutamatergic system as a therapeutic target for GWI.

5. Key words

Gulf War illness

Glutamatergic system

Therapy

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Intro

Gulf War illness

Nearly one-third of veterans who served in the first Gulf War in 1990-1991 have experienced a variety of symptoms that are difficult to explain by a typical medical illness, commonly known as "Gulf War illness" (GWI)[1]. GWI affects each individual differently, but overall, evidence indicates that multiple organ systems are involved, including the nervous system, digestive system, and respiratory system[2]. The typical symptoms reported in GWI veterans include fatigue, joint pain, memory loss, sleep difficulties, headaches, concentration loss, depression and anxiety, skin rashes, gastrointestinal problems, and breathing problems[3]. Subsets of the symptoms reported by GWI veterans are also frequently observed in other disorders, such as anxiety disorder, major depression disorder (MDD), chronic fatigue syndrome (CFS), fibromyalgia, and irritable bowel syndrome (IBS)[3]. The wide variety in the symptomatology makes it hard to identify a single entity as the cause. Epidemiological investigation of large cohorts revealed that a combination of war-related traumatic stress and exposure to chemical agents, such as pyridostigmine bromide (given to soldiers as an anti-nerve agent pretreatment), sarin nerve agent, pesticides, and smoke from burning oil wells may account for these multiple symptoms[4,5]. However, the detailed mechanism linking the GWI-related exposure to multi-symptomatic health outcomes remains unclear.

Glutamatergic system

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system (CNS). It is released by nerve cells and is responsible for sending signals between nerve cells. A typical glutamate transmission contains three steps. The first step is glutamate release through vesicular glutamate transporters (vGLUT1-3), which function to package glutamate for exocytotic release. The second step is receptor signaling through glutamate receptors. There are two different types of glutamate receptors in the CNS: ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). The iGluRs mediate the majority of excitatory synaptic transmission and include N-methyl-D-aspartate (NMDA) receptor, α-amino-3 hydroxy- 5-methyl-4-isoxazolepropionic acid (AMPA) receptor, and kainate receptor. The mGluRs participate in the modulation of excitatory synaptic transmission and are subclassified into three groups[6]. Group I receptors include mGluR1 and mGluR5, which postsynaptically facilitate NMDA receptor activity and increase synaptic plasticity. Group II receptors include mGluR2 and mGluR3. Group III receptors include mGluR4, mGluR6, mGluR7, and mGluR8. Both Group II and Group III receptors presynaptically inhibit glutamate release and decrease neuronal excitability[7]. The third step is glutamate uptake through plasma membrane excitatory amino-acid transporters (EAAT1-5), which are mainly located at glial cell processes and are responsible for clearing glutamate in the synaptic cleft. Under normal conditions, glutamate plays an important role in regulating emotional and cognitive function. However, when glutamate accumulates in the brain, excess glutamate activates glutamate receptors, and exerts structural and functional effects, including axonal degeneration, dendritic remodeling, elimination of synapses, and possibly volumetric reductions[8-11]. These

effec been implicated in many neurological disorders.

Glutamatergic dysfunction and GWI

Substantial evidence indicates that GW-related agents and traumatic stress (GW-related exposures) affect glutamate transmission in the brain. Pyridostigmine bromide (PB), pesticides, and sarin are organophosphate or carbamate acetylcholinesterase inhibitors[12,13]. In normal situations, they cannot pass the blood-brain barrier. However, under stress conditions, these agents can enter the brain and inhibit acetylcholinesterase activity, leading to elevated acetylcholine levels, which results in increased spontaneous glutamate release via overactivation of muscarinic receptors located on presynaptic terminals[14,15]. Moreover, accumulating evidence indicates that stress increases the readily releasable pool of glutamate vesicles, and further results in increased glutamate release in the brain[16-18]. In our previous study, we observed elevated glutamate levels in the hippocampi of mice subjected to GW-related exposures; a similar result was also reported in a rat model of GWI[19,20]. Elevated glutamate could lead to excitotoxic damage and synaptic dysfunction[21]. Studies have demonstrated that exposure to an organophosphate pesticide, individually or together with other GW agents, results in hippocampal synaptic integrity impairment[22,23]. Additionally, stress has been reported to result in losses of dendrites and their synapses, resulting in decreased hippocampal grey matter volume[24]. Decreased thickness of hippocampal CA3 and CA1 has been reported in a rat model of GWI[25], which is consistent with GWI veterans[26-28]. Taken together, current literature suggests that the abnormal glutamate transmission caused by chronic GW-related exposures may lead to structural and functional alterations in the brain, and consequently result in the development of GWI symptoms. In the following section, we discuss the potential roles of glutamatergic system dysfunction in different symptoms of GWI. A summary diagram is shown in Fig. 1.

Targeting glutamatergic system as a potential therapy for GWI

Mood problems

Epidemiological studies have demonstrated a prevalence of 15%-35% for anxiety or depression in GWI veterans[5,29]. This could result from the brain structure alteration. By structural magnetic resonance imaging (MRI) scans, Zhang *et al.* found that GWI veterans exhibited significant brainstem atrophy; the resulting atrophy (especially of midbrain volume) could in turn mediate or moderate symptoms shown in GWI veterans, such as depression[30]. Interestingly, decreased volume of hippocampus, which is commonly reported in patients with MDD, has also been observed in GWI veterans[26-28,31,32].

GW-related exposures increase the glutamate levels and trigger anxiety and depression in the animal models[33-36]. Abdullah *et al.* and Carreras *et al.* reported that rodent model of GWI exhibited significant anxiety and depression phenotype at three-months post-exposure[33,34]. These symptoms are often accompanied by increased glutamate levels, impaired synaptic integrity, reduced neurogenesis, damaged neuronal

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differ bited significant anxiety and depression at both three- and six- months post-exposure, concomitant with higher glutamate levels and impaired structure and function of tripartite glutamatergic synapses. Importantly, normalizing the glutamate level by LDN/OSU-215111, a pyridazine derivative that can strengthen the structure and function of tripartite synapses[37], restored the glutamatergic synapses and significantly reduced the anxiety and depression symptoms[19]. The tripartite glutamatergic synapses, comprising a presynaptic terminal, a postsynaptic spine, and an astrocytic process, play a critical role in glutamate transmission[37]. Furthermore, in a recent clinic trial, Brandley *et al*. demonstrated that one-month of low glutamate diet was sufficient to reduce anxiety and depression in GWI veterans[38]. These studies suggest that targeting glutamatergic system abnormalities may play an anxiolytic and antidepressant role in GWI.

Both pre-clinical and clinical studies have demonstrated that normalizing glutamatergic dysfunction ameliorated anxiety or depression symptoms in different psychiatric disorders. Riluzole, which is thought to block glutamate release, significantly reduced anxiety symptoms in patients with generalized anxiety disorder and obsessive-compulsive disorder (OCD)[39,40]. Riluzole also exhibits antidepressant effect in MDD[41]. Currently, increasing interests have been put on drugs that block the NMDAR activity, of which ketamine has received the most attention. Ketamine is an NMDAR antagonist, which was originally approved by the FDA as an anesthetic. However, it is increasingly being used as a treatment for anxiety and depression symptoms in different psychiatric disorders. Glue *et al.* reported that ketamine reduced the anxiety in patients with refractory anxiety disorder within an hour of dosing, and this effect persisted for up to 7 days[42]. It also improves depression scores in patients with anxious bipolar depression[43]. The mechanism underlying ketamine-induced anxiolytic and antidepressant effect is not only inhibition of NMDAR activity, but also enhanced function of AMPAR[44]. Enhancement of AMPAR function has been considered as a novel strategy for antidepressant-like effects[45]. Besides the ionotropic glutamate receptors NMDAR and AMPAR, alteration of metabotropic glutamate receptors has also reported in anxiety or depression disorders. Feyissa *et al*. showed a significantly elevated mGluR2/3 level in the prefrontal cortex of patients with MDD, which is believed to modulate glutamatergic neurotransmission by sensing glutamate spillover and regulating transmitter release[46]. Blockade of mGluR2/3 with antagonists exerted antidepressant and anxiolytic effects in different rodent models[47-49]. Anxiolytic or antidepressant-like effects have also been reported with group I mGluRs antagonists[50,51]. Similar effects have been observed with group III mGluRs antagonist CPPG[52]. In addition, drugs that enhance glutamate uptake via increasing EAAT2 expression, such as ceftriaxone and LDN/OSU-215111, have also been demonstrated to significantly reduce anxiety and depression symptoms in different models[19,53-55]. Overall, these studies suggest that targeting glutamatergic system dysfunction could be an effective approach to treat anxiety and depression symptoms in GWI.

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Epidemiological studies revealed that 25-40% of GWI veterans experienced cognitive impairment[5,29]. The symptoms vary within individuals, and the most frequently reported include forgetfulness, difficulties with short- and long-term memory, and difficulties with thinking/reasoning problems[3]. This cognitive impairment could be an outcome of brain structure alteration. MRI studies have revealed changes in the structure or function of specific brain regions that are associated with cognition in GWI veterans. Chao *et al.* reported a positive correlation between total white matter volume and executive function and visuospatial abilities, which decreased in GWI veterans exposed to sarin/cyclosarin[56]. Later, they identified a significant reduction of total gray and white matter volumes in veterans exposed to sarin/cyclosarin, which is positively related to distractibility[57]. In addition, Odegard *et al.* reported a decreased amount of activation in the left hippocampus in GWI veterans, which is related to decreased memory performance[31]. Significant differences in prefrontal cortex activity were also recorded in GWI veterans during a working memory task, indicating allocation of high demand to working memory loads[58]. Recently, Zhang *et al.* also identified a strong correlation between brainstem atrophy and memory impairment in GWI veterans[30].

Cognitive difficulties have also been observed in animal models of GWI. In our previous study, we found that GWI mice exhibited recognition and spatial memory deficits. These deficits occurred at three-months post-exposure and worsened at six-months post-exposure, which is consistent with GWI veterans, as cognition declined with time[19]. Zakirova *et al.* and Parihar *et al.* reported similar results in different rodent models[35,36,59]. Interestingly, Abdel-Rahman *et al.* reported that GW-related exposures significantly decrease the thickness of hippocampus in rats[25]. Smaller total hippocampal volume was also found in GWI veterans[26].

Dysfunction of glutamate transmission is considered as one of the contributors to cognitive deficits. Researchers have demonstrated that insufficient glutamate release leads to cognition impairment. Knockout of vGLUT1 in mice leads to reduced long-term memory, impaired visual attention, and reduction of the dynamic range of short-term plasticity[60,61]. Blockade of glutamate receptor activity could also contribute to cognition impairment. Ketamine, an NMDA receptor antagonists, produced decrements in free recall, recognition memory, and attention in healthy volunteers[62]. Similar results have also been observed in animal models. Stefani *et al.* reported that systemic administration of MK-801, another NMDA receptor antagonist, impaired set-shifting ability in rats[63]. In the same study mentioned above, we observed that GW-related exposures also significantly impaired the recognition and spatial memory, accompanied by abnormal glutamatergic transmission. Importantly, restoring the glutamate transmission by LDN/OSU-215111 significantly normalized the cognitive deficits[19]. In a clinical trial, Kirkland *et al.* reported that after one-month of low glutamate diet, several cognitive domains, including neurocognitive function, executive functioning, cognitive flexibility, motor speed, processing speed, and psychomotor speed, were significantly improved in GWI veterans[64]. These studies strongly indicate a critical role of the glutamatergic system in cognitive functions.

Positive modulation of glutamate transmission has been proven as a strategy for treating cognitive dysfunction. Levin *et al*. reported that oral administration of D-serine, an NMDAR-glycine site obligatory co-

agon ects[65]. NMDAR participate in dendritic synaptic integration and are critical for generating persistent activity of neural assemblies[66], while AMPAR mediate fast excitatory neurotransmission and are selectively recruited during activity-dependent plasticity to increase synaptic strength[67]. In a rat model of perinatal stress, Fletcherah *et al.* showed that S 47445, a positive modulator of AMPAR, significantly improved social memory, concomitant with increased depolarization-evoked glutamate release and trafficking of synaptic vesicles[68]. Studies have also demonstrated an important role of mGluR modulators in restoring cognitive function. Nikiforuk *et al.* reported that a positive allosteric modulator of group II metabotropic glutamate receptors, LY487379, increased cognitive flexibility and impulsive-like responses in rats[69]. While a negative allosteric modulator of mGluR3 reversed the positive effects of the mGluR2/3 orthosteric agonist LY379268 on synaptic plasticity and learning abilities, suggesting an important role of mGluR3 in cognitive function[70]. In addition, Horio *et al.* demonstrated that a 14-day administration of CDPPB (3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide), a positive allosteric modulator of mGluR5, restored the cognitive deficits[71]. Furthermore, drugs that increase glutamate uptake, such as ceftriaxone and LDN/OSU-215111, have also been reported to significantly normalize the cognitive deficits in different models[19,72-75]. Taken together, these studies suggest that enhancing glutamate transmission could be an effective approach to treating cognitive deficits in GWI.

Muscle and joint pain

Widespread chronic pain is the most prevalent symptom reported in GWI veterans, in which muscle pain and joint pain are often reported[76,77]. Alteration of brain activity may contribute to the pain in GWI. Rayhan *et al.* reported that increased brain white matter axial diffusivity in the right inferior fronto-occipital fasciculus positively correlated to the pain in GWI[78]. In addition, Riper *et al.* has demonstrated that the cerebral white matter structure is disrupted in GWI veterans with chronic musculoskeletal pain[79]. Furthermore, Lindheimer *et al.* demonstrated that GWI veterans with chronic musculoskeletal pain are more sensitive to pain anticipation, as they observed that these subjects displayed a significant incremental linear decrease in the middle temporal gyrus under pain stimuli condition[80].

GW-related exposures induce long-lasting musculoskeletal pain in animal models[77,81]. Lacagnina *et al.* reported that rat subjected to GW-agent and a 7-day of corticosterone (to mimic high physiological stress) developed long-lasting, bilateral allodynia[81]. Nutter *et al.* reported a delay of pain symptom in rats subjected to GW-agents, and these symptoms worsen with increased time or intensity of exposure[77]. They observed that 4-8 weeks of GW-agents treatment did not produce consistent delayed pain syndrome similar to the pain in GWI veterans[82,83]. However, rats exposed to intensified GW-agents for 30 days exhibited significant increases in resting at 8 weeks post-exposure, indicating more severe pain symptoms. In addition, much more severe pain phenotype has been observed in rat exposed to GW-agents for 60 days[77].

Glutamate may be involved in pain from the following two aspects: i) glutamate plays a leading role in neurotransmission, which could transduce the pain from the periphery to the brain; ii) glutamate is involved in

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centr..., the author has demonstrated that one-month of a low glutamate diet is sufficient to reduce pain in GWI veterans[84]. Pain is a common symptom shared by GWI, fibromyalgia, and chronic tendon pain, in which increased brain glutamate level have been reported[85-87]. Thus, normalizing the glutamate transmission may be an attractive target for reducing pain in GWI veterans. Ketamine, as mentioned above, was originally approved by the FDA as an anesthetic. Ketamine has both acute and prolonged effects on chronic neuropathic pain syndromes. A single administration of ketamine can rapidly (5-10 minutes) and transiently (2-3 hours) reduce ongoing pain of neuropathic origin, as well as symptoms of allodynia and hyperalgesia[88]. Therapy combining ketamine and opioids helps to control pain without the risk of opioid-induced high blood pressure[89], suggesting an effective and safe role of inhibiting glutamate transmission in pain relief. Besides blockade of NMDAR activity with ketamine, microinjection of AMPA receptor inhibitors has also been reported to reduce visceral and spontaneous pain behaviors in a mouse model of chronic visceral pain[90]. In addition, growing evidence has suggested an important role of mGluRs in pain modulation. Studies have concluded that activation of group I mGluRs induces hyperalgesic effects, while activation of group II and III mGluRs induces analgesic effects in chronic pain[91]. Kolber *et al.* reported that pharmacological activation of group I mGluRs with DHPG in the amygdala of mice is sufficient to induce peripheral hypersensitivity, while pharmacological blockade or genetic disruption of mGluR5 reduced the peripheral hypersensitivity, suggesting that activation of mGluR5 in the amygdala facilitates the pain-like behavior[92]. Activation of mGluR5 also contributes to increased hypersensitivity, a feature underlying pain pathology[93-95]. Contrastingly, activation of mGluR2/3 decreased nociceptive behavior in an acute model of pain in a dose-dependent manner, and the effect was blocked by a group II antagonist[96]. Interestingly, N-acetyl-cysteine, a supplement that enhances the endogenous activation of group II mGluRs, inhibits nociceptive transmission in humans[97]. Peripheral group III mGluRs are also involved in the regulation of hyperalgesia. Genetic deletion of mGluR4 in mice increased the sensitivity to noxious mechanical stimuli and accelerated the onset of the nociceptive behavior[98]. While activation of mGluR4 by its agonist AMN082 inhibited inflammatory pain-induced and incision-induced hypersensitivity in rat[99]. More studies have reported to support the role of group III mGluRs in pain. Administration of the group III mGluRs agonist in the knee joint is able to reduce hyperalgesia in an arthritic pain model[100], and intrathecal injection of group III mGluRs agonist dose-dependently inhibited nociceptive behavior of different animal models of inflammatory or neuropathic pain. Importantly, the anti-hyperalgesia effect was blocked by either a nonselective antagonist of mGluRs or a selective group III antagonist[101]. Moreover, upregulation of EAAT2 level by ceftriaxone has also been demonstrated to alleviate the pain in several rodent models[102-104]. These studies support that normalizing the glutamatergic transmission could be an effective approach to control pain in GWI.

Migraine and headache

jects report suffering from migraines[105]. One study demonstrated a positive correlation between headache symptoms and durations of service in the Persian Gulf War region[106]. Differing from typical headache, migraine is a medical condition that involves severe and recurring headaches, usually triggered by sensory stimuli, such as odors, visual stimuli, and sounds. Sensory hypersensitivity is one of the critical contributors to migraine[107]. Currently, no study has been conducted relating functional or structural alterations in the brain to migraine or headache symptoms in GWI veterans. However, many studies have investigated the mechanisms for migraine and headache development, independently of GWI.

Again, sensory hypersensitivity could be one main contributor to migraine, as headache attacks are usually triggered by sensory stimuli[107]. This sensory hypersensitivity, also known as central sensitization, involves increased sensitivity even to normal stimuli due to greater function of nociceptive circuitry as well as reduced inhibition[108]. Enhanced glutamatergic activity could contribute to this hypersensitivity. Zielman *et al.* directly investigated glutamatergic activity in visual cortices and found higher glutamate levels in patients who suffered from migraine (without aura)[109]. Similarly, Zukerman *et al.* measured cerebrospinal fluid glutamate levels in chronic migraine and concluded that headache intensity positively correlated with glutamate levels[110]. Elevated blood levels of glutamate have also been recorded in migraine patients[111]. Furthermore, Hansen *et al.* reported that oral administration of monosodium glutamate significantly increased headache and pericranial muscle tenderness in healthy volunteers[112].

Animal models of migraine also implicate glutamatergic mechanisms in migraine development. Nitroglycerin is commonly used to generate animal model of migraine. Nagy-Grócz and colleagues reported that in rat injected with nitroglycerin, l-kynurenine hydrolase (KYNU) and l-kynurenine 3-monooxygenase (KMO) were significantly decreased. Down-regulation of KYNU and KMO leads to decreased kynurenic acid, which is an endogenous glutamate receptor antagonist. Decreased kynurenic acid potentially contributes to an increase in glutamatergic function through hyperactivation of NMDA receptors, suggesting a pathogenic role of glutamate in migraine[113].

Importantly, downregulation of plasma glutamate levels has been reported following effective migraine preventive therapies[114], and treatments that target the glutamatergic system have effectively reduced migraine or headache symptoms in patients with various disorders. For example, memantine, an NMDA receptor antagonist, has significantly reduced headache frequency in patients with migraine[115,116]. Several studies have demonstrated that ketamine, another NMDA receptor antagonist, has a beneficial effect in patients with headache[117,118]. Furthermore, topiramate, a kainate receptor antagonist, significantly reduced the frequency of headache in patients with migraine[119-121]. Migraine and headache may have a similar pathway to pain in GWI veterans: they share positive correlations with glutamate levels, hypersensitivity, and disrupted white matter intensity. Meanwhile, most of the treatments (for example, ketamine) that are beneficial for pain can also reduce migraine and headache symptoms. It is well established that group I mGluRs are predominantly pronociceptive, while groups II and III mGluRs are antinociceptive[91]. In a clinical trial with a negative allosteric

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modu ently attenuated dural vasodilator responses to meningeal stimulation in subjects affected by episodic migraine, suggesting that inhibition of mGluR5 activity may be an effective strategy in the treatment of migraine[122]. Considering the relationship between increased glutamate function and migraine/headache symptoms, as well as the effectiveness of different glutamate receptor antagonists in providing preventive or therapeutic effects in migraine/headache patients, it is plausible to propose that targeting glutamatergic deficits could help to reduce migraine/headache symptoms in GWI.

Chronic fatigue

Chronic fatigue is one of the primary symptoms of GWI; a majority of GWI veterans report suffering from moderate to severe fatigue[123]. The fatigue symptoms of GWI significantly overlap with the symptoms of CFS, with over half of GWI veterans also meeting the criteria for CFS[124]. One possible explanation for this fatigue (which may be related to glutamatergic dyshomeostasis) could be mitochondrial dysfunction. Mitochondria play a vital role in various metabolic processes and in ATP production, so it is plausible that impairment of their function could lead to fatigue. Studies on the subject have reported results indicating mitochondrial dysfunction in GWI veterans[125,126]. The link between glutamatergic dysfunction and mitochondrial impairment could be mediated by oxidative stress and/or excessive calcium influx in neurons. Human studies have shown glutamate excitotoxicity tends to induce oxidative stress[127,128]. Oxidative stress, in turn, can cause damage to mitochondria and mitochondrial DNA[129,130]. Excessive calcium influx could also contribute to mitochondrial impairment. Excessive glutamatergic activation of ionotropic receptors can lead to significant influx of calcium. This calcium, in turn, can be sequestered by the mitochondria, and in excess can contribute to mitochondrial death perhaps through collapse of mitochondrial membrane potential. Furthermore, excess Na^+ can also enter the cell during hyperactivation of glutamate ionotropic receptors, which places a high demand on Na^+/K^+-ATPase activity. These pumps consume cellular ATP rapidly, eventually resulting in depletion of ATP. Accumulated intracellular Na^+ alters the osmotic movement of water, consequently leading to microtubule depolymerization and mitochondrial collapse[131].

Fatigue has also been found in animal models of GWI[19]. In our previously mentioned GWI mouse model, we observed that glutamate levels were significantly raised in the hippocampus, while ATP levels were significantly reduced in sub-cortex and muscle tissue. The observed decrease in ATP levels could be a major contributing factor to the central fatigue that is reported in GW veterans, and could further lead to muscle weakness[19]. Furthermore, Li *et al.* demonstrated that rats subjected to a fatigue-loading procedure exhibited significantly higher glutamate levels in the whole brain; this suggests that glutamate may play an important role in fatigue development[132]. Shetty *et al.* reported that GW-related exposures leads to mitochondrial impairment in a rat model of GWI, accompanied by increased inflammation[133]. It is possible that the mitochondrial dysfunction and decreased ATP levels in GWI models may result from excess glutamate in the brain, by either of the mechanisms mentioned above (oxidative stress or calcium influx).

treatments which target this system. In a study mentioned above, low glutamate diet significantly decreased fatigue symptoms in GW veterans[84]. Additionally, amantadine, a low-affinity NMDA antagonist, has proven to be an effective treatment for fatigue in multiple sclerosis[134,135]. Furthermore, in a GWI mouse model mentioned above, we found that LDN/OSU-215111 not only decreased glutamate levels in the hippocampal CA1 region, but also reduced fatigue and restored the ATP levels, suggesting that targeting the glutamatergic system provides an effective means to reducing fatigue symptoms in GWI via restoring the ATP level[19]. Increased levels of glutamate and decreased levels of glutamate transporter EAAT2 could directly contribute to the development of fatigue. In rats subjected to exercise-induced fatigue, the author reported a significant downregulation of EAAT2 level. Functional inhibition of EAAT2 activity led to a decrease in exercise endurance and an increase in extracellular glutamate concentration[136]. Considering the above studies together suggests a potential glutamatergic mechanism for fatigue in GWI, which could also be targeted for therapeutic benefits.

Gastrointestinal complaints

Around 25% of GWI veterans experienced persistent, chronic gastrointestinal (GI) symptoms[137]. The majority of complaints include diarrhea and abdominal pain, which are commonly reported in IBS[5,138]. GWI veterans with chronic GI symptoms endured significantly more chemical weapons exposure during the war and reported significantly greater chronic pain, fatigue, and sleep difficulties[139]. They also reported a higher visceral, cutaneous, and somatic hypersensitivity, as studies have demonstrated significantly higher abdominal pain ratings and decreased thresholds to different unpleasant stimuli[137,140]. The GI problem can be affected by brain structure alteration. Zhang *et al*. has demonstrated a strong correlation between medullar atrophy and increased abdominal pain by MRI[30]. Decreased size of the brain stem has also been observed in patients with IBS[141].

In a mouse model of GWI, Hernandez *et al.* reported that exposure to PB alters gut function by disrupting the neural and immune systems of the intestine, as they observed that PB caused alterations to colonic motility and structure, accompanied by major shifts in the expression of proinflammatory cytokines and chemokines in the colon and brain[142]. Similarly, Alhasson *et al.* reported that GW-agents caused significant neuroinflammation and intestinal dysbiosis in the gut of mice, accompanied by upregulation of toll like receptor 4 (TLR4) and claudin-2, and reduction of occludin[143]. TLR4 belongs to the pattern recognition receptor family, and activates the innate immune system[144]. Claudin-2 is a mediator of a leaky gut barrier during intestinal inflammation[145]; and occludin is a tight junction protein[146]. All these observations suggest that the GWI model exhibits intestinal hyperpermeability.

A positive correlation has been found between intestinal hyperpermeability and abdominal pain[147]. Interestingly, studies have demonstrated that glutamate and glutamine play an important role in intestinal hyperpermeability. One study has demonstrated that glutamate reduces intestinal hyperpermeability in cell cultures and facilitates glutamine support of gut integrity[148]. Similar to glutamate, depletion of glutamine also

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resul bility, and glutamine supplementation can improve gut barrier function[149]. In addition, reduced hippocampal glutamate-glutamine levels have also been reported in IBS patients, suggesting that abnormal glutamatergic neurotransmission may contribute to GI problems[150].

As mentioned above, visceral hypersensitivity is another symptom in functional GI disorders[102,105]. In a chronic visceral hypersensitivity rat model, Su *et al.* reported that microinjection of different doses of glutamate into the paraventricular nucleus reduced the visceral sensitivity in a dose-dependent manner[151]. Similar results were also reported by injection of glutamate into the cerebellum fastigial nucleus[152]. Furthermore, in a stress-induced visceral hypersensitivity rat model, Gosselin *et al.* reported that riluzole significantly counteracted the stress-induced visceral hypersensitivity, without affecting visceral response in control rats[153]. Accumulating evidence has suggested an important role of mGluRs in hypersensitivity regulation. Administration of AMN082, a mGluR7 agonist, significantly attenuated the colorectal distension induced visceral hypersensitivity in neonatal maternal separation rats, accompanied by reduced the abnormal immune cytokine response[154], which was frequently reported in GWI rodents model with GI problems[142,143]. mGluR5 has been implicated in regulating intestinal inflammation, visceral pain and the epithelial barrier function in the intestine; the underlying mechanism could be that mGluR5 enhances the interaction between TLR4 and NMDAR subunit 1 (GluN1), which initiates the inflammatory process, and this effect can be reversed by mGluR5 antagonist, MTEP[143,155]. Interestingly, increasing the expression of the glutamate transporter EAAT2 inhibits the visceral hypersensitivity, as observed from a EAAT2-overexpression transgenic mouse[156]. These results strongly support that target glutamatergic dysfunction may be beneficial for addressing GI problems in GWI.

Conclusions

To our knowledge, this is the first review to focus on understanding the potential contributors that linking the initial wartime exposures to different aspects of symptoms that are observed in GWI veterans. Previous reviews of GWI, which were mainly focused on summarizing the initial causes, the population structure of GWI veterans, or the health outcome of GWI veterans couple decades after deployment, fail to explain the potential contributors that lead to the symptoms of GWI. In this review, by breaking down the multi-symptoms into individual symptom, it becomes clear that glutamatergic dysfunction is involved in GWI.

Many symptoms of GWI are shared with other disorders, such as anxiety in anxiety disorder, depression in MDD, pain in fibromyalgia, migraine/headache in patients with migraine/headache, fatigue in CFS, and GI problems in IBS, in which glutamatergic dysfunction have been extensively reported[39,46,85,110,132,150]. Overall, increased glutamate level or impaired glutamatergic transmission are commonly reported in patients of GWI or related disorders. Increased glutamate level is frequently reported in patients with anxiety, depression, migraine/headache, and pain. Elevated glutamate activates glutamate receptors and leads to neuro-excitotoxicity. And impaired glutamatergic transmission is frequently reported in patients with cognition impairment, fatigue, and GI problems, the impaired transmission could be an outcome of neuro-excitotoxicity.

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In co and normalization of the glutamatergic system is a fruitful therapeutic approach for the veterans who are suffering from GWI.

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Cred

Xueqin Wang: Conceptualization, Resources, Writing - original draft, Writing - review & editing.

Noor Ali: Resources, Writing - original draft, Writing - review & editing.

Chien-liang Glenn Lin: Conceptualization, Supervision, Writing - review & editing.

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Conflict of Interest statement

The authors declare that there are no conflicts of interest.

Figure

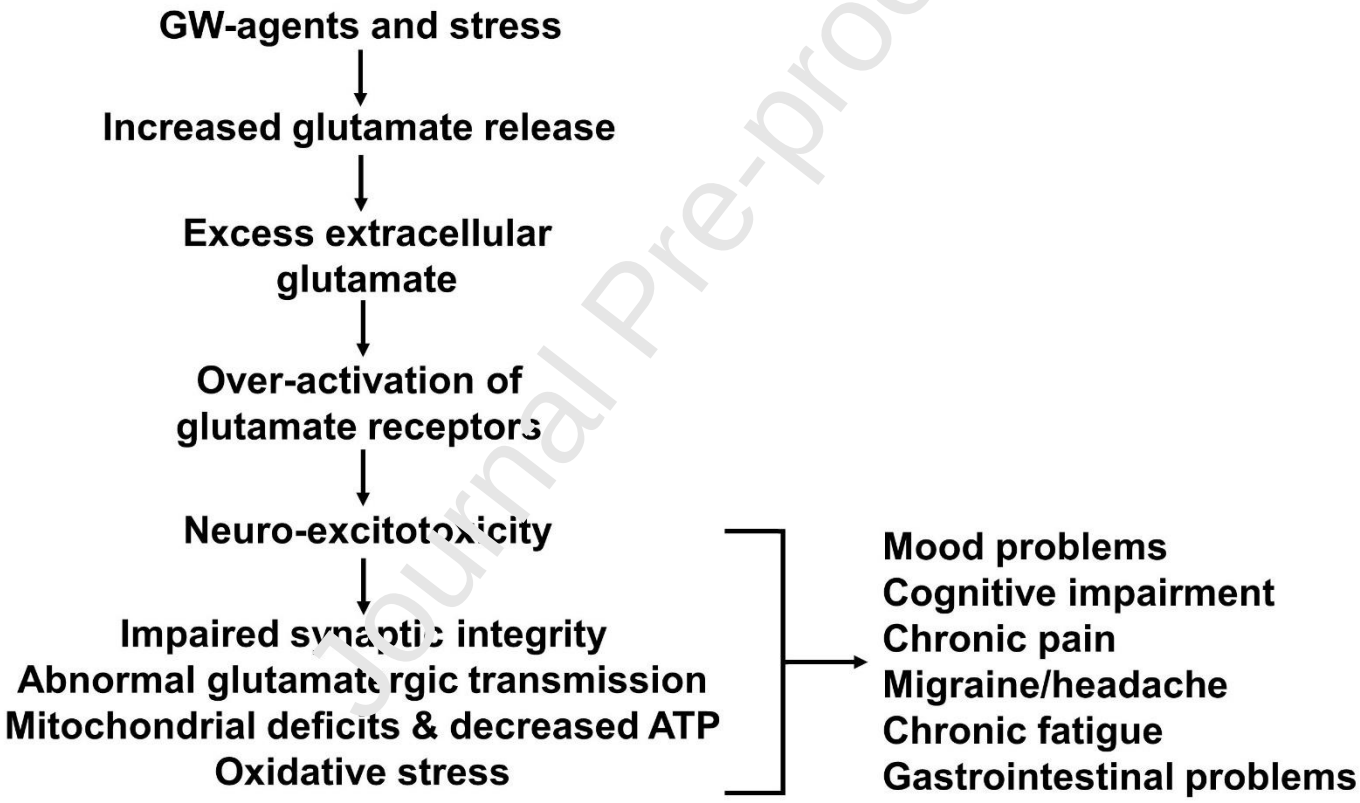

Fig 1: The role of glutamatergic system dysfunction in Gulf War Illness.

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1. Nettleman, M. Gulf War Illness: Challenges Persist. *Transactions of the American Clinical and Climatological Association* **126**, 237-247 (2015). PMCID: 4530672
2. Cory-Slechta, D. & Wedge, R. *Gulf War and health: Volume 10: Update of health effects of serving in the Gulf War, 2016*, (National Academies Press, 2016). https://doi.org/10.17226/21840
3. Joyce, M.R. & Holton, K.F. Neurotoxicity in Gulf War Illness and the potential role of glutamate. *Neurotoxicology* **80**, 60-70 (2020). https://doi.org/10.1016/j.neuro.2020.06.008
4. Wolfe, J., Proctor, S.P., Erickson, D.J. & Hu, H. Risk factors for multisymptom illness in US Army veterans of the Gulf War. *Journal of occupational and environmental medicine* **44**, 271-281 (2002). https://doi.org/10.1097/00043764-200203000-00015
5. Gray, G.C., Reed, R.J., Kaiser, K.S., Smith, T.C. & Gastañaga, V.M. Self-reported symptoms and medical conditions among 11,868 Gulf War-era veterans: the Seabee Health Study. *American Journal of Epidemiology* **155**, 1033-1044 (2002). https://doi.org/10.1093/aje/155.11.1033
6. Sanacora, G., Zarate, C.A., Krystal, J.H. & Manji, H.K. Targeting the glutamatergic system to develop novel, improved therapeutics for mood disorders. *Nature reviews. Drug discovery* **7**, 426-437 (2008). https://doi.org/10.1038/nrd2462
7. Conn, P.J. & Pin, J.-P. Pharmacology and functions of metabotropic glutamate receptors. *Annual review of pharmacology and toxicology* **37**, 205-237 (1997). https://doi.org/10.1146/annurev.pharmtox.37.1.205
8. Hernández, D.E.*, et al.* Axonal degeneration induced by glutamate excitotoxicity is mediated by necroptosis. *Journal of cell science* **131**, jcs214684 (2018).
9. Garzón, F.*, et al.* NeuroEPO preserves neurons from glutamate-induced excitotoxicity. *Journal of Alzheimer's Disease* **65**, 1469-1483 (2018).
10. Zárate, S.C., Traetta, M.E., Codagnone, M.G., Seilicovich, A. & Reinés, A.G. Humanin, a mitochondrial-derived peptide released by astrocytes, prevents synapse loss in hippocampal neurons. *Frontiers in aging neuroscience* **11**, 123 (2019).
11. Plitman, E.*, et al.* Glutamate-mediated excitotoxicity in schizophrenia: a review. *European Neuropsychopharmacology* **24**, 1591-1605 (2014).
12. Golomb, B.A. Acetylcholinesterase inhibitors and Gulf War illnesses. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 4295-4300 (2008). https://doi.org/10.1073/pnas.0711986105
13. Colovic, M.B., Krstic, D.Z., Lazarevic-Pasti, T.D., Bondzic, A.M. & Vasic, V.M. Acetylcholinesterase inhibitors: pharmacology and toxicology. *Current neuropharmacology* **11**, 315-335 (2013). https://doi.org/10.2174/1570159X11311030006
14. Meyer, D.A. & Shafer, T.J. Permethrin, but not deltamethrin, increases spontaneous glutamate release from hippocampal neurons in culture. *Neurotoxicology* **27**, 594-603 (2006). https://doi.org/10.1016/j.neuro.2006.03.016
15. Pavlovsky, L., Brownie, E.O. & Friedman, A. Pyridostigmine enhances glutamatergic transmission in hippocampal CA1 neurons. *Experimental neurology* **179**, 181-187 (2003). https://doi.org/10.1016/s0014-4886(02)00016-x
16. Treccani, G.*, et al.* Stress and corticosterone increase the readily releasable pool of glutamate vesicles in synaptic terminals of prefrontal and frontal cortex. *Molecular psychiatry* **19**, 433-443 (2014). https://doi.org/10.1038/mp.2014.5
17. Musazzi, L.*, et al.* Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: the dampening action of antidepressants. *PloS one* **5**, e8566 (2010). https://doi.org/10.1371/journal.pone.0008566
18. Musazzi, L., Tornese, P., Sala, N. & Popoli, M. Acute stress is not acute: sustained enhancement of glutamate release after acute stress involves readily releasable pool size and synapsin I activation. *Molecular psychiatry* **22**, 1226-1227 (2017). https://doi.org/10.1038/mp.2016.175
19. Wang, X.*, et al.* Restoring tripartite glutamatergic synapses: A potential therapy for mood and cognitive deficits in Gulf War illness. *Neurobiology of stress* **13**, 100240 (2020). https://doi.org/10.1016/j.ynstr.2020.100240

Journal Pre-proof

20. ergic neurochemistry: Insights from a rat model of Gulf War Illness. *Neurobiology of stress* **12**, 100210 (2020). https://doi.org/10.1016/j.ynstr.2019.100210
21. Li, N.*, et al.* Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biological psychiatry* **69**, 754-761 (2011). https://doi.org/10.1016/j.biopsych.2010.12.015
22. Ojo, J.O.*, et al.* Exposure to an organophosphate pesticide, individually or in combination with other Gulf War agents, impairs synaptic integrity and neuronal differentiation, and is accompanied by subtle microvascular injury in a mouse model of Gulf War agent exposure. *Neuropathology : official journal of the Japanese Society of Neuropathology* **34**, 109-127 (2014). https://doi.org/10.1111/neup.12061
23. Abdel-Rahman, A., Shetty, A.K. & Abou-Donia, M.B. Subchronic dermal application of N,N-diethyl m-toluamide (DEET) and permethrin to adult rats, alone or in combination, causes diffuse neuronal cell death and cytoskeletal abnormalities in the cerebral cortex and the hippocampus, and Purkinje neuron loss in the cerebellum. *Experimental neurology* **172**, 153-171 (2001). https://doi.org/10.1006/exnr.2001.7807
24. Kassem, M.S.*, et al.* Stress-induced grey matter loss determined by MRI is primarily due to loss of dendrites and their synapses. *Molecular neurobiology* **47**, 645-661 (2013). https://doi.org/10.1007/s12035-012-8365-7
25. Abdel-Rahman, A., Abou-Donia, S., El-Masry, E., Shetty, A. & Abou-Donia, M. Stress and combined exposure to low doses of pyridostigmine bromide, DEET, and permethrin produce neurochemical and neuropathological alterations in cerebral cortex, hippocampus, and cerebellum. *Journal of toxicology and environmental health. Part A* **67**, 163-192 (2004). https://doi.org/10.1080/15287390490264802
26. Chao, L.L., Raymond, M.R., Leo, C.K. & Abadjian, L.R. Evidence of Hippocampal Structural Alterations in Gulf War Veterans With Predicted Exposure to the Khamisiyah Plume. *Journal of occupational and environmental medicine* **59**, 923-929 (2017). https://doi.org/10.1097/JOM.0000000000001082
27. Apfel, B.A.*, et al.* Hippocampal volume differences in Gulf War veterans with current versus lifetime posttraumatic stress disorder symptoms. *Biological psychiatry* **69**, 541-548 (2011). https://doi.org/10.1016/j.biopsych.2010.09.044
28. Vythilingam, M.*, et al.* Smaller head of the hippocampus in Gulf War-related posttraumatic stress disorder. *Psychiatry research* **139**, 89-99 (2005). https://doi.org/10.1016/j.pscychresns.2005.04.003
29. Doebbeling, B.N.*, et al.* Is there a Persian Gulf War syndrome? Evidence from a large population-based survey of veterans and nondeployed controls. *Am J Med* **108**, 695-704 (2000). https://doi.org/10.1016/S0002-9343(00)00405-8
30. Zhang, Y.*, et al.* Brainstem atrophy in Gulf War Illness. *Neurotoxicology* **78**, 71-79 (2020). https://doi.org/10.1016/j.neuro.2020.02.006
31. Odegard, T.N.*, et al.* Memory impairment exhibited by veterans with Gulf War Illness. *Neurocase* **19**, 316-327 (2013). https://doi.org/10.1080/13554794.2012.667126
32. Frodl, T.*, et al.* Reduced hippocampal volume correlates with executive dysfunctioning in major depression. *Journal of Psychiatry and Neuroscience* **31**, 316 (2006). https://doi.org/PMID: 16951734
33. Carreras, I.*, et al.* Anxiety, neuroinflammation, cholinergic and GABAergic abnormalities are early markers of Gulf War illness in a mouse model of the disease. *Brain research* **1681**, 34-43 (2018). https://doi.org/10.1016/j.brainres.2017.12.030
34. Abdullah, L.*, et al.* Lipidomic profiling of phosphocholine-containing brain lipids in mice with sensorimotor deficits and anxiety-like features after exposure to Gulf War agents. *Neuromolecular Med* **14**, 349-361 (2012). https://doi.org/10.1007/s12017-012-8192-z
35. Parihar, V.K., Hattiangady, B., Shuai, B. & Shetty, A.K. Mood and memory deficits in a model of Gulf War illness are linked with reduced neurogenesis, partial neuron loss, and mild inflammation in the hippocampus. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **38**, 2348-2362 (2013). https://doi.org/10.1038/npp.2013.158
36. Hattiangady, B.*, et al.* Object location and object recognition memory impairments, motivation deficits and depression in a model of Gulf War illness. *Frontiers in behavioral neuroscience* **8**, 78 (2014). https://doi.org/10.3389/fnbeh.2014.00078

Journal Pre-proof

37. artite Synapse Via Activation of Local Translation in Astrocytic Processes. *Neuroscience* **388**, 224-238 (2018). https://doi.org/10.1016/j.neuroscience.2018.07.028
38. Brandley, E., Kirkland, A. & Holton, K. Gulf War Veterans with Psychiatric Symptoms (Anxiety, Depression, and PTSD) Significantly Improve on a Low Glutamate Diet. *Current Developments in Nutrition* **4**, 1192-1192 (2020). https://doi.org/10.1093/cdn/nzaa057_008
39. Mathew, S.J.*, et al.* Open-label trial of riluzole in generalized anxiety disorder. *American Journal of Psychiatry* **162**, 2379-2381 (2005). https://doi.org/10.1176/appi.ajp.162.12.2379
40. Coric, V.*, et al.* Riluzole augmentation in treatment-resistant obsessive–compulsive disorder: an open-label trial. *Biological psychiatry* **58**, 424-428 (2005). https://doi.org/10.1016/j.biopsych.2005.04.043
41. Zarate Jr, C.A.*, et al.* An open-label trial of riluzole in patients with treatment-resistant major depression. *American Journal of Psychiatry* **161**, 171-174 (2004). https://doi.org/10.1016/j.jad.2019.06.065
42. Glue, P.*, et al.* Ketamine's dose-related effects on anxiety symptoms in patients with treatment refractory anxiety disorders. *Journal of Psychopharmacology* **31**, 1302-1305 (2017). https://doi.org/10.1177/0269881117705089
43. Ionescu, D.F., Luckenbaugh, D.A., Niciu, M.J., Richards, E.M. & Zarate Jr, C.A. A single infusion of ketamine improves depression scores in patients with anxious bipolar depression. *Bipolar disorders* **17**, 438-443 (2015). https://doi.org/10.1111/bdi.12277
44. Maeng, S.*, et al.* Cellular mechanisms underlying the antidepressant effects of ketamine: role of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biological psychiatry* **63**, 349-352 (2008). https://doi.org/10.1016/j.biopsych.2007.05.028
45. Farley, S., Apazoglou, K., Witkin, J.M., Giros, B. & Tzavara, E.T. Antidepressant-like effects of an AMPA receptor potentiator under a chronic mild stress paradigm. *International Journal of Neuropsychopharmacology* **13**, 1207-1218 (2010). https://doi.org/10.1017/S1461145709991076
46. Feyissa, A.M.*, et al.* Elevated level of metabotropic glutamate receptor 2/3 in the prefrontal cortex in major depression. *Progress in neuro-psychopharmacology & biological psychiatry* **34**, 279-283 (2010). https://doi.org/10.1016/j.pnpbp.2009.11.018
47. Yoshimizu, T., Shimazaki, T., Ito, A. & Chaki, S. An mGluR2/3 antagonist, MGS0039, exerts antidepressant and anxiolytic effects in behavioral models in rats. *Psychopharmacology* **186**, 587-593 (2006). https://doi.org/10.1007/s00213-006-0390-7
48. Chaki, S.*, et al.* MGS0039: a potent and selective group II metabotropic glutamate receptor antagonist with antidepressant-like activity. *Neuropharmacology* **46**, 457-467 (2004). https://doi.org/10.1016/j.neuropharm.2003.10.009
49. Kim, S.*, et al.* Proneurogenic Group II mGluR antagonist improves learning and reduces anxiety in Alzheimer Aβ oligomer mouse. *Molecular psychiatry* **19**, 1235-1242 (2014). https://doi.org/10.1038/mp.2014.57
50. Belozertseva, I., Kos, T., Popik, P., Danysz, W. & Bespalov, A. Antidepressant-like effects of mGluR1 and mGluR5 antagonists in the rat forced swim and the mouse tail suspension tests. *European neuropsychopharmacology* **17**, 172-179 (2007). https://doi.org/10.1016/j.euroneuro.2006.03.002
51. Pałucha, A.*, et al.* Potential antidepressant-like effect of MTEP, a potent and highly selective mGluR5 antagonist. *Pharmacology Biochemistry and Behavior* **81**, 901-906 (2005). https://doi.org/10.1016/j.pbb.2005.06.015
52. Ebrahimi-Ghiri, M., Khakpai, F. & Zarrindast, M.-R. Combined treatment of scopolamine and group III mGluR antagonist, CPPG, exerts antidepressant activity without affecting anxiety-related behaviors. *Physiology & behavior* **224**, 113034 (2020). https://doi.org/10.1016/j.physbeh.2020.113034
53. Agostini, J.F.*, et al.* Ceftriaxone attenuated anxiety-like behavior and enhanced brain glutamate transport in zebrafish subjected to alcohol withdrawal. *Neurochemical research*, 1-10 (2020). https://doi.org/10.1007/s11064-020-03008-z
54. Kang, S., Li, J., Bekker, A. & Ye, J.-H. Rescue of glutamate transport in the lateral habenula alleviates depression-and anxiety-like behaviors in ethanol-withdrawn rats. *Neuropharmacology* **129**, 47-56 (2018). https://doi.org/10.1016/j.neuropharm.2017.11.013
55. Mineur, Y.S., Picciotto, M.R. & Sanacora, G. Antidepressant-like effects of ceftriaxone in male C57BL/6J mice. *Biological psychiatry* **61**, 250-252 (2007). https://doi.org/10.1016/j.biopsych.2006.04.037

Journal Pre-proof

56. -level exposure to sarin and cyclosarin during the 1991 Gulf War on brain function and brain structure in US veterans. *Neurotoxicology* **31**, 493-501 (2010). https://doi.org/10.1016/j.neuro.2010.05.006
57. Chao, L.L., Abadjian, L., Hlavin, J., Meyerhoff, D.J. & Weiner, M.W. Effects of low-level sarin and cyclosarin exposure and Gulf War Illness on brain structure and function: a study at 4T. *Neurotoxicology* **32**, 814-822 (2011). https://doi.org/10.1016/j.neuro.2011.06.006
58. Hubbard, N.A.*, et al.* Central Executive Dysfunction and Deferred Prefrontal Processing in Veterans with Gulf War Illness. *Clinical psychological science : a journal of the Association for Psychological Science* **2**, 319-327 (2014). https://doi.org/10.1177/2167702613506580
59. Zakirova, Z.*, et al.* Gulf War agent exposure causes impairment of long-term memory formation and neuropathological changes in a mouse model of Gulf War Illness. *PloS one* **10**, e0119579 (2015). https://doi.org/10.1371/journal.pone.0119579
60. Lindstrom, S.H.*, et al.* VGluT1 Deficiency Impairs Visual Attention and Reduces the Dynamic Range of Short-Term Plasticity at Corticothalamic Synapses. *Cerebral cortex* **30**, 1813-1829 (2020). https://doi.org/10.1093/cercor/bhz204
61. Inta, D.*, et al.* Sensorimotor gating, working and social memory deficits in mice with reduced expression of the vesicular glutamate transporter VGLUT1. *Behavioural brain research* **228**, 328-332 (2012). https://doi.org/10.1016/j.bbr.2011.12.012
62. Malhotra, A.K.*, et al.* NMDA receptor function and human cognition: the effects of ketamine in healthy volunteers. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **14**, 301-307 (1996). https://doi.org/10.1016/0893-133X(95)00137-3
63. Stefani, M.R. & Moghaddam, B. Activation of type 5 metabotropic glutamate receptors attenuates deficits in cognitive flexibility induced by NMDA receptor blockade. *Eur J Pharmacol* **639**, 26-32 (2010). https://doi.org/10.1016/j.ejphar.2010.01.028
64. Kirkland, A., Brandley, E. & Holton, K. A Low Glutamate Diet Improves Cognitive Functioning in Veterans with Gulf War Illness. *Current Developments in Nutrition* **4**, 1217-1217 (2020). https://doi.org/10.1093/cdn/nzaa057_033
65. Levin, R.*, et al.* Behavioral and cognitive effects of the N-methyl-D-aspartate receptor co-agonist D-serine in healthy humans: initial findings. *Journal of psychiatric research* **61**, 188-195 (2015). https://doi.org/10.1016/j.jpsychires.2014.12.007
66. Hunt, D.L. & Castillo, P.E. Synaptic plasticity of NMDA receptors: mechanisms and functional implications. *Current opinion in neurobiology* **22**, 496-508 (2012). https://doi.org/10.1016/j.conb.2012.01.007
67. O'Neill, M.J. & Dix, S. AMPA receptor potentiators as cognitive enhancers. *IDrugs: the investigational drugs journal* **10**, 185-192 (2007). PMID: 17351873
68. Morley-Fletcher, S.*, et al.* The reduction in glutamate release is predictive of cognitive and emotional alterations that are corrected by the positive modulator of AMPA receptors S 47445 in perinatal stressed rats. *Neuropharmacology* **135**, 284-296 (2018). https://doi.org/10.1016/j.neuropharm.2018.03.018
69. Nikiforuk, A.*, et al.* Effects of a positive allosteric modulator of group II metabotropic glutamate receptors, LY487379, on cognitive flexibility and impulsive-like responding in rats. *Journal of Pharmacology and Experimental Therapeutics* **335**, 665-673 (2010). https://doi.org/10.1124/jpet.110.170506
70. Walker, A.G.*, et al.* Metabotropic glutamate receptor 3 activation is required for long-term depression in medial prefrontal cortex and fear extinction. *Proceedings of the National Academy of Sciences* **112**, 1196-1201 (2015).
71. Horio, M., Fujita, Y. & Hashimoto, K. Therapeutic effects of metabotropic glutamate receptor 5 positive allosteric modulator CDPPB on phencyclidine-induced cognitive deficits in mice. *Fundamental & clinical pharmacology* **27**, 483-488 (2013). https://doi.org/10.1111/j.1472-8206.2012.01045.x
72. Takahashi, K.*, et al.* Restored glial glutamate transporter EAAT2 function as a potential therapeutic approach for Alzheimer's disease. *The Journal of experimental medicine* **212**, 319-332 (2015). https://doi.org/10.1084/jem.20140413

73. glial glutamate transporter in a mouse model of Alzheimer's disease. *Neurobiology of aging* **36**, 2260-2271 (2015). https://doi.org/10.1016/j.neurobiolaging.2015.04.005
74. Tikhonova, M.A.*, et al.* Neuroprotective effects of ceftriaxone treatment on cognitive and neuronal deficits in a rat model of accelerated senescence. *Behavioural brain research* **330**, 8-16 (2017). https://doi.org/10.1016/j.bbr.2017.05.002
75. Fan, S.*, et al.* Ceftriaxone improves cognitive function and upregulates GLT-1-related glutamate-glutamine cycle in APP/PS1 mice. *Journal of Alzheimer's Disease* **66**, 1731-1743 (2018). https://doi.org/10.3233/JAD-180708
76. Thomas, H.V., Stimpson, N.J., Weightman, A., Dunstan, F. & Lewis, G. Pain in veterans of the Gulf War of 1991: a systematic review. *BMC musculoskeletal disorders* **7**, 74 (2006). https://doi.org/10.1186/1471-2474-7-74
77. Nutter, T.J., Johnson, R.D. & Cooper, B.Y. A delayed chronic pain like condition with decreased Kv channel activity in a rat model of Gulf War Illness pain syndrome. *Neurotoxicology* **51**, 67-79 (2015). https://doi.org/10.1016/j.neuro.2015.09.010
78. Rayhan, R.U.*, et al.* Increased Brain White Matter Axial Diffusivity Associated with Fatigue, Pain and Hyperalgesia in Gulf War Illness. *PloS one* **8**(2013). https://doi.org/10.1371/journal.pone.0058493
79. Van Riper, S.M.*, et al.* Cerebral white matter structure is disrupted in Gulf War Veterans with chronic musculoskeletal pain. *Pain* **158**, 2364-2375 (2017). https://doi.org/10.1097/j.pain.0000000000001038
80. Lindheimer, J.B.*, et al.* Influence of pain anticipation on brain activity and pain perception in Gulf War Veterans with chronic musculoskeletal pain. *Psychophysiology* **56**, e13452 (2019). https://doi.org/10.1111/psyp.13452
81. Lacagnina, M.J.*, et al.* A role for neuroimmune signaling in a rat model of Gulf War Illness-related pain. *Brain, behavior, and immunity* **91**, 418-428 (2021). https://doi.org/10.1016/j.bbi.2020.10.022
82. Nutter, T.J., Jiang, N. & Cooper, B.Y. Persistent Na+ and K+ channel dysfunctions after chronic exposure to insecticides and pyridostigmine bromide. *Neurotoxicology* **39**, 72-83 (2013). https://doi.org/10.1016/j.neuro.2013.08.006
83. Nutter, T.J. & Cooper, B.Y. Persistent modification of Nav1.9 following chronic exposure to insecticides and pyridostigmine bromide. *Toxicology and applied pharmacology* **277**, 298-309 (2014). https://doi.org/10.1016/j.taap.2014.04.005
84. Holton, K.F.*, et al.* The Low Glutamate Diet Effectively Improves Pain and Other Symptoms of Gulf War Illness. *Nutrients* **12**, 2593 (2020). https://doi.org/10.3390/nu12092593
85. Pyke, T.L., Osmotherly, P.G. & Baines, S. Measuring glutamate levels in the brains of fibromyalgia patients and a potential role for glutamate in the pathophysiology of fibromyalgia symptoms. *The Clinical journal of pain* **33**, 944-954 (2017). https://doi.org/10.1097/AJP.0000000000000474
86. Valdés, M.*, et al.* Increased glutamate/glutamine compounds in the brains of patients with fibromyalgia: a magnetic resonance spectroscopy study. *Arthritis & Rheumatism* **62**, 1829-1836 (2010). https://doi.org/10.1002/art.27430
87. Alfredson, H. & Lorentzon, R. Chronic tendon pain: no signs of chemical inflammation but high concentrations of the neurotransmitter glutamate. Implications for treatment? *Current drug targets* **3**, 43-54 (2002). https://doi.org/10.2174/1389450023348028
88. Backonja, M., Arndt, G., Gombar, K.A., Check, B. & Zimmermann, M. Response of chronic neuropathic pain syndromes to ketamine: a preliminary study. *Pain* **56**, 51-57 (1994). https://doi.org/10.1016/0304-3959(94)90149-X
89. Dickey, N., Jenkins, D. & Butler, F.K. Prehospital use of ketamine in battlefield analgesia. (Defense Health Agency/Defense Health Board Falls Church United States, 2012).
90. Liu, S.-B.*, et al.* Long-term upregulation of cortical glutamatergic AMPA receptors in a mouse model of chronic visceral pain. *Molecular brain* **8**, 1-13 (2015). https://doi.org/10.1186/s13041-015-0169-z
91. Chiechio, S. & Nicoletti, F. Metabotropic glutamate receptors and the control of chronic pain. *Current opinion in pharmacology* **12**, 28-34 (2012). https://doi.org/10.1016/j.coph.2011.10.010
92. Kolber, B.J.*, et al.* Activation of metabotropic glutamate receptor 5 in the amygdala modulates pain-like behavior. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **30**, 8203-8213 (2010). https://doi.org/10.1523/JNEUROSCI.1216-10.2010

Journal Pre-proof

93. ce of cold hypersensitivity following a peripheral mononeuropathy in the rat. *Neuropharmacology* **44**, 983-993 (2003). https://doi.org/10.1016/s0028-3908(03)00118-7
94. Hama, A.T. Acute activation of the spinal cord metabotropic glutamate subtype-5 receptor leads to cold hypersensitivity in the rat. *Neuropharmacology* **44**, 423-430 (2003). https://doi.org/10.1016/S0028-3908(03)00026-1
95. Dolan, S. & Nolan, A.M. Blockade of metabotropic glutamate receptor 5 activation inhibits mechanical hypersensitivity following abdominal surgery. *European Journal of Pain* **11**, 644-651 (2007). https://doi.org/10.1016/j.ejpain.2006.10.002
96. Simmons, R.M., Webster, A.A., Kalra, A.B. & Iyengar, S. Group II mGluR receptor agonists are effective in persistent and neuropathic pain models in rats. *Pharmacology, biochemistry, and behavior* **73**, 419-427 (2002). https://doi.org/10.1016/s0091-3057(02)00849-3
97. Truini, A*., et al.* N-acetyl-cysteine, a drug that enhances the endogenous activation of group-II metabotropic glutamate receptors, inhibits nociceptive transmission in humans. *Molecular pain* **11**, s12990-12015-10009-12992 (2015). https://doi.org/10.1186/s12990-015-0009-2
98. Vilar, B*., et al.* Alleviating pain hypersensitivity through activation of type 4 metabotropic glutamate receptor. *Journal of Neuroscience* **33**, 18951-18965 (2013). https://doi.org/10.1523/JNEUROSCI.1221-13.2013
99. Dolan, S., Gunn, M.D., Biddlestone, L. & Nolan, A.M. The selective metabotropic glutamate receptor 7 allosteric agonist AMN082 inhibits inflammatory pain-induced and incision-induced hypersensitivity in rat. *Behavioural pharmacology* **20**, 596-604 (2009). https://doi.org/0.1097/FBP.0b013e32832ec5d1
100. Lee, K.S., Park, E.H., Cho, H.Y., Kim, Y.I. & Han, H.C. Peripheral group II and III metabotropic glutamate receptors in the knee joint attenuate carrageenan-induced nociceptive behavior in rats. *Neuroscience letters* **542**, 21-25 (2013). https://doi.org/10.1016/j.neulet.2013.03.006
101. Goudet, C*., et al.* Group III metabotropic glutamate receptors inhibit hyperalgesia in animal models of inflammation and neuropathic pain. *Pain* **137**, 112-124 (2008). https://doi.org/10.1016/j.pain.2007.08.020
102. Nicholson, K., Gilliland, T. & Winkelstein, B. Upregulation of GLT-1 by treatment with ceftriaxone alleviates radicular pain by reducing spinal astrocyte activation and neuronal hyperexcitability. *Journal of neuroscience research* **92**, 116-129 (2014). https://doi.org/10.1002/jnr.23295
103. Amin, B., Hajhashemi, V., Hosseinzadeh, H. & Abnous, K. Antinociceptive evaluation of ceftriaxone and minocycline alone and in combination in a neuropathic pain model in rat. *Neuroscience* **224**, 15-25 (2012). https://doi.org/10.1016/j.neuroscience.2012.07.058
104. Gunduz, O., Oltulu, C., Buldum, D., Guven, R. & Ulugol, A. Anti-allodynic and anti-hyperalgesic effects of ceftriaxone in streptozocin-induced diabetic rats. *Neuroscience letters* **491**, 23-25 (2011). https://doi.org/10.1016/j.neulet.2010.12.063
105. Rayhan, R.R., Ravindran, M.K. & Baraniuk, J.N. Migraine in gulf war illness and chronic fatigue syndrome: prevalence, potential mechanisms, and evaluation. *Frontiers in physiology* **4**, 181 (2013). https://doi.org/10.3389/fphys.2013.00181
106. Lei, K., Metzger-Smith, V., Golshan, S., Javors, J. & Leung, A. The prevalence of headaches, pain, and other associated symptoms in different Persian Gulf deployment periods and deployment durations. *SAGE open medicine* **7**, 2050312119871418 (2019). https://doi.org/10.1177/2050312119871418
107. Schwedt, T.J., Chiang, C.-C., Chong, C.D. & Dodick, D.W. Functional MRI of migraine. *The Lancet Neurology* **14**, 81-91 (2015). https://doi.org/10.1016/S1474-4422(14)70193-0
108. Latremoliere, A. & Woolf, C.J. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *The journal of pain* **10**, 895-926 (2009). https://doi.org/10.1016/j.jpain.2009.06.012
109. Zielman, R*., et al.* Cortical glutamate in migraine. *Brain* **140**, 1859-1871 (2017). https://doi.org/10.1093/brain/awx130
110. Peres, M*., et al.* Cerebrospinal fluid glutamate levels in chronic migraine. *Cephalalgia* **24**, 735-739 (2004). https://doi.org/10.1111/j.1468-2982.2004.00750.x
111. Campos, F*., et al.* Glutamate oxaloacetate transaminase: a new key in the dysregulation of glutamate in migraine patients. *Cephalalgia* **33**, 1148-1154 (2013). https://doi.org/10.1177/0333102413487444

Journal Pre-proof

112. [illegible] mate (MSG) on headache and pericranial muscle sensitivity. *Cephalalgia*, no-no (2009). https://doi.org/10.1111/j.1468-2982.2009.01881.x
113. Nagy-Grócz, G.*, et al.* The effect of systemic nitroglycerin administration on the kynurenine pathway in the rat. *Frontiers in neurology* **8**, 278 (2017). https://doi.org/10.3389/fneur.2017.00278
114. Ferrari, A., Spaccalopelo, L., Pinetti, D., Tacchi, R. & Bertolini, A. Effective prophylactic treatments of migraine lower plasma glutamate levels. *Cephalalgia* **29**, 423-429 (2009).
115. Charles, A., Flippen, C., Reyes, M.R. & Brennan, K.C. Memantine for prevention of migraine: a retrospective study of 60 cases. *The journal of headache and pain* **8**, 248-250 (2007). https://doi.org/10.1007/s10194-007-0406-7
116. Noruzzadeh, R.*, et al.* Memantine for prophylactic treatment of migraine without aura: A randomized double-blind placebo-controlled study. *Headache: The Journal of Head and Face Pain* **56**, 95-103 (2016). https://doi.org/10.1111/head.12732
117. Pomeroy, J.L., Marmura, M.J., Nahas, S.J. & Viscusi, E.R. Ketamine infusions for treatment refractory headache. *Headache: The Journal of Head and Face Pain* **57**, 276-282 (2017). https://doi.org/10.1111/head.13013
118. Lauritsen, C., Mazuera, S., Lipton, R.B. & Ashina, S. Intravenous ketamine for subacute treatment of refractory chronic migraine: a case series. *The journal of headache and pain* **17**, 1-5 (2016). https://doi.org/10.1186/s10194-016-0700-3
119. Brandes, J.L.*, et al.* Topiramate for migraine prevention: a randomized controlled trial. *Jama* **291**, 965-973 (2004). https://doi.org/10.1001/jama.291.8.965
120. Silberstein, S.D., Neto, W., Schmitt, J. & Jacobs, D. Topiramate in migraine prevention: results of a large controlled trial. *Archives of Neurology* **61**, 490-495 (2004). https://doi.org/10.1001/archneur.61.4.490
121. Diener, H.*, et al.* Topiramate reduces headache days in chronic migraine: a randomized, double-blind, placebo-controlled study. *Cephalalgia* **27**, 814-823 (2007). https://doi.org/10.1111/j.1468-2982.2007.01326.x
122. Waung, M.W., Akerman, S., Wakefield, M., Keywood, C. & Goadsby, P.J. Metabotropic glutamate receptor 5: a target for migraine therapy. *Annals of clinical and translational neurology* **3**, 560-571 (2016). https://doi.org/10.1002/acn3.[illegible]
123. Fappiano, C.M. & Baraniuk, J.N. Gulf War Illness Symptom Severity and Onset: A Cross-Sectional Survey. *Military medicine* **185**, e1120-e1127 (2020). https://doi.org/10.1093/milmed/usz471
124. Wylie, G.*, et al.* Fatigue in Gulf War Illness is associated with tonically high activation in the executive control network. *NeuroImage: Clinical* **21**, 101641 (2019). https://doi.org/10.1016/j.nicl.2018.101641
125. Koslik, H.J., Hamilton, G. & Golomb, B.A. Mitochondrial dysfunction in Gulf War illness revealed by 31Phosphorus Magnetic Resonance Spectroscopy: a case-control study. *PloS one* **9**, e92887 (2014). https://doi.org/10.1371/journal.pone.0092887
126. Chen, Y.*, et al.* Role of mitochondrial DNA damage and dysfunction in veterans with Gulf War Illness. *PloS one* **12**, e0184832 (2017). https://doi.org/10.1371/journal.pone.0184832
127. Savolainen, K.M., Loikkanen, J. & Naarala, J. Amplification of glutamate-induced oxidative stress. *Toxicology letters* **82**, 399-405 (1995). https://doi.org/10.1016/0378-4274(95)03490-0
128. Pereira, C.F. & de Oliveira, C.R. Oxidative glutamate toxicity involves mitochondrial dysfunction and perturbation of intracellular Ca2+ homeostasis. *Neuroscience research* **37**, 227-236 (2000). https://doi.org/10.1016/s0168-0102(00)00124-3
129. Sastre, J., Pallardó, F.V., García de la Asunción, J. & Viña, J. Mitochondria, oxidative stress and aging. *Free radical research* **32**, 189-198 (2000). https://doi.org/10.1080/10715760000300201
130. Yakes, F.M. & Van Houten, B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proceedings of the National Academy of Sciences* **94**, 514-519 (1997). https://doi.org/10.1073/pnas.94.2.514
131. Greenwood, S.M. & Connolly, C.N. Dendritic and mitochondrial changes during glutamate excitotoxicity. *Neuropharmacology* **53**, 891-898 (2007). https://doi.org/10.1016/j.neuropharm.2007.10.003
132. Li, R.*, et al.* Mapping the alterations of glutamate using Glu-weighted CEST MRI in a rat model of fatigue. *Frontiers in Neurology* **11**, 1408 (2020). https://doi.org/10.3389/fneur.2020.589128

133. and Inflammation in the Hippocampus Accompany Heightened Systemic Inflammation and Oxidative Stress in an Animal Model of Gulf War Illness. *Frontiers in molecular neuroscience* **10**, 182 (2017). https://doi.org/10.3389/fnmol.2017.00182
134. Shaygannejad, V., Janghorbani, M., Ashtari, F. & Zakeri, H. Comparison of the effect of aspirin and amantadine for the treatment of fatigue in multiple sclerosis: a randomized, blinded, crossover study. *Neurological research* **34**, 854-858 (2012). https://doi.org/10.1179/1743132812Y.0000000081
135. Foroughipour, M.*, et al.* Amantadine and the place of acupuncture in the treatment of fatigue in patients with multiple sclerosis: an observational study. *Acupuncture in Medicine* **31**, 27-30 (2013). https://doi.org/10.1136/acupmed-2012-010199
136. Wang, D. & Wang, X. GLT-1 mediates exercise-induced fatigue through modulation of glutamate and lactate in rats. *Neuropathology : official journal of the Japanese Society of Neuropathology* **38**, 237-246 (2018). https://doi.org/10.1111/neup.12465
137. Zhou, Q., Verne, M.L., Zhang, B. & Verne, G.N. Evidence for somatic hypersensitivity in veterans with Gulf War Illness and gastrointestinal symptoms. *The Clinical journal of pain* **34**, 944 (2018). https://doi.org/10.1097/AJP.0000000000000611
138. Hotopf, M. & Wessely, S. Can epidemiology clear the fog of war? Lessons from the 1990-91 Gulf War. *International journal of epidemiology* **34**, 791-800 (2005). https://doi.org/10.1093/ije/dyi102
139. Janulewicz, P.A.*, et al.* The Gut-Microbiome in Gulf War Veterans: A Preliminary Report. *Int J Environ Res Public Health* **16**(2019). https://doi.org/10.3390/ijerph16193751
140. Dunphy, R.C.*, et al.* Visceral and cutaneous hypersensitivity in Persian Gulf war veterans with chronic gastrointestinal symptoms. *Pain* **102**, 79-85 (2003). https://doi.org/10.1016/s0304-3959(02)00342-1
141. Labus, J.S.*, et al.* Irritable bowel syndrome in female patients is associated with alterations in structural brain networks. *Pain*® **155**, 137-149 (2014). https://doi.org/10.1016/j.pain.2013.09.020
142. Hernandez, S., Fried, D.E., Grubišić, V., McClain, J.L. & Gulbransen, B.D. Gastrointestinal neuroimmune disruption in a mouse model of Gulf War illness. *The FASEB Journal* **33**, 6168-6184 (2019). https://doi.org/10.1096/fj.201802572R
143. Alhasson, F.*, et al.* Altered gut microbiome in a mouse model of Gulf War Illness causes neuroinflammation and intestinal injury via leaky gut and TLR4 activation. *PloS one* **12**, e0172914 (2017). https://doi.org/10.1371/journal.pone.0172914
144. Vaure, C. & Liu, Y. A comparative review of toll-like receptor 4 expression and functionality in different animal species. *Frontiers in immunology* **5**, 316 (2014). https://doi.org/10.3389/fimmu.2014.00316
145. Luettig, J., Rosenthal, R., Barmeyer, C. & Schulzke, J. Claudin-2 as a mediator of leaky gut barrier during intestinal inflammation. *Tissue barriers* **3**, e977176 (2015).
146. McCarthy, K.M.*, et al.* Occludin is a functional component of the tight junction. *Journal of cell science* **109**, 2287-2298 (1996). PMID: 8886979
147. Zhang, B., Verne, M.L., Fields, J.Z., Verne, G.N. & Zhou, Q. Intestinal Hyperpermeability in Gulf War Veterans With Chronic Gastrointestinal Symptoms. *Journal of clinical gastroenterology* **53**, e298-e302 (2019). https://doi.org/10.1097/MCG.0000000000001135
148. Vermeulen, M.A., de Jong, J., Vaessen, M.J., van Leeuwen, P.A. & Houdijk, A.P. Glutamate reduces experimental intestinal hyperpermeability and facilitates glutamine support of gut integrity. *World journal of gastroenterology* **17**, 1569-1573 (2011). https://doi.org/10.3748/wjg.v17.i12.1569
149. Achamrah, N., Dechelotte, P. & Coeffier, M. Glutamine and the regulation of intestinal permeability: from bench to bedside. *Current opinion in clinical nutrition and metabolic care* **20**, 86-91 (2017). https://doi.org/10.1097/MCO.0000000000000339
150. Niddam, D.M., Tsai, S.-Y., Lu, C.-L., Ko, C.-W. & Hsieh, J.-C. Reduced hippocampal glutamate–glutamine levels in irritable bowel syndrome: preliminary findings using magnetic resonance spectroscopy. *American Journal of Gastroenterology* **106**, 1503-1511 (2011). https://doi.org/10.1038/ajg.2011.120
151. Su, Z.*, et al.* Protective effect of microinjection of glutamate into hypothalamus paraventricular nucleus on chronic visceral hypersensitivity in rats. *Brain research* **1747**, 147048 (2020). https://doi.org/10.1016/j.brainres.2020.147048

Journal Pre-proof

152. ...stigial nucleus on chronic visceral hypersensitivity in rats. *Life sciences* **203**, 184-192 (2018). https://doi.org/10.1016/j.lfs.2018.04.043
153. Gosselin, R.D.*, et al.* Riluzole normalizes early-life stress-induced visceral hypersensitivity in rats: role of spinal glutamate reuptake mechanisms. *Gastroenterology* **138**, 2418-2425 (2010). https://doi.org/10.1053/j.gastro.2010.03.003
154. Shao, L.*, et al.* Activating metabotropic glutamate receptor-7 attenuates visceral hypersensitivity in neonatal maternally separated rats. *International journal of molecular medicine* **43**, 761-770 (2019). https://doi.org/10.3892/ijmm.2018.4022
155. Cui, J., Yu, S., Li, Y., Li, P. & Liu, F. Direct binding of Toll-like receptor 4 to ionotropic glutamate receptor N-methyl-D-aspartate subunit 1 induced by lipopolysaccharide in microglial cells N9 and EOC 20. *International journal of molecular medicine* **41**, 1323-1330 (2018). https://doi.org/10.3892/ijmm.2017.3331
156. Lin, Y.*, et al.* Increased glial glutamate transporter EAAT2 expression reduces visceral nociceptive response in mice. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **296**, G129-G134 (2009). https://doi.org/10.1152/ajpgi.90556.2008

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