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EFFECT OF CANNABIS ON GLUTAMATE SIGNALLING IN THE BRAIN: A SYSTEMATIC REVIEW OF HUMAN AND ANIMAL EVIDENCE

Running title: Delta-9-tetrahydrocannabinol effect on glutamate signalling

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HIGHLIGHTS

- Use of cannabis or its main active ingredient Δ9-THC is associated with psychosis
- Evidence of the effects of Δ9-THC or cannabis on the dopamine system in man is equivocal
- Chronic cannabis use reduces glutamate or its metabolites levels in human brain
- Δ9-THC alters glutamate signalling in animal brain

ABSTRACT

Use of cannabis or delta-9-tetrahydrocannabinol (Δ9-THC), its main psychoactive ingredient, is associated with psychotic symptoms or disorder. However, the neurochemical mechanism that may underlie this psychotomimetic effect is poorly understood. Although dopaminergic dysfunction is generally recognized as the final common pathway in psychosis, evidence of the effects of Δ9-THC or cannabis use on dopaminergic measures in the brain is equivocal. In fact, it is thought that cannabis or Δ9-THC may not act on dopamine firing directly but indirectly by altering glutamate neurotransmission. Here we systematically review all studies examining acute and chronic effects of cannabis or Δ9-THC on glutamate signalling in both animals and man. Limited research carried out in humans tends to support the evidence that chronic cannabis use reduces levels of glutamate-derived metabolites in both cortical and subcortical brain areas. Research in animals tends to consistently suggest that Δ9-THC depresses glutamate synaptic transmission via CB1 receptor activation, affecting glutamate release, inhibiting receptors and transporters function, reducing enzyme activity, and disrupting glutamate synaptic plasticity after prolonged exposure.

Keywords: Cannabis; Delta-9-tetrahydrocannabinol; Glutamate signalling; Glutamatergic synaptic transmission; Cannabinoid receptor type 1

1. INTRODUCTION

Convergent and replicated findings suggest that use of cannabis or delta-9-tetrahydrocannabinol (Δ9-THC), the main psychoactive ingredient in cannabis, is associated with psychotic symptoms (Bhattacharyya et al., 2012a; Bhattacharyya et al., 2009a) or disorder (Moore et al., 2007; Murray et al., 2007), and its relapse (Colizzi et al., in press; Patel et al., in press; Schoeler et al., in press), consistent with independent evidence of endocannabinoid abnormalities in psychosis (summarized in Appiah-Kusi et al., 2016). However, the neurochemical abnormality that may underlie this psychotomimetic effect is poorly understood. Whilst evidence for the involvement of presynaptic dopamine dysfunction in psychosis is compelling (Howes et al., 2015), data from candidate gene-interaction studies seem to suggest vulnerability to the psychotomimetic and cognitive effects of cannabis use involving genes that control dopamine signalling particularly postsynaptically (Bhattacharyya et al., 2012a; Bhattacharyya et al., 2014; Colizzi et al., 2015a; Colizzi et al., 2015b). Also, evidence from human studies of the effect of cannabis use on acute striatal dopamine release or chronic dopamine receptor status is equivocal (summarized in Sami et al., 2015), though studies using functional neuroimaging techniques provide indirect evidence of the acute effect of Δ9-THC on dopaminergic regions such as striatum or midbrain across a range of different cognitive tasks (Bhattacharyya et al., 2009a; Bhattacharyya et al, 2009b; Bhattacharyya et al., 2012a; Bhattacharyya et al., 2012b; Bhattacharyya et al., 2015a).

Δ9-THC is a cannabinoid receptor type 1 (CB1) and 2 (CB2) partial agonist and the strength of its effects that are mediated by CB1 or CB2 receptors can be affected by endogenous cannabinoids and by the density and signalling efficiency of these receptors (Pertwee, 2008; Colizzi et al., 2015c). For example, if CB1 and CB2 are down-regulated or being targeted by endogeneous agonists, Δ9-THC may act as an antagonist whereas it will exert its partial agonist effect if receptors are up-regulated or endogeneous agonist levels are low. Interestingly, evidence is emerging that continued cannabis use may be associated with CB1 receptor alteration in the brain (Ceccarini et al.,

2015; Hirvonen et al., 2012). Evidence has also emerged suggesting that Δ9-THC affects dopamine release indirectly via a CB1-dependent inhibition of glutamate release onto gamma-aminobutyric acid (GABA) neurons in the nucleus accumbens and the ventral tegmental area (Pertwee, 2008). This model might explain the modest striatal dopamine release observed in Δ9-THC challenge studies (reviewed in Sami et al., 2015), offering a possible key to the understanding of the biology underlying cannabis-induced psychosis.

This effect of Δ9-THC on glutamate signalling represents a promising neurochemical mechanism that may underlie the psychotomimetic effect of this cannabinoid. Although only a few studies have investigated the effects of cannabis exposure on glutamate signalling in humans (Chang et al., 2006; Muetzel et al., 2013; Prescot et al., 2011; Prescot et al., 2013; Sung et al., 2013), a large body of evidence has accumulated from animal studies on the effect of exogenous cannabinoids on different glutamate-related outcome measures. The purpose of this review is to bring together and discuss all available data generated by human and animal studies that have investigated the acute and chronic effects of cannabis and Δ9-THC on the glutamatergic neurotransmitter system by carrying out a systematic literature search for all such data.

1.1. Objectives

Our main objective was to carry out a systematic review of all available literature concerning effects of cannabis and Δ9-THC on the glutamate system in humans as well as animal models. We aimed to review all interventional and observational studies, employing both retrospective and prospective methodologies with any reported neurochemical outcomes related to the glutamate system after exposure to cannabis or Δ9-THC.

2. EXPERIMENTAL PROCEDURES

2.1. Inclusion/exclusion criteria

Inclusion criteria for studies were: (1) human or animal studies, (2) studies investigating the acute and/or long-term effects of cannabis use/administration or Δ9-THC administration/application, (3) studies measuring molecular markers related to glutamate neurotransmission, including (a) glutamate metabolites, (b) synaptic transmission, (c) enzyme activity, (d) neurotransmitter release, (e) neurotransmitter uptake, (f) transporters, (g) receptors, (h) brain neurotransmitter levels. Exclusion criteria were (1) studies where cannabis or Δ9-THC were not the intervention or exposure of interest (i.e. studies including only synthetic cannabinoid agonists/antagonists) and (2) studies in which the neurochemical outcomes were not directly reported upon.

2.2. Search strategy

A final search was undertaken on the 29th of October 2015. The search terms used were: (cannabis or Δ9-THC or marijuana or marihuana or tetrahydrocannabinol or dronabinol) and (glu* or glutamate(s) or glutamine or glutamic acid). This search was undertaken in Medline, EMBASE, and PsychInfo using the OvidSP platform. All studies published in any language and indexed in the above databases were included. Reference lists from all identified relevant studies, reviews and conference abstracts were screened for any additional relevant studies.

2.3. Data extraction

Demographic and methodological variables and outcome data for studies identified were extracted into a spreadsheet. Primary outcomes of interest were neurochemical markers pertaining to the glutamatergic system. These were compared within interventional/exposed cohorts and/or between exposed and non-exposed control groups. Studies were grouped based on the investigated

population (humans or animal models); animal studies were further grouped depending on the Δ9-THC challenge strategy [application (in vitro studies) or administration (in vivo studies)] and the animal model stage of development (embryonal or postnatal).

2.4. Risk of bias

Risk of bias and quality assessment of the methodologically heterogeneous group of studies (Table 1) reviewed here required a suitably inclusive and flexible approach. For this purpose, an adapted set of criteria suggested by the Agency for Healthcare Research and Quality (AHRQ) guidance (West et al., 2002), amended as appropriate for observational studies in humans was used (Table 2a). Risk of systematic bias across human studies was further identified by assessing all papers for possible confounding factors such as mental health comorbidity, tobacco, alcohol, and other substance use in cannabis users (Table 2b). Also, in order to evaluate potential factors that may explain similarities/differences in results across animal studies, information was derived regarding study characteristics, such as animal model (e.g. mouse or rat), stage of development (i.e. embryonal or postnatal), sex, weight, and dosage of cannabinoids used (Table 3-6).

3. RESULTS

3.1. Study selection

In total 268 records were identified. All abstracts of the records were screened against the inclusion and exclusion criteria. A final list of 41 studies (5 human; 36 animal) was identified for systematic analysis in this review (Table 1). Cumulatively, the included studies investigated different aspects of the glutamate signalling pathway (Table 1) in response to cannabis or Δ9-THC exposure. These include (1) in vivo glutamate-related metabolites in chronic human cannabis users (Table 2a); (2) synaptic transmission, enzyme activity, neurotransmitter release and uptake, and extracellular levels in animal models after in vitro application of Δ9-THC and other cannabinoids

(Table 3); (3) enzyme activity, neurotransmitter release and uptake, extracellular levels, transporters, and receptors function in animal models after in vivo Δ9-THC embryonal exposure (Table 4); (4) synaptic transmission, enzyme activity, neurotransmitter release, intracerebral levels, and receptor function in animal models after in vivo Δ9-THC postnatal exposure (Table 5); (5) synaptic transmission, transporters, and receptor function in animal models after in vivo Δ9-THC postnatal exposure, followed by additional in vitro Δ9-THC exposure (Table 6). Further information on methodological quality of studies carried out in humans is reported in Table 2b. A brief synopsis of the key findings is reported below.

3.2. In vivo changes in levels of glutamate-related metabolites in response to cannabis use in humans

Five studies have addressed this area in humans, all using in vivo Proton Magnetic Resonance Spectroscopy (1H-MRS; Table 2a, Table 2b). Three studies investigated metabolites in the cerebral cortex, with particular attention to the anterior cingulate cortex (Prescot et al., 2011; Prescot et al., 2013; Sung et al., 2013), while the other two studies investigated metabolites in subcortical areas, in particular basal ganglia (Chang et al., 2006; Muetzel et al., 2013). One study additionally investigated metabolites in the frontal white matter (Chang et al., 2006).

Chang and colleagues (2006) conducted the first study in humans investigating brain glutamate levels in healthy individuals with a chronic history of cannabis use. They found that subjects who have used cannabis almost daily for at least one year (*n*=24) show a 9.5% reduction in their basal ganglia glutamate metabolite levels compared to cannabis-naive subjects (*n*=30). This evidence was even more robust when they also considered 42 individuals with Human Immunodeficiency Virus (HIV) infection, 21 of them with a history of chronic cannabis use. This second analysis (*n*=96) indicated that chronic cannabis users with and without comorbid HIV have a 12-13% decrease in their glutamate metabolite levels. HIV infection alone did not have a

significant effect on basal ganglia glutamate levels. The same model of analysis was used in order to investigate the effects of chronic cannabis use on glutamate metabolite levels in the frontal white matter. While an initial analysis failed to show a significant main effect of chronic cannabis use on glutamate metabolite levels (*n*=54, 24 cannabis users and 30 cannabis naive subjects), a second analysis including the 42 HIV positive individuals (*n*=96, 24 healthy cannabis users, 21 HIV individuals, 21 HIV cannabis users, and 30 cannabis naive subjects) revealed that healthy cannabis users have lower glutamate levels compared to cannabis naive subjects.

In a similarly sized study (*n*=53), Muetzel and colleagues (2013) found that glutamate metabolite levels in the dorsal striatum are non-significantly decreased in chronic cannabis users (*n*=27) compared to controls (*n*=26). However, their study suggested a group by sex interaction, with female cannabis users having a significant (12.5%) decrease in their glutamate metabolite levels.

In two different studies (2011, *n*=34; 2013, *n*=29) Prescot and colleagues found that, compared to cannabis-naive subjects (*n*=17 and *n*=16 respectively), chronic cannabis users (*n*=17 and *n*=13 respectively) exhibited a significant (14-15%) reduction in glutamate metabolite levels in the anterior cingulate cortex. These findings were not replicated by Sung et al. (2013, *n*=27) using a similar region of interest (ROI) approach. However, this last study investigated a modestly sized sample of cannabis users with comorbid methamphetamine use (*n*=8). Methamphetamine alone (*n*=9) also did not show any main effect on glutamate metabolites levels in the anterior cingulate cortex.

3.2.1. Risk of systematic bias across human studies

While generally well-designed, the results mentioned above (Section 3.2.) need to be considered in light of certain limitations. All of the studies investigated effects of chronic cannabis use. As long-term exposure to Δ9-THC, the psychoactive ingredient of cannabis, has been reported to block synaptic plasticity and reduce the sensitivity of glutamatergic synapses to cannabinoids in animal models (Hoffman et al., 2003), acute Δ9-THC challenge studies are needed to better elucidate the effects of cannabis on baseline glutamate responses. Moreover, across the studies that investigated chronic cannabis use, individuals differed in terms of their last cannabis use. While one study investigated cannabis users who were abstinent for at least 12 hours before scanning (Muetzel et al., 2013), another reported a wide range of abstinence, from no abstinence to 20 years (Chang et al., 2006), and a third reported that 54% of subjects had used cannabis within 24 hours before scanning (Prescot et al., 2013). No information on period of abstinence was available for the other two studies. These differences could be associated with considerable inter-individual variation in cannabis bio-availability in the brain, which may potentially affect spectroscopic measures. Also, the outcome measure was not the same for all studies, involving glutamate + glutamine metabolite measurement for two studies (Muetzel et al., 2013; Sung et al., 2013) but only glutamate metabolite measurement for the others (Chang et al., 2006; Prescot et al., 2011; Prescot et al., 2013). In two of the studies (Prescot et al., 2011; Prescot et al., 2013) metabolite levels were corrected for water while correction was carried out for cerebrospinal fluid or total creatine or phosphocreatine + creatine in the other studies (Chang et al., 2006; Muetzel et al., 2013; Sung et al., 2013), making comparisons difficult across studies. Moreover, three studies also included participants with a mental health problem (Muetzel et al., 2013; Prescot et al., 2011; Prescot et al., 2013), and in two of them this involved participants undergoing treatment for depression (Prescot et al., 2011; 2013) which was not accounted for in analyses in one of these studies (Prescot et al., 2011). Finally, while substance use represented an exclusion criterion in all the studies, alcohol use was an exclusion criterion only in one study (Chang et al., 2006); similarly,

tobacco use was an exclusion criterion only in one study and only for cannabis users (Muetzel et al., 2013). All the other studies registered an increase in tobacco and alcohol use (or abuse/dependence) in cannabis users compared to controls and in one report this information was not provided or corrected for (Prescot et al., 2011).

3.3. Glutamate-related outcome measures in response to cannabis/Δ9-THC application/administration in animal models

Thirty-six studies addressed this area in animal models, employing different strategies: embryonal/postnatal in vitro Δ9-THC exposure (Table 3, 7 studies), embryonal (Table 4, 7 studies) and postnatal (Table 5, 17 studies) in vivo Δ9-THC exposure; and postnatal in vivo Δ9-THC exposure followed by in vitro Δ9-THC (Table 6, 5 studies).

3.3.1. Animal studies of glutamatergic effects of in vitro Δ9-THC exposure

The first in vitro study was conducted by Shen and Thayer (1999), who explored hippocampal glutamatergic synaptic transmission after application of Δ9-THC in embryonal rat cells. Additional information was obtained by the use of WIN55,212-2 and SR141716A, a potent CB1 receptor agonist and a CB1-selective antagonist respectively. Results from this study suggest that Δ9-THC can reduce, but not block, excitatory neurotransmission, by acting presynaptically in the glutamatergic system. In particular, Δ9-THC exhibited high potency, but rather modest efficacy compared to the WIN55,212-2; also its action was prevented by SR141716A; finally, it appeared that Δ9-THC has both agonist and antagonist properties, being able to partially reverse the WIN55,212-2-induced full block of the excitatory neurotransmission. A second study (Brown et al., 2003) indicated that Δ9-THC reduces both the release and uptake of glutamate in rat striatal slices in a dose-dependent manner that is mediated by CB1 receptor activation; these effects were prevented in slices pre-treated with selective CB1 receptor antagonists but only partially reversed

by post-treatment. It was also found in this study that both activation of CB1 receptors and inhibition of glutamate uptake reduce corticostriatal synaptic transmission in a mutually inhibitory manner and that both forms of depression are dependent on metabotropic glutamate receptor (mGluR) activation (Brown et al., 2003). Straiker and Mackie (2005) were able to show temporary and long-lasting effects of different cannabinoids resulting in CB receptor-mediated reduction in glutamate transmission in mice. Authors confirmed the involvement of the CB1 receptor activation (Brown et al., 2003; Shen and Thayer, 1999) in the reduction of glutamate neurotransmission as all these effects were absent in CB1 knockout (KO) mice and/or reversed by the antagonist SR141716A, and that Δ9-THC antagonizes CB1 receptors in the short term (Shen and Thayer, 1999), but desensitizes them in the long term. However, in their studies Δ9-THC did not inhibit glutamate neurotransmission, even when using higher dosages compared to previous studies (Shen and Thayer, 1999; Brown et al., 2003). A more recent study (Hoffman et al., 2010) identified a likely cause of this discrepancy, showing different sensitivities among rodent species (rat vs mouse) to Δ9-THC at excitatory and inhibitory axon terminals in the hippocampus. Consistently, Δ9-THC was shown to induce depression of glutamate neurotransmission in rats but not in mice, as previously reported (Brown et al., 2003; Shen and Thayer, 1999; Straiker and Mackie, 2005). Interestingly, this difference seemed to be attributable to species differences in brain adenosine systems. Finally, Irie and colleagues (2015) suggested that Δ9-THC inhibits glutamatergic synaptic transmission also in the cerebellum by the same molecular mechanism; in particular, Δ9-THC seems to suppress the synaptic input to cerebellar Purkinje cells via activation of presynaptic CB1 receptors. Again, Δ9-THC was a less potent inhibitor than a potent synthetic CB1 receptor agonist (Irie et al., 2015). In contrast to this evidence suggesting a Δ9-THC-induced reduction in glutamate synaptic transmission, Tomasini et al. (2002) found that in vitro Δ9-THC application increases glutamate release and extracellular levels, suggesting an increase in cortical glutamatergic neurotransmission can be induced by CB1 receptor activation.

Only one in vitro study focused on glutamate enzyme activity (Monnet-Tschudi et al., 2008), and this showed that in aggregating brain cell cultures Δ9-THC reduces the activity both of glutamic acid decarboxylase (GAD), which catalyzes the decarboxylation of glutamate to GABA, and of glutamine synthetase (GS), which catalyzes the condensation of glutamate (Glu) and ammonia to form glutamine (Gln). Interestingly, use of both a CB1 receptor-selective agonist and a CB2 receptor-selective agonist in this study yielded data suggesting that the effects of Δ9-THC on GAD and GS activity are more likely caused by CB2 than CB1 receptor activation.

3.3.2. Animal studies of glutamatergic effects of embryonal in vivo Δ9-THC exposure

All these studies investigated the effect of prenatal exposure to Δ9-THC on glutamate signalling, using overlapping methodology (mode of administration, period of exposure, and dosage of Δ9-THC), even if investigating different brain regions of interest. Apart from one study (Castaldo et al., 2007), which confined Δ9-THC exposure to the embryonal period, all others prolonged it to postnatal age.

Castaldo and colleagues (2007) showed that adolescent rats which were exposed to Δ9-THC during gestation had a reduction in their basal extracellular glutamate levels. Prenatal exposure to WIN55,212-2 induced the same alteration. By studying the effects of this CB1 agonist on glutamate uptake and transporters, it was found that a possible explanation accounting for CB1-mediated glutamate outflow reduction may be an increase of glutamate uptake as a consequence of enhanced expression of the glutamate transporter 1 (GLT1) and excitatory amino acid carrier (EAAC1) protein.

Three studies assessed the activity of different enzymes in the glutamate metabolic pathway after embryonal exposure to Δ9-THC. Garcia-Gil et al. (1999) found that Δ9-THC does not produce any changes in glutamic acid decarboxylase (GAD) activity across several rat brain areas. On the contrary, glutamine synthetase (GS) activity has been reported to be reduced in the

cerebellar cortex of rats exposed to Δ9-THC; interestingly, GS expression increased progressively after Δ9-THC withdrawal, but did not reach control levels in male rats even two months after withdrawal (Suarez et al., 2002). Finally, Campolongo et al. (2007) showed that early exposure to Δ9-THC might interfere with the rigidly ordered temporal sequence of events that occur during the ontogeny of the central nervous system, leading to long-lasting neurodevelopmental alterations, including prefrontal enzyme activity. In addition, this study both confirmed a reduction in basal extracellular glutamate levels (Castaldo et al., 2007) and showed an alteration in glutamate receptors in offspring exposed to Δ9-THC during gestation.

Suarez et al. (2004a; 2004b) carried out two studies on the long-term effects of Δ9-THC exposure during development in rat cerebellum, indicating down-regulation of both glial (glutamate aspartate transporter, GLAST) and neuronal (excitatory amino acid carrier, EAAC1) glutamate transporter expression, and decreased expression of glutamate receptors 1 and 2/3 (GluR1; GluR2/3). Collectively, these findings suggest an abnormal maturation of the glutamatergic neuron-glia circuitry leading to a long-lasting inhibition of glutamatergic neurotransmission.

Finally, Castaldo and colleagues (2010) confirmed previous findings by Brown et al., (2003) or by Suarez et al. (2004b), that glutamate release at synapses is inhibited by prenatal exposure to Δ9-THC (Brown et al., 2003) with reduction in the functional activity and expression of glutamate transporters (Suarez et al., 2004b) (glutamate transporter 1, GLT1; GLAST). However, Castaldo et al. (2010) demonstrated that further in vitro application of low Δ9-THC concentrations enhanced hippocampal glutamate release in the hippocampus of vehicle-exposed rats, while this effect was selectively lost in rats exposed to Δ9-THC during gestation, suggesting that alteration in glutamatergic neurotransmission could partially depend on the loss of a similar control by endocannabinoids.

3.3.3. Animal studies of effect of postnatal in vivo Δ9-THC exposure on glutamate

Out of 17 studies carried out to investigate the effects of postnatal in vivo Δ9-THC administration on glutamate, 4 investigated glutamatergic synaptic transmission, 3 of them also providing information on glutamate receptors, while one also assessed neurotransmitter release and intracerebral levels. The remaining 13 studies assessed a single outcome: glutamate receptors (*n*=3), release (*n*=2), intracerebral levels (*n*=4), and enzyme activity (*n*=4).

Fan and colleagues (2010) confirmed that Δ9-THC impairs hippocampal glutamate synaptic transmission even when administered postnatally. Once again, Δ9-THC-induced depression of glutamatergic synaptic transmission seemed to require CB1 receptor activation, since it was prevented by administration of the CB1 receptor antagonist SR141716A, and absent in CB1 KO mice. Also, this alteration was associated with Δ9-THC-induced down-regulation of the expression of glutamate receptors and increases in glutamate release and intracellular levels. Good and Lupica (2010) obtained evidence that GluR2-lacking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors could be selectively increased at subcortical pedunculopontine nucleus (PPN) synapses following postnatal exposure to Δ9-THC, and that this can provide a substrate to permit long-term depression (LTD) in glutamatergic synaptic transmission. These effects were prevented by in vivo pretreatment with the cannabinoid CB1 receptor antagonist AM251, in line with other studies (Fan et al., 2010; Han et al., 2012; Liu et al., 2010). Another study explored Δ9-THC effects on the Ventral Tegmental Area (VTA), and showed that chronic exposure to Δ9-THC activates VTA CB1 receptors to induce transient depression of neurotransmission at VTA Glutamate-Dopamine (Glu-DA) synapses through the activation of N-methyl-D-aspartate (NMDA) receptors (Liu et al., 2010). This finding was confirmed by Han and colleagues (2012) who described cannabinoid-mediated long term depression in the hippocampus in relation to the activation of NMDA receptors containing GluN2B subunits.

Two recent studies did not find any glutamate receptor alteration following postnatal Δ9-THC exposure (Long et al., 2013; Spencer et al., 2013); however, both studies detected an aberrant NMDA receptor expression in Δ9-THC-treated mice carrying a genetic modification of the neuregulin 1 (NRG1) gene considered a schizophrenia relevant behavioral phenotype. In particular, adolescent Δ9-THC-treated NRG1 HET mice displayed increased NMDA receptor expression (Long et al., 2013) and altered expression of proteins involved in the NMDA receptor trafficking to the synaptic membrane (Spencer et al., 2013). Furthermore, Rubino et al. (2015) found that postnatal Δ9-THC exposure alters the maturational fluctuations of NMDA and AMPA units in the prefrontal cortex during adolescence.

Two studies showed that Δ9-THC decreases striatal glutamate release, and that SR141716A is able to reverse this decrease (Orru et al., 2011; Sano et al., 2008). Interestingly, as γ-aminobutyric acid (GABA)-ergic neurons did not appear to be particularly sensitive to Δ9-THC, it is noteworthy that evidence has been obtained for a selective CB1 receptor-mediated Δ9-THC action on glutamate-containing neurons in the nucleus accumbens (NAc; Sano et al., 2008).

The effect of cannabis on intracerebral glutamate levels was studied for the first time more than 30 years ago, when it was found that no significant changes in glutamine or glutamate levels occurred in adult chacma baboons after they were fed with Cannabis sativa (Ames et al., 1979). Similarly, Hikal et al. (1988) did not report any change in glutamine or glutamic acid in rats after Δ9-THC exposure. However, a more recent study indicated that Δ9-THC increases extracellular glutamate levels; this was also associated with increased dopamine levels and decreased GABA levels (Pistis et al., 2002). Galanopoulos et al. (2011) described a dose-dependent Δ9-THC induced increases in glutamate tissue levels across several brain areas (dorsal striatum; NAc; hippocampus; prefrontal cortex, PFC) which presumably reflects a reduction in synaptic glutamate levels.

Studies on glutamate enzyme activity have focused mainly on GAD activity: Edery and Gottesfeld (1975) were the first to detect a GAD activity reduction in cerebellum and motor

cortex, but not striatum, following Δ9-THC exposure. The absence of effect in the striatum was confirmed by Romero and colleagues (1998). More recently, Zamberletti et al (2014) described a time and dose-dependent ability of Δ9-THC to reduce prefrontal GAD activity, and showed that hippocampal GAD activity was not affected. Only one study has assessed glutamate dehydrogenase (GLDH), which appeared to be peripherally increased in rabbits fed with hashish for a month (Ghoneim et al., 1980).

3.3.4. Animal studies of effect of postnatal in vivo Δ9-THC exposure on glutamate, followed by additional in vitro Δ9-THC exposure

This study design was used to evaluate the sensitivity to cannabinoids in animals chronically exposed to Δ9-THC. Hoffman et al (2003) reported that an electrical stimulation of glutamatergic afferents to the NAc is able to induce long-term depression (LTD) in vehicle-treated animals, and that LTD can be completely blocked by the CB1 receptor antagonist SR141716A. On the contrary, electrical stimulation was not able to induce LTD in brain slices from rats chronically treated with Δ9-THC or the CB1 receptor agonist WIN55,212-2; also, in vitro WIN55,212-2 application was less effective in inducing LTD in chronically Δ9-THC -treated rats compared to controls. These data demonstrate functional tolerance, as long-term exposure to the psychoactive ingredient of cannabis can alter the modulatory effect of endogenous and exogenous cannabinoids on synaptic transmission. Another study confirmed that the ability of a cannabinoid agonist to inhibit glutamatergic transmission at PFC–NAc synapses is reduced after repeated exposure to Δ9-THC (Mato et al., 2005). However, it was also found in this study that in Δ9-THC-treated mice, LTD can still be induced because a presynaptic mGluR2/3 (metabotropic glutamate receptor 2/3)-dependent mechanism replaces the impaired endocannabinoid system. According to this study, eCB–LTD and mGluR2/3–LTD in the NAc can inhibit each other (a mGluR2/3 antagonist completely abolished Δ9-THC-induced LTD), despite being mediated by different receptors. Two

additional studies confirmed both impaired glutamatergic synaptic neurotransmission and functional tolerance as a consequence of prolonged cannabinoid receptor occupation by Δ9-THC (Hoffman et al., 2007; Tonini et al., 2006). Finally, Chen et al (2013) demonstrated that impaired synaptic function induced by repeated Δ9-THC exposure is associated with reduced uptake of glutamate by glutamate transporters in astrocytes; as a consequence, the resulting sustained elevation and accumulation of extracellular glutamate induces downregulation and internalization of glutamate receptor subunits and reduction in the density of dendritic spines in hippocampal neurons, leading to deficits in long-term synaptic plasticity.

4. DISCUSSION

This is the first systematic review of all studies examining the acute as well as chronic effect of cannabis or its main psychoactive ingredient, Δ9-THC, on the glutamate system in humans as well as animals. Previous reviews have mainly focused on the effects of cannabis use on dopamine transmission, in both animals (Kuepper et al., 2010) and humans (Ghazzaoui and Abi-Dargham, 2014; Sami et al., 2015; van Winkel and Kuepper, 2014), indicating that although there is consistent evidence for time and dose-dependent effects of Δ9-THC on psychosis risk (Di Forti et al., 2009), the relationship between Δ9-THC and dopaminergic abnormalities is still a matter of contention (Sami et al., 2015). Overall, this review demonstrates that cannabis or Δ9-THC have definite effects on glutamate in preclinical models, affecting glutamate signalling at different levels. Also, taking into account the paucity of observational studies and the absence of experimental studies in humans, cannabis use seems to consistently affect glutamate signalling in the human brain. These effects are discussed in greater detail below.

4.1. In vivo human studies

4.1.1. Chronic cannabis use reduces glutamate-related metabolite levels in the brain

The largest study supported an association between chronic cannabis use and reduced levels of glutamate-related metabolites in basal ganglia (Chang et al., 2006). However, when focusing specifically on the dorsal striatum, another study suggested a gender-related vulnerability to cannabis-induced glutamate abnormalities (Muetzel et al., 2013). In particular, female subjects had lower Glx (glutamate + glutamine) metabolite levels in the context of cannabis use, while male individuals did not show any difference in their Glx levels with reference to their history of cannabis use. This neurobiological finding is not in line with the clinical evidence from a recent review that males admitted to hospital with a diagnosis of cannabis psychosis outnumber females by 4:1 (Hamilton et al., 2015). However, the available data are too limited to draw any firm conclusions about a possible gender-related vulnerability to cannabis-induced glutamate impairment.

Two studies supported the evidence of a glutamate-related metabolite reduction in the anterior cingulate cortices of cannabis users (Prescot et al., 2011; Prescot et al., 2013). This finding was not replicated by Sung et al (2013). This study applied more rigorous criteria in order to control for the confounding effects of substance use and psychiatric comorbidity; however, the negative findings could be explained by the small sample size compared to the other studies and the inclusion of individuals with comorbid methamphetamine use which appeared to have no effect on glutamate signalling.

Taken together, the 5 studies provide a limited amount of evidence that cannabis use affects the cortical and subcortical levels of glutamate-related metabolites. However, work in this area is limited by a heterogeneity of outcome measures (different spectral fitting methods and glutamatergic indices) and methods to exclude/adjust for possible confounding factors, such as substance use and psychiatric comorbidity. Research suggests that even low alcohol concentrations can inhibit the excitatory activity of NMDA receptors (Gonzales and Jaworski, 1997); also, the glutamatergic system is critically involved in nicotine dependence (Liechti et al., 2008) and its

dysfunction has been robustly associated with depression (Sanacora et al., 2012). Most importantly, cannabis available in the street has many different ingredients, some of which such as cannabidiol may oppose the effects of Δ9-THC (Bhattacharyya et al., 2010; Bhattacharyya et al., 2012c; Bhattacharyya et al., 2015b), perhaps accounting for inconsistencies in the results from different studies. Perhaps acute challenge studies using different cannabinoids (Δ9-THC or cannabidiol) are first needed in order to further understand the effects of cannabis on glutamate in man. Also, no post-mortem/in vitro study has been carried out in humans; this work could add important information to the topic, in light of the important inter-species difference (human vs rat) in the effect of cannabis on dopaminergic signalling (Sami et al., 2015). Finally, all the studies identified used ^{1}H magnetic resonance spectroscopy (^{1}H-MRS), which has been reported to have limited spatial resolution, and present a whole tissue neurochemical measure, rather than distinguishing between intrasynaptic, extrasynaptic, or intracellular compartments (Poels et al., 2014). In particular, the quantification of glutamate (Glu) and glutamine (Gln) using MRS at 3Tesla field strength is challenging because of the overlapping multiplet structure of the coupled resonances (Gu et al., 2013). Even if constant-time point-resolved spectroscopy (CT-PRESS) has been optimized at 3 T to detect Glu, it did not resolve Gln (Gu et al., 2013).

4.2. Animal studies

4.2.1. Δ9-THC depresses glutamate synaptic transmission via CB1 receptor activation

Evidence from animal studies indicates that Δ9-THC depresses glutamate transmission in cerebellar (Irie et al., 2015), striatal (Brown et al., 2003; Mato et al., 2005) and midbrain (Good and Lupica, 2010; Liu et al., 2010) preparations as well as hippocampal slices (Chen et al., 2013; Fan et al., 2010; Han et al., 2012; Hoffman et al., 2010; Hoffman et al., 2007; Shen and Thayer, 1999; Straiker and Mackie, 2005). This has been consistently shown to be mediated by the CB1 receptor, using different methodologies, such as potent CB1 receptor agonists (Han et al., 2012;

Irie et al., 2015; Liu et al., 2010; Shen and Thayer, 1999; Straiker and Mackie, 2005) and antagonists (Brown et al., 2003; Fan et al., 2010; Good and Lupica, 2010; Han et al., 2012; Hoffman et al., 2010; Irie et al., 2015; Liu et al., 2010; Shen and Thayer, 1999; Straiker and Mackie, 2005) or CB1 receptor genetic silencing (Fan et al., 2010; Han et al., 2012; Hoffman et al., 2010; Liu et al., 2010; Straiker and Mackie, 2005). Also, Han and colleagues (2012) suggested that in vitro cannabinoid application decreases excitatory synaptic transmission via activation of "glutamatergic" CB1 receptors, whereas *in vivo* cannabinoid administration induces endocannabinoid mediated long-term depression (eCB-LTD) via astroglial CB1 receptor.

4.2.2. Chronic Δ9-THC exposure reduces the CB1 receptor-mediated inhibition of glutamate synaptic transmission

Another important finding obtained in the identified animal studies is that chronic treatment with Δ9-THC reduces the CB1 receptor-mediated inhibition of excitatory synaptic transmission (Hoffman et al., 2003; Mato et al., 2005). Impairment of eCB-LTD is attributable to prolonged CB1 receptor occupation and the resulting functional tolerance. Consistently, prolonged Δ9-THC exposure caused decreased sensitivity of glutamatergic synapses to the inhibitory effects of cannabinoids, even to potent CB1R agonists (Hoffman et al., 2003; Mato et al., 2005). Interestingly, results support an alternative presynaptic mGluR2/3–LTD at the excitatory synapses between the prefrontal cortex and the nucleus accumbens (PFC-NAc) in animals chronically exposed to Δ9-THC, which inhibit each other in occluding eCB-LTD (Mato et al., 2005).

4.2.3. Δ9-THC exhibits both CB1 receptor agonist and antagonist properties, resulting in opposite effects on glutamate synaptic transmission

In vitro studies clarified the relevance of the Δ9-THC partial agonist profile with reference to glutamatergic synaptic transmission (Shen and Thayer, 1999; Straiker and Mackie, 2005). More

specifically, Δ9-THC has been reported to have high potency but modest efficacy in affecting glutamate synaptic transmission, if compared to the potent CB1 receptor agonist WIN55,212-2 (Shen and Thayer, 1999). Most importantly, it has been suggested that Δ9-THC can block the activation of CB1 receptors. Indeed, Δ9-THC has been found to prevent (Straiker and Mackie, 2005) and partially reverse (Shen and Thayer, 1999) WIN55,212-2-induced glutamate synaptic depression.

4.2.4. Effects of Δ9-THC on glutamate release differ under in vivo and in vitro conditions and depending on the duration of the exposure

The blockade of glutamate synaptic plasticity after prolonged Δ9-THC exposure has been related to an increase in glutamate release probability that occurs in Δ9-THC-tolerant mice in both the cerebellum (Tonini et al., 2006) and the hippocampus (Hoffman et al., 2007). A more recent study has proposed that impairment in hippocampal glutamate synaptic function following chronic Δ9-THC exposure is associated with a CB1 receptor-G beta gamma complex-protein kinase B-extracellular-signal-regulated kinase-mitogen activated protein kinase-nuclear factor kappa-light-chain-enhancer of activated B cells- cyclooxygenase 2 (CB1R-Gβγ-Akt-ERK/MAPK-NF-κB-COX-2) signalling pathway (Chen et al., 2013). Upon repeated Δ9-THC exposure, glutamate released from astroglial cells triggered by cyclooxygenase 2-derived prostaglandine E2 (COX2-derived PGE2) and reduced uptake of glutamate by glutamate transporters in astrocytes would cause extracellular glutamate accumulation. Sustained elevation and accumulation of extracellular glutamate would induce down-regulation and internalization of glutamate receptor subunits and reduction in the density of dendritic spines, leading to deficits in long-term synaptic plasticity (Chen et al., 2013).

However, the finding of a Δ9-THC-induced increase in glutamate release probability after prolonged in vivo exposure (Fan et al., 2010; Hoffman et al., 2007; Tonini et al., 2006) is

apparently in contrast with results from other in vivo studies involving administration of a single dose of Δ9-THC, as well as with results from in vitro studies. In particular, in vivo exposure to a single dose of Δ9-THC has been associated with CB1 receptor-mediated inhibition of glutamate release (Orru et al., 2011; Sano et al., 2008). Similarly, it has been suggested that in vitro Δ9-THC application induces a decrease in presynaptic glutamate release via activation of presynaptic CB1 receptors (Brown et al., 2003; Shen and Thayer, 1999), an effect that has been observed in cells treated with potent CB1 receptor agonists (Irie et al., 2015). Brown and colleagues (2003) proposed that striatal CB1 receptor activation decreases glutamate transporter activity and that the resulting increase in synaptic cleft glutamate concentration causes the activation of presynaptic mGluRs, which then decrease glutamate release. Consistently, this study reported that blockade of glutamate uptake mimics the actions of Δ9-THC in vitro: blockade of glutamate transport and activation of CB1 receptors act to depress glutamate transmission by a similar mechanism, and mGluR antagonism abolishes the depression induced by Δ9-THC (Brown et al., 2003).

Castaldo and colleagues (2010) tried to identify the cause of the discrepancy between the effects of Δ9-THC on glutamate release under in vivo and in vitro conditions or prolonged and single dose exposure. This study indicated that in vitro application of low concentrations of Δ9-THC was able to paradoxically promote hippocampal glutamate release in rats with no previous exposure. On the contrary, perinatal exposure to Δ9-THC suppressed the ability of low cannabinoid concentrations to promote glutamate release (Castaldo et al., 2010). The authors proposed that low cannabinoid concentrations enhance glutamate release in response to depolarization (as also shown by Tomasini et al., 2002), and that this effect is selectively lost in Δ9-THC-treated rats, suggesting that impaired glutamatergic neurotransmission could partially depend on the loss of a similar control by endocannabinoids (Castaldo et al., 2010).

4.2.5. Δ9-THC reduces glutamate enzyme activity affecting the normal central nervous system ontogeny

A few studies addressed glutamate enzyme activity in response to Δ9-THC. The only in vitro study in this field reported that Δ9-THC reduces both GAD and GS activity; interestingly, while JWH-015, a selective CB2 receptor agonist had the same effects as Δ9-THC on GAD and GS activity, Arachidonyl-2'-chloroethylamide (ACEA), a selective CB1 agonist, had no effects. Therefore, the authors concluded that the adverse effects of Δ9-THC on glutamate enzyme activity are likely caused by CB2 receptor activation (Monnet-Tschudi et al., 2008). Suarez and colleagues (2002) confirmed a decrease in GS expression after perinatal Δ9-THC exposure. According to the authors, a possible explanation for this finding is that the release of glutamate is decreased, a hypothesis that needs further investigation. Impaired activity of the glutamate enzymes, glutamate cysteine ligase, modifier subunit (GCLM), glutamate dehydrogenase 1 (GLUD1) and glutamate oxaloacetate transaminase 1 (GOT1), has been proposed to be related to aberrant events occurring during the ontogeny of the central nervous system in animal embryos exposed to Δ9-THC (Campolongo et al., 2007). In vivo studies indicate that GAD activity is reduced in cerebellum, motor and prefrontal cortex, but not in the striatum and ventral midbrain (Edery and Gottesfeld; Garcia-Gil et al., 1999; Romero et al., 1998; Zamberletti et al., 2014). According to Zamberletti et al (2014), reduced GAD expression may be directly related to the altered behavior observed in adult Δ9-THC-treated rats.

4.2.6. Δ9-THC alters expression, function, and maturational fluctuations of glutamate receptors

It has been proposed that the impaired long-term synaptic plasticity induced by Δ9-THC is associated with altered expression, function, and maturational fluctuations of glutamate receptors

(Fan et al., 2010; Han et al., 2012; Liu et al., 2010; Rubino et al., 2015; Suarez et al., 2004b) and that this alteration is more pronounced in animal models of schizophrenia (Long et al., 2013; Spencer et al., 2013). Spencer and colleagues (2013) have suggested that increased expression of NMDA receptors in Δ9-THC-treated Nrg1 HET mice might increase the expression of the apoptotic marker PCD2 and the anandamide synthesizing enzyme NAPE-PLD, protein changes reflecting heightened excitotoxicity and apoptosis, and a homeostatic attempt to dampen increased NMDA receptor activation. Moreover, it has been proposed that Δ9-THC-induced CB1 receptor activation causes the insertion of higher conducting GluR2-lacking AMPA receptors into subcortical glutamate synapses (Good and Lupica, 2010).

4.2.7. Δ9-THC decreases expression and functional activity of glutamate transporters

Studies which have assessed the effect of prenatal exposure to Δ9-THC on expression and functional activity of glutamate transporters suggest a Δ9-THC-induced reduction of glial and/or neuronal transporters in hippocampus (GLAST; GLUT1; Castaldo et al., 2010) and cerebellum (GLAST; EAAC1; Suarez et al., 2004b). Collectively, these studies suggest that Δ9-THC exposure during brain development may alter the glutamatergic system not only during the period of drug exposure but also in the postnatal stage following withdrawal, supporting an association between abnormal maturation of the glutamatergic neuron-glia circuitry and downregulation of glutamate transporters. Chen and colleagues (2013) indicated that postnatal Δ9-THC exposure is able to affect hippocampal GLAST and GLUT1 transporters levels in a similar way. Interestingly, Castaldo et al (2007) reported that prenatal exposure to the cannabinoid CB1 receptor agonist WIN55,212-2 exhibits opposite effects to those of Δ9-THC, increasing expression and functional activity of GLT1 and EAAC1 glutamate transporters in rat frontal cerebral cortex, possibly due to one or more differences between the mode(s) of action of WIN55,212-2 (a full agonist at the CB1 receptor) and Δ9-THC (a partial CB1R agonist).

4.2.8. Less conclusive evidence of an effect of Δ9-THC on brain glutamate levels

Studies on brain neurotransmitter levels tend to support an alteration following Δ9-THC exposure, even if the direction of this alteration is still unclear. First studies conducted in the 1970's and 1980's failed to show altered glutamate, glutamine or glutamic acid levels after in vivo exposure to Δ9-THC, possibly due to technical limitations (Edery and Gottesfeld, 1975; Hikal et al., 1998). Furthermore, while perinatal exposure has been related to reduced cortical basal extracellular glutamate levels (Campolongo et al., 2007; Castaldo et al., 2007), studies exploring effects of postnatal Δ9-THC exposure indicate an increase in cortical extracellular (Pistis et al., 2002) and hippocampal intracellular glutamate levels (Fan et al., 2010). Galanopoulos et al (2011) found cortical, striatal, as well as hippocampal increased glutamate tissue content (Galanopoulos et al., 2011), inferring a dose-dependent Δ9-THC-induced reduction in glutamate synaptic levels, possibly due to the activation of CB1 receptors at the glutamatergic presynapses in cortical and subcortical brain regions.

4.3. Conclusion

Together, the 41 studies discussed in this review suggest that cannabis use or exposure to its main psychoactive constituent, Δ9-THC, have the ability to disrupt glutamate signalling in preclinical models as well as humans. There is reasonably consistent evidence from animal models that in vitro application as well as in vivo embryonal or postnatal administration of Δ9-THC depresses endocannabinoid-mediated glutamate synaptic transmission, affecting glutamate release, enzyme activity and the expression and activity of both receptors and transporters. Also Δ9-THC seems to be able to agonize as well as antagonize CB1 receptors depending on the extent of receptor occupation, resulting in opposite effects on glutamate synaptic transmission. Finally, prolonged Δ9-THC exposure affects glutamate synaptic plasticity by a functional tolerance

mechanism, reducing the ability of CB1R agonists to inhibit glutamate synaptic transmission (Figure 1).

In light of all this evidence, the finding that chronic cannabis use reduces glutamate-related metabolite levels in man could well be a result of excessive down-regulation of glutamate signalling. However, research in humans is still too limited and further investigations are needed in order to address this issue and its relevance for the clinical phenotype of cannabis-associated schizophrenia. Convergent and replicated findings from several studies suggest that dopamine dysregulation is the final common biological pathway for all the psychosis risk factors (Howes and Kapur, 2009). This hypothesis suggests that many neurochemical pathways could induce dopamine sensitisation. Consistently, hypofunctioning glutamate neurotransmission has been postulated to be the primary deficit determining the dopamine abnormalities consistently reported in schizophrenia (Howes et al., 2015; Snyder and Murphy, 2008). Indeed, the biological distance between Δ9-THC effects and glutamate abnormalities is shorter compared to that of dopamine dysregulation. Therefore, glutamate rather than dopamine correlates may best capture the effects of cannabis on the brain.

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REFERENCES

Ames, F.R., Brownell, B., Zuurmond, T.J., 1979. Effects of the oral administration of Cannabis sativa (dagga) on chacma baboons (Papio ursinus). S. Afr. Med. J. 55, 1127-1132.

Appiah-Kusi, E., Leyden, E., Parmar, S., Mondelli, V., McGuire, P., Bhattacharyya, S., 2016. Abnormalities in neuroendocrine stress response in psychosis: the role of endocannabinoids. Psychol. Med. 46, 27-45.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J.A., Kambeitz, J., Malhi, S., Giampietro, V., Williams, S., Brammer, M., Rubia, K., Collier, D.A., McGuire, P.K., 2015a. Impairment of inhibitory control processing related to acute psychotomimetic effects of cannabis. Eur. Neuropsychopharmacol. 25, 26-37.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J.A., Kambeitz, J., Prata, D., Williams, S., Brammer, M., Collier, D.A., McGuire, P.K., 2012a. Preliminary report of biological basis of sensitivity to the effects of cannabis on psychosis: AKT1 and DAT1 genotype modulates the effects of delta-9-tetrahydrocannabinol on midbrain and striatal function. Mol. Psychiatry 17, 1152-1155.

Bhattacharyya, S., Atakan, Z., Martin-Santos, R., Crippa, J.A., McGuire, P.K., 2012c. Neural mechanisms for the cannabinoid modulation of cognition and affect in man: a critical review of neuroimaging studies. Curr Pharm Des. 2012c;18(32):5045-54.

Bhattacharyya, S., Crippa, J.A., Allen, P., Martin-Santos, R., Borgwardt, S., Fusar-Poli, P., Rubia, K., Kambeitz, J., O'Carroll, C., Seal, M.L., Giampietro, V., Brammer, M., Zuardi, A.W., Atakan, Z., McGuire, P.K., 2012b. Induction of psychosis by Δ9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. Arch. Gen. Psychiatry 69, 27-36.

Bhattacharyya, S., Crippa, J.A., Martin-Santos, R., Winton-Brown, T., Fusar-Poli, P., 2009b. Imaging the neural effects of cannabinoids: current status and future opportunities for psychopharmacology. Curr. Pharm. Des. 15, 2603-2614.

Bhattacharyya, S., Falkenberg, I., Martin-Santos, R., Atakan, Z., Crippa, J.A., Giampietro, V., Brammer, M., McGuire, P., 2015b. Cannabinoid modulation of functional connectivity within regions processing attentional salience. Neuropsychopharmacology 40, 1343-1352.

Bhattacharyya, S., Fusar-Poli, P., Borgwardt, S., Martin-Santos, R., Nosarti, C., O'Carroll, C., Allen, P., Seal, M.L., Fletcher, P.C., Crippa, J.A., Giampietro, V., Mechelli, A., Atakan, Z., McGuire, P., 2009a. Modulation of Mediotemporal and Ventrostriatal Function in Humans by Delta 9-Tetrahydrocannabinol A Neural Basis for the Effects of Cannabis sativa on Learning and Psychosis. Arch. Gen. Psychiatry 66, 442-451.

Bhattacharyya, S., Iyegbe, I., Atakan, Z., Martin-Santos, R., Crippa, J.A., Xu, X., Williams, S., Brammer, M., Rubia, K., Prata, D., Collier, D.A., McGuire, P.K., 2014. Protein kinase B (AKT1) genotype mediates sensitivity to cannabis-induced impairments in psychomotor control. Psychol. Med. 44, 3315-3328.

Bhattacharyya, S., Morrison, P.D., Fusar-Poli, P., Martin-Santos, R., Borgwardt, S., Winton-Brown, T., Nosarti, C., O' Carroll, C.M., Seal, M., Allen, P., Mehta, M.A., Stone, J.M., Tunstall, N., Giampietro, V., Kapur, S., Murray, R.M., Zuardi, A.W., Crippa, J.A., Atakan, Z., McGuire, P.K., 2010. Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. Neuropsychopharmacology 35, 764-774.

Brown, T.M., Brotchie, J.M., Fitzjohn, S.M., 2003. Cannabinoids decrease corticostriatal synaptic transmission via an effect on glutamate uptake. J. Neurosci. 23, 11073-11077.

Campolongo, P., Trezza, V., Cassano, T., Gaetani, S., Morgese, M.G., Ubaldi, M., Soverchia, L., Antonelli, T., Ferraro, L., Massi, M., Ciccocioppo, R., Cuomo, V., 2007. Perinatal exposure to delta-9-tetrahydrocannabinol causes enduring cognitive deficits associated with alteration of cortical gene expression and neurotransmission in rats. Addict. Biol. 12, 485-495.

Castaldo, P., Magi, S., Cataldi, M., Arcangeli, S., Lariccia, V., Nasti, A.A., Ferraro, L., Tomasini, M.C., Antonelli, T., Cassano, T., Cuomo, V., Amoroso, S., 2010. Altered regulation of glutamate

release and decreased functional activity and expression of GLT1 and GLAST glutamate transporters in the hippocampus of adolescent rats perinatally exposed to Delta(9)-THC. Pharmacol. Res. 61, 334-341.

Castaldo, P., Magi, S., Gaetani, S., Cassano, T., Ferraro, L., Antonelli, T., Amoroso, S., Cuomo, V., 2007. Prenatal exposure to the cannabinoid receptor agonist WIN 55,212-2 increases glutamate uptake through overexpression of GLT1 and EAAC1 glutamate transporter subtypes in rat frontal cerebral cortex. Neuropharmacology 53, 369-378.

Ceccarini, J., Kuepper, R., Kemels, D., van Os, J., Henquet, C., Van Laere, K., 2015. [F-18]MK-9470 PET measurement of cannabinoid CB1 receptor availability in chronic cannabis users. Addict. Biol. 20, 357-367.

Chang, L., Cloak, C., Yakupov, R., Ernst, T., 2006. Combined and independent effects of chronic marijuana use and HIV on brain metabolites. J. Neuroimmune. Pharmacol. 1, 65-76.

Chen, R., Zhang, J., Fan, N., Teng, Z.Q., Wu, Y., Yang, H., Tang, Y.P., Sun, H., Song, Y., Chen, C., 2013. Delta9-THC-caused synaptic and memory impairments are mediated through COX-2 signaling. Cell 155, 1154-1165.

Colizzi, M., Carra, E., Fraietta, S., Lally, J., Quattrone, D., Bonaccorso, S., Mondelli, V., Ajnakina, O., Dazzan, P., Trotta, A., Sideli, L., Kolliakou, A., Gaughran, F., Khondoker, M., David, A.S., Murray, R.M., MacCabe, J.H., Di Forti, M., in press. Substance use, medication adherence and outcome one year following a first episode of psychosis. Schizophr. Res.

Colizzi, M., Fazio, L., Ferranti, L., Porcelli, A., Masellis, R., Marvulli, D., Bonvino, A., Ursini, G., Blasi, G., Bertolino, A., 2015c. Functional genetic variation of the cannabinoid receptor 1 and cannabis use interact on prefrontal connectivity and related working memory behavior. Neuropsychopharmacology 40, 640-649.

Colizzi, M., Iyegbe, C., Powell, J., Blasi, G., Bertolino, A., Murray, R.M., Di Forti, M., 2015a. Interaction between DRD2 and AKT1 genetic variations on risk of psychosis in cannabis users: a case–control study. Npj Schizophrenia 1, 15025.

Colizzi, M., Iyegbe, C., Powell, J., Ursini, G., Porcelli, A., Bonvino, A., Taurisano, P., Romano, R., Masellis, R., Blasi, G., Morgan, C., Aitchison, K., Mondelli, V., Luzi, S., Kolliakou, A., David, A., Murray, R.M., Bertolino, A., Di Forti, M., 2015b. Interaction Between Functional Genetic Variation of DRD2 and Cannabis Use on Risk of Psychosis. Schizophr. Bull. 41, 1171-1182.

Di Forti, M., Morgan, C., Dazzan, P., Pariante, C., Mondelli, V., Marques, T.R., Handley, R., Luzi, S., Russo, M., Paparelli, A., Butt, A., Stilo, S.A., Wiffen, B., Powell, J., Murray, R.M., 2009. High-potency cannabis and the risk of psychosis. Br. J. Psychiatry 195, 488-491.

Edery, H., Gottesfeld, Z., 1975. The gamma-aminobutyric acid system in rat cerebellum during cannabinoid-induced cataleptoid state. Br. J. Pharmacol. 54, 406-408.

Fan, N., Yang, H., Zhang, J., Chen, C., 2010. Reduced expression of glutamate receptors and phosphorylation of CREB are responsible for in vivo Delta9-THC exposure-impaired hippocampal synaptic plasticity. J. Neurochem. 112, 691-702.

Galanopoulos, A., Polissidis, A., Papadopoulou-Daifoti, Z., Nomikos, G.G., Antoniou, K., 2011. Δ(9)-THC and WIN55,212-2 affect brain tissue levels of excitatory amino acids in a phenotype-, compound-, dose-, and region-specific manner. Behav. Brain Res. 224, 65-72.

Garcia-Gil, L., de Miguel, R., Romero, J., Perez, A., Ramos, J.A., Fernandez-Ruiz, J.J., 1999. Perinatal delta9-tetrahydrocannabinol exposure augmented the magnitude of motor inhibition caused by GABA(B), but not GABA(A), receptor agonists in adult rats. Neurotoxicol. Teratol. 21, 277-283.

Ghazzaoui, R., Abi-Dargham, A., 2014. Imaging dopamine transmission parameters in cannabis dependence. Prog. Neuropsychopharmacol. Biol. Psychiatry 52, 28-32.

Ghoneim, M.T., Mikhail, M.M., Mahfouz, M., Makar, A.B., 1980. Effect of hashish smoke on some blood and serum parameters in rabbits. Pharmazie 35, 226-228.

Gonzales, R.A., Jaworski, J.N., 1997. Alcohol and glutamate. Alcohol Health Res W 21, 120-127.

Good, C.H., Lupica, C.R., 2010. Afferent-specific AMPA receptor subunit composition and regulation of synaptic plasticity in midbrain dopamine neurons by abused drugs. J. Neurosci. 30, 7900-7909.

Gu, M., Zahr, N.M., Spielman, D.M., Sullivan, E.V., Pfefferbaum, A., Mayer, D., 2013. Quantification of glutamate and glutamine using constant-time point-resolved spectroscopy at 3 T. NMR Biomed. 26, 164-172.

Hamilton, I., Galdas, P., Essex, H., 2015. Cannabis psychosis, gender matters. Adv. Dual Diagn. 8, 153-162.

Han, J., Kesner, P., Metna-Laurent, M., Duan, T., Xu, L., Georges, F., Koehl, M., Abrous, D.N., Mendizabal-Zubiaga, J., Grandes, P., Liu, Q., Bai, G., Wang, W., Xiong, L., Ren, W., Marsicano, G., Zhang, X., 2012. Acute cannabinoids impair working memory through astroglial CB1 receptor modulation of hippocampal LTD. Cell 148, 1039-1050.

Hikal, A.H., Lipe, G.W., Slikker, W., Jr., Scallet, A.C., Ali, S.F., Newport, G.D., 1988. Determination of amino acids in different regions of the rat brain. Application to the acute effects of tetrahydrocannabinol (THC) and trimethyltin (TMT). Life Sci. 42, 2029-2035.

Hirvonen, J., Goodwin, R.S., Li, C.T., Terry, G.E., Zoghbi, S.S., Morse, C., Pike, V.W., Volkow, N.D., Huestis, M.A., Innis, R.B., 2012. Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. Mol. Psychiatry 17, 642-649.

Hoffman, A.F., Laaris, N., Kawamura, M., Masino, S.A., Lupica, C.R., 2010. Control of cannabinoid CB1 receptor function on glutamate axon terminals by endogenous adenosine acting at A1 receptors. J. Neurosci. 30, 545-555.

Hoffman, A.F., Oz, M., Caulder, T., Lupica, C.R., 2003. Functional tolerance and blockade of long-term depression at synapses in the nucleus accumbens after chronic cannabinoid exposure. J. Neurosci. 23, 4815-4820.

Hoffman, A.F., Oz, M., Yang, R., Lichtman, A.H., Lupica, C.R., 2007. Opposing actions of chronic Delta9-tetrahydrocannabinol and cannabinoid antagonists on hippocampal long-term potentiation. Learn. Mem. 14, 63-74.

Howes, O., McCutcheon, R., Stone, J., 2015. Glutamate and dopamine in schizophrenia: an update for the 21st century. J. Psychopharmacol. 29, 97-115.

Howes, O.D., Kapur, S., 2009. The dopamine hypothesis of schizophrenia: version III--the final common pathway. Schizophr. Bull. 35, 549-562.

Irie, T., Kikura-Hanajiri, R., Usami, M., Uchiyama, N., Goda, Y., Sekino, Y., 2015. MAM-2201, a synthetic cannabinoid drug of abuse, suppresses the synaptic input to cerebellar Purkinje cells via activation of presynaptic CB1 receptors. Neuropharmacology 95, 479-491.

Kuepper, R., Morrison, P.D., van Os, J., Murray, R.M., Kenis, G., Henquet, C., 2010. Does dopamine mediate the psychosis-inducing effects of cannabis? A review and integration of findings across disciplines. Schizophr. Res. 121, 107-117.

Liechti, M., Kuczenski, R., Markou, A., 2008. Glutamate and the reinforcing effects of nicotine. Eur. Neuropsychopharm. 18, S56-S56.

Liu, Z., Han, J., Jia, L., Maillet, J.C., Bai, G., Xu, L., Jia, Z., Zheng, Q., Zhang, W., Monette, R., Merali, Z., Zhu, Z., Wang, W., Ren, W., Zhang, X., 2010. Synaptic neurotransmission depression in ventral tegmental dopamine neurons and cannabinoid-associated addictive learning. PloS one 5, e15634.

Long, L.E., Chesworth, R., Huang, X.F., McGregor, I.S., Arnold, J.C., Karl, T., 2013. Transmembrane domain Nrg1 mutant mice show altered susceptibility to the neurobehavioural actions of repeated THC exposure in adolescence. Int. J. Neuropsychopharmacol. 16, 163-175.

Mato, S., Robbe, D., Puente, N., Grandes, P., Manzoni, O.J., 2005. Presynaptic homeostatic plasticity rescues long-term depression after chronic Delta 9-tetrahydrocannabinol exposure. J. Neurosci. 25, 11619-11627.

Monnet-Tschudi, F., Hazekamp, A., Perret, N., Zurich, M.G., Mangin, P., Giroud, C., Honegger, P., 2008. Delta-9-tetrahydrocannabinol accumulation, metabolism and cell-type-specific adverse effects in aggregating brain cell cultures. Toxicol. Appl. Pharmacol. 228, 8-16.

Moore, T.H., Zammit, S., Lingford-Hughes, A., Barnes, T.R., Jones, P.B., Burke, M., Lewis, G., 2007. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet 370, 319-328.

Muetzel, R.L., Marjanska, M., Collins, P.F., Becker, M.P., Valabregue, R., Auerbach, E.J., Lim, K.O., Luciana, M., 2013. In vivo H magnetic resonance spectroscopy in young-adult daily marijuana users. NeuroImage Clin. 2, 581-589.

Murray, R.M., Morrison, P.D., Henquet, C., Di Forti, M., 2007. Cannabis, the mind and society: the hash realities. Nat. Rev. Neurosci. 8, 885-895.

Orru, M., Quiroz, C., Guitart, X., Ferre, S., 2011. Pharmacological evidence for different populations of postsynaptic adenosine A2A receptors in the rat striatum. Neuropharmacology 61, 967-974.

Patel, R., Wilson, R., Jackson, R., Ball, M., Shetty, H., Broadbent, M., Stewart, R., McGuire, P., Bhattacharyya, S., in press. Association of cannabis use with hospital admission and antipsychotic treatment failure in first episode psychosis: an observational study. BMJ Open

Pertwee, R.G., 2008. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br. J. Pharmacol. 153, 199-215.

Pistis, M., Ferraro, L., Pira, L., Flore, G., Tanganelli, S., Gessa, G.L., Devoto, P., 2002. Delta(9)-tetrahydrocannabinol decreases extracellular GABA and increases extracellular glutamate and

dopamine levels in the rat prefrontal cortex: an in vivo microdialysis study. Brain Res. 948, 155-158.

Poels, E.M., Kegeles, L.S., Kantrowitz, J.T., Javitt, D.C., Lieberman, J.A., Abi-Dargham, A., Girgis, R.R., 2014. Glutamatergic abnormalities in schizophrenia: a review of proton MRS findings. Schizophr. Res. 152, 325-332.

Prescot, A.P., Locatelli, A.E., Renshaw, P.F., Yurgelun-Todd, D.A., 2011. Neurochemical alterations in adolescent chronic marijuana smokers: a proton MRS study. Neuroimage 57, 69-75.

Prescot, A.P., Renshaw, P.F., Yurgelun-Todd, D.A., 2013. γ-Amino butyric acid and glutamate abnormalities in adolescent chronic marijuana smokers. Drug Alcohol Depend. 129, 232-239.

Romero, J., de Miguel, R., Ramos, J.A., Fernandez-Ruiz, J.J., 1998. The activation of cannabinoid receptors in striatonigral GABAergic neurons inhibited GABA uptake. Life Sci. 62, 351-363.

Rubino, T., Prini, P., Piscitelli, F., Zamberletti, E., Trusel, M., Melis, M., Sagheddu, C., Ligresti, A., Tonini, R., Di Marzo, V., Parolaro, D., 2015. Adolescent exposure to THC in female rats disrupts developmental changes in the prefrontal cortex. Neurobiol. Dis. 73, 60-69.

Sami, M.B., Rabiner, E.A., Bhattacharyya, S., 2015. Does cannabis affect dopaminergic signaling in the human brain? A systematic review of evidence to date. Eur. Neuropsychopharmacol. 25, 1201-1224.

Sanacora, G., Treccani, G., Popoli, M., 2012. Towards a glutamate hypothesis of depression An emerging frontier of neuropsychopharmacology for mood disorders. Neuropharmacology 62, 63-77.

Sano, K., Mishima, K., Koushi, E., Orito, K., Egashira, N., Irie, K., Takasaki, K., Katsurabayashi, S., Iwasaki, K., Uchida, N., Egawa, T., Kitamura, Y., Nishimura, R., Fujiwara, M., 2008. Delta 9-tetrahydrocannabinol-induced catalepsy-like immobilization is mediated by decreased 5-HT neurotransmission in the nucleus accumbens due to the action of glutamate-containing neurons. Neuroscience 151, 320-328.

Schoeler, T., Monk, A., Sami, M., Klamerus, E., Foglia, E., Brown, R., Camuri, G., Altamure, A.,

Murray, R. and Bhattacharyya, S., in press. Continued versus discontinued cannabis use in patients with psychosis: a systematic review and meta-analysis. Lancet Psychiatry.

Shen, M., Thayer, S.A., 1999. Delta9-tetrahydrocannabinol acts as a partial agonist to modulate glutamatergic synaptic transmission between rat hippocampal neurons in culture. Mol. Pharmacol. 55, 8-13.

Snyder, E.M., Murphy, M.R., 2008. Schizophrenia therapy: beyond atypical antipsychotics. Nature reviews. Nat. Rev. Drug Discov. 7, 471-472.

Spencer, J.R., Darbyshire, K.M., Boucher, A.A., Kashem, M.A., Long, L.E., McGregor, I.S., Karl, T., Arnold, J.C., 2013. Novel molecular changes induced by Nrg1 hypomorphism and Nrg1-cannabinoid interaction in adolescence: a hippocampal proteomic study in mice. Front. Cell. Neurosci. 7, 15.

Straiker, A., Mackie, K., 2005. Depolarization-induced suppression of excitation in murine autaptic hippocampal neurones. J. Physiol. 569, 501-517.

Suarez, I., Bodega, G., Fernandez-Ruiz, J., Ramos, J.A., Rubio, M., Fernandez, B., 2004a. Down-regulation of the AMPA glutamate receptor subunits GluR1 and GluR2/3 in the rat cerebellum following pre- and perinatal delta9-tetrahydrocannabinol exposure. Cerebellum 3, 66-74.

Suarez, I., Bodega, G., Fernandez-Ruiz, J.J., Ramos, J.A., Rubio, M., Fernandez, B., 2002. Reduced glial fibrillary acidic protein and glutamine synthetase expression in astrocytes and Bergmann glial cells in the rat cerebellum caused by delta(9)-tetrahydrocannabinol administration during development. Dev. Neurosci. 24, 300-312.

Suarez, I., Bodega, G., Rubio, M., Fernandez-Ruiz, J.J., Ramos, J.A., Fernandez, B., 2004b. Prenatal cannabinoid exposure down- regulates glutamate transporter expressions (GLAST and EAAC1) in the rat cerebellum. Dev. Neurosci. 26, 45-53.

Sung, Y.H., Carey, P.D., Stein, D.J., Ferrett, H.L., Spottiswoode, B.S., Renshaw, P.F., Yurgelun-Todd, D.A., 2013. Decreased frontal N-acetylaspartate levels in adolescents concurrently using both methamphetamine and marijuana. Behav. Brain Res. 246, 154-161.

Tomasini, M.C., Ferraro, L., Bebe, B.W., Tanganelli, S., Cassano, T., Cuomo, V., Antonelli, T., 2002. Delta(9)-tetrahydrocannabinol increases endogenous extracellular glutamate levels in primary cultures of rat cerebral cortex neurons: involvement of CB(1) receptors. J Neurosci. Res. 68, 449-453.

Tonini, R., Ciardo, S., Cerovic, M., Rubino, T., Parolaro, D., Mazzanti, M., Zippel, R., 2006. ERK-dependent modulation of cerebellar synaptic plasticity after chronic Delta9-tetrahydrocannabinol exposure. J Neurosci. 26, 5810-5818.

van Winkel, R., Kuepper, R., 2014. Epidemiological, neurobiological, and genetic clues to the mechanisms linking cannabis use to risk for nonaffective psychosis. Annu. Rev. Clin. Psychol. 10, 767-791.

West, S., King, V., Carey, T.S., Lohr, K.N., McKoy, N., Sutton, S.F., Lux, L., 2002. Systems to rate the strength of scientific evidence. Evid Rep Technol Assess (Summ). 1-11.

Zamberletti, E., Beggiato, S., Steardo, L., Jr., Prini, P., Antonelli, T., Ferraro, L., Rubino, T., Parolaro, D., 2014. Alterations of prefrontal cortex GABAergic transmission in the complex psychotic-like phenotype induced by adolescent delta-9-tetrahydrocannabinol exposure in rats. Neurobiol. Dis. 63, 35-47.

Figure Captions

Figure 1. Summary of the main effects of delta-9-tetrahydrocannabinol (Δ9-THC) on glutamate signalling

1a. Glutamate synaptic transmission
- In vitro studies
↓↓↓↓
→
- Postnatal in vivo studies
↓↓↓↓
- Postnatal in vivo studies followed by in vitro experiments
↓↓↓

1b. Glutamate synaptic release
- In vitro studies
↓
↑
- Embryonal in vivo studies
↓
- Postnatal in vivo studies
↓↓
↑

1c. Glutamate synaptic uptake
- In vitro studies
↓
→
- Embryonal in vivo studies
↓

1d. Glutamate extracellular levels
- In vitro studies
↑
- Embryonal in vivo studies
↓↓
- Postnatal in vivo studies
↑

2. Glutamate synaptic transmission functional tolerance
- In vitro studies
+
- Embryonal in vivo studies
+
- Postnatal in vivo studies followed by in vitro experiments
++++

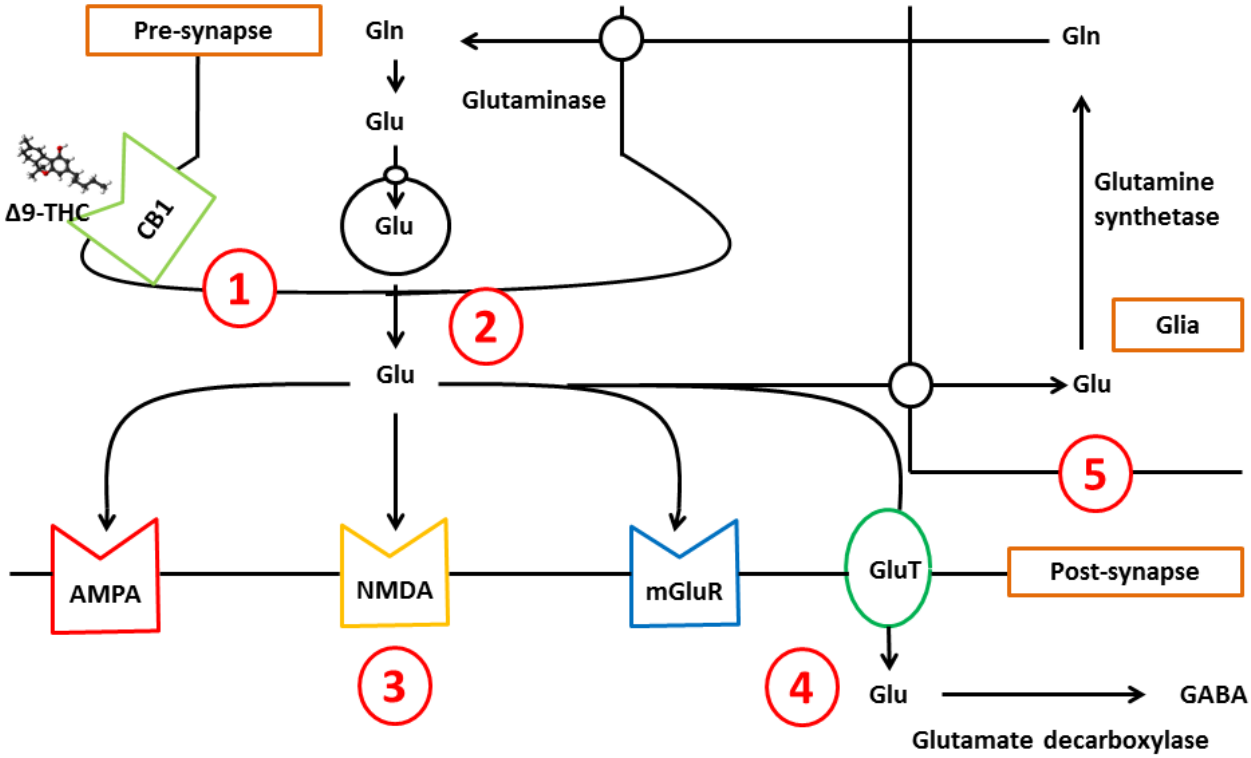
3. Glutamate receptors
- Embryonal studies
AMPA GluR1
↓
AMPA GluR2/3
↓
Disrupted maturation
+
- Postnatal studies
AMPA GluR1
↓↓↓
→→
AMPA GluR2
↓↓
→→→
NMDA GluN1
→→→
NMDA GluN2A
↓
→→
NMDA GluN2B
↓
→→
Disrupted maturation
+
Activation of NMDA GluN2B
++
- Postnatal studies followed by in vitro experiments
AMPA GluR1
↓
NMDA GluN2A
↓
NMDA GluN2B
↓

4. Glutamate transporters
- Embryonal studies
GLAST
↓↓
EAAC1
↓
→
GLT1
↓
- Postnatal studies followed by in vitro experiments
GLAST
↓
EAAC1

→
GLT1
↓

5. Glutamate enzyme activity
- In vitro studies
GAD
↓
GS
↓
- Embryonal in vivo studies
GAD
→
GS
↓
Disrupted maturation
+
- Postnatal studies
GAD
↓↓
→
GLDH
↑

↓, reduction; ↑, increase; →, no effect; +, present; Number of arrows (↓ or ↑) indicate number of studies; Δ9-THC, delta-9-tetrahydrocannabinol; CB1, cannabinoid receptor type 1; Gln, glutamine; Glu, glutamate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GluR1, glutamate receptor 1; GluR2/3, glutamate receptor 2/3; NMDA, N-methyl-d-aspartate; GluT, glutamate transporters; GLAST, L-glutamate/L-aspartate transporter; EAAC1, excitatory amino acid carrier 1; GLT1, glutamate transporter 1; GAD, glutamic acid decarboxylase; GS, glutamine synthetase; GLDH, glutamate dehydrogenase; GABA, gamma-aminobutyric acid



Tables

Table 1. Studies included in review

Study	Type of study	Region of Interest	Site of abnormality in Glutamate Pathway
Chang et al., 2006	MR Imaging studies in chronic human users	Basal ganglia, Frontal white matter	Glutamate-related metabolites
Prescot et al., 2011	MR Imaging studies in chronic human users	Anterior cingulate cortex	Glutamate-related metabolites
Muetzel et al., 2013	MR Imaging studies in chronic human users	Dorsal striatum	Glutamate-related metabolites
Prescot et al., 2013	MR Imaging studies in chronic human users	Anterior cingulate cortex	Glutamate-related metabolites
Sung et al., 2013	MR Imaging studies in chronic human users	Anterior cingulate cortex	Glutamate-related metabolites
Shen and Tayer, 1999	In vitro experiment in embryonally exposed animal cells	Hippocampus	Glutamate synaptic transmission
Monnet-Tschudi et al., 2008	In vitro experiment in embryonally exposed animal cells	Mixed brain cell aggregates	Glutamate enzyme activity
Tomasini et al., 2002	In vitro experiment in postnatally exposed animal cells	Cerebral Cortex	Extracellular glutamate levels, glutamate uptake, uptake
Brown et al., 2003	In vitro experiment in postnatally exposed animal cells	Striatum	Glutamate release, uptake, synaptic transmission
Straiker and Mackie, 2005	In vitro experiment in postnatally exposed animal cells	Hippocampus	Glutamate synaptic transmission
Hoffman et al., 2010	In vitro experiment in postnatally exposed animal cells	Hippocampus	Glutamate synaptic transmission
Irie et al., 2015	In vitro experiment in postnatally exposed animal cells	Cerebellum	Glutamate synaptic transmission
Castaldo et al., 2007	In vivo embryonal exposure in animals	Right frontal cortex	Extracellular glutamate levels, glutamate uptake, transporters
Garcia-Gil et al., 1999	In vivo embryonal and postnatal exposure in animals	Basal ganglia	Glutamate enzyme activity
Suarez et al., 2002	In vivo embryonal and postnatal exposure in animals	Cerebellum (cortex)	Glutamate enzyme activity
Suarez et al., 2004 (a)	In vivo embryonal and postnatal exposure in animals	Cerebellum	Glutamate transporters
Suarez et al., 2004 (b)	In vivo embryonal and postnatal exposure in animals	Cerebellum	Glutamate receptors
Campolongo et al., 2007	In vivo embryonal and postnatal exposure in animals	Prefrontal cortex	Glutamate receptors, enzyme activity, synaptic transmission
Castaldo et al., 2010	In vivo embryonal and postnatal exposure in animals	Hippocampus	Glutamate release, uptake, transporters
Edery and Gottesfeld, 1975	In vivo postnatal exposure in animals	Cerebellum, striatum, motor cortex	Glutamate enzyme activity
Ames et al., 1979	In vivo postnatal exposure in animals	Right temporal lobe	Glutamate, glutamine
Ghoneim et al., 1980	In vivo postnatal exposure in animals	Peripheral	Glutamate enzyme activity
Hikal et al., 1988	In vivo postnatal exposure in animals	Hippocampus	Glutamic acid, glutamine
Romero et al., 1998	In vivo postnatal exposure in animals	Striatum, ventral midbrain	Glutamate enzyme activity

Pistis et al., 2002	In vivo postnatal exposure in animals	Prefrontal cortex	Extracellular glutamate levels
Sano et al., 2008	In vivo postnatal exposure in animals	Nucleus accumbens	Glutamate release
Fan et al., 2010	In vivo postnatal exposure in animals	Hippocampus	Glutamate receptors, synaptic transmission
Good and Lupica, 2010	In vivo postnatal exposure in animals	PPN, ventral tegmental area	Glutamate synaptic transmission
Liu et al., 2010	In vivo postnatal exposure in animals	Ventral tegmental area	Glutamate synaptic transmission
Galanopoulos et al., 2011	In vivo postnatal exposure in animals	PFC, DS, NAc, HPC, AMYG, HTH	Glutamate tissue levels
Orrú et al., 2011	In vivo postnatal exposure in animals	Striatum	Glutamate release
Han et al., 2012	In vivo postnatal exposure in animals	Hippocampus	Glutamate synaptic transmission
Long et al., 2013	In vivo postnatal exposure in animals	HPC, Cg, Aco, PMCo	Glutamate receptors
Spencer et al., 2013	In vivo postnatal exposure in animals	Hippocampus	Glutamate receptors
Zamberletti et al., 2014	In vivo postnatal exposure in animals	Prefrontal cortex, hippocampus	Glutamate enzyme activity
Rubino et al., 2015	In vivo postnatal exposure in animals	Prefrontal cortex	Glutamate receptors
Chen et al., 2013	In vivo postnatal exposure in animals + in vitro experiment	Hippocampus	Glutamate receptors, synaptic transmission
Hoffman et al., 2007	In vivo postnatal exposure in animals + in vitro experiment	Hippocampus	Glutamate receptors, synaptic transmission
Tonini et al., 2006	In vivo postnatal exposure in animals + in vitro experiment	Cerebellum	Glutamate synaptic transmission
Mato et al., 2005	In vivo postnatal exposure in animals + in vitro experiment	Nucleus accumbens	Glutamate synaptic transmission
Hoffman et al., 2003	In vivo postnatal exposure in animals + in vitro experiment	Nucleus accumbens	Glutamate release, synaptic transmission

MR, Magnetic Resonance; PPN, pedunculopontine nucleus; PFC, prefrontal cortex; DS, dorsal striatum; NAc, nucleus accumbens; HPC, hippocampus; AMYG, amygdala; HTH, hypothalamus; Cg. Cingulate cortex; Aco, Auditory cortex; PMCo, posteromedial cortical amygdaloid nucleus

Table 2A. Summary of human neuroimaging studies of effect of chronic cannabis use on glutamate-related metabolites

Study	Aim of study	Population	n	Cannabis use	Controls	Region of interest	Results	Imaging analysis
Chang et al (2006)	Glu metabolite concentration in chronic CBS and HIV	1. 24 CBS users 2a. 24 only CBS 2b. 21 HIV+CBS 2c. 21 HIV subjects	96	CBS interview	30 healthy controls	Basal ganglia _____ Frontal white matter	**1. ↓(-9.5%, p=0.05)** **2a. ↓(-12%, p = 0.03)** **2b. ↓(-13%, p = 0.03)** 2c. NS _____ 1. NS **2a.↓** 2b. NS **2c.↓**	1H magnetic resonance spectroscopy 4T MRI system
Prescot et al (2011)	Glu metabolite concentration in chronic CBS	17 CBS users	34	urine analysis + CBS interview	17 healthy controls	Anterior cingulate cortex	**↓ (-15%, p<0.01)**	1H magnetic resonance spectroscopy 3T MRI system
Muetzel et al (2013)	Glu metabolite concentration in chronic CBS	27 CBS users	53	Personal Experience Inventory (PEI)	26 healthy controls	Dorsal striatum	NS overall group; **group by sex interaction, p=0.01: ↓ in F (-12.5%, p=0.04)**, NS in M	1H magnetic resonance spectroscopy 3T MRI system
Prescot et al (2013)	Glu metabolite concentration in chronic CBS	13 CBS users	29	urine analysis + CBS interview	16 healthy controls	Anterior cingulate cortex	**↓ (-14%, p=0.01)**	1H magnetic resonance spectroscopy 3T MRI system
Sung et al (2013)	Glu metabolite concentration in chronic CBS and MA	1. 8 CBS+MA users 2. 9 MA users	27	K-SADS diagnostic interview	10 healthy controls	Anterior cingulate cortex	1. NS 2. NS	1H magnetic resonance spectroscopy 3T MRI system

Glu, glutamate; CBS, cannabis; HIV, Human Immunodeficiency Virus; MA, methamphetamine; K-SADS, Kiddie-Schedule for Affective Disorders and Schizophrenia; M, male; F, female; MRI, magnetic resonance system

Table 2B. Methodological quality of imaging studies of effect of chronic cannabis use on glutamate-related metabolites

Study	Defined study population	Adequate exposure	Control	Comparability of Subjects	Statistical analysis	Outcome measurement	Mental health comorbidity	Excluded/adjusted for tobacco use	Excluded/adjusted for alcohol use	Excluded/ adjusted for substance use	Funding or sponsorship
Chang et al (2006)	✓ History of chronic CBS use - last CBS use between 0 and 240 months before scanning	✓ use of CBS>4days/week for at least 12 months	✓	✓ Demographic characteristics matched (sex, age, neuropsychological tests)	✓ ANOVA, ANCOVA	✓ Glu metabolite levels corrected for the partial volume of cerebrospinal fluid	✓ Exclusion criterion	✓ Trend for difference in daily tobacco use (CBS>HC), but this was corrected in analyses	✓ Exclusion criterion	✓ Exclusion criterion	✓ Declared
Prescot et al (2011)	✓ Current chronic CBS users	✓ use of CBS at least 100 times in the last 12 months	✓	✓ Significant differences in sex and age, but these were corrected	✓ ANOVA, ANCOVA	✓ Glu metabolite levels/water	✓/✕ 2 CBS users with treated depression, not corrected	✕	✕ 3 CBS users with history of alcohol abuse and/or dependence, not corrected	✓ Exclusion criterion	✓ Declared
Muetzel et al (2013)	✓ Current chronic CBS users; briefly abstinent (at least 12h before scanning)	✓ use of CBS>4days/week for at least 12 months, age at first use<17years, DSM-IV criteria for CBS abuse or dependence	✓	✓ Demographic characteristics matched (sex, age, IQ), however age corrected and sex differences examined	✓ ANCOVA	✓ Glx (Glu+Gln) metabolite levels/tCr	✓ Most participants were free of a DSM-IV Axis I diagnosis and none had psychosis	✓/✕Exclusion criterion only in CBS users	✓ Significant difference in alcohol use (CBS>HC), but this was corrected	✓ Exclusion criterion	✓ Declared
Prescot et al (2013)	✓ Current chronic CBS users; 7 (54%) having used CBS within the 24h before scanning	✓ use of CBS at least 100 times in the last 12 months	✓	✓ Significant differences in sex and age, but these were corrected	✓ ANOVA, ANCOVA	✓ Glu metabolite levels/water	✓Significant difference in HAM-D (CBS > HC) and 2 CBS users with treated depression, but these were corrected/excluded	✓ 3 CBS users with history of past/current nicotine use, but this was corrected/excluded	✓ 3 CBS users with history of alcohol abuse and/or dependence, but this was corrected/excluded	✓ Exclusion criterion	✓ Declared
Sung et al (2013)	✓ Current chronic CBS users	✓ diagnostic criteria for current MA/MA+CBS dependence	✓	✓ Demographic characteristics matched (sex, language, income, repeated grades); trend for differences in age and education, but these were corrected	✓ ANOVA, ANCOVA	✓ Glx (Glu+Gln) metabolite levels/PCr+Cr	✓ No individual met diagnostic criteria for a DSM-IV Axis I disorder	✓ No significant difference in tobacco use (lifetime n cigarettes, duration in months), however corrected	✓ Exclusion criterion, no significant difference, however corrected	✓ Exclusion criterion	✓ Declared

CBS, cannabis; HC, healthy controls; h, hours; DSM-IV, The Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; MA, methamphetamine; IQ, intelligence quotient; ANOVA, analysis of variance; ANCOVA, analysis of covariance; Glu, glutamate; Gln, glutamine; tCr, total creatine; PCr, phosphocreatine; HC, healthy controls; ✕, no information provided

Table 3. Summary of animal studies of effect of in vitro Δ9-THC exposure on glutamate

Study	Animal model	Stage of development	Sex	Weight	Genetics	Δ9-THC application dosage	Other used cannabinoids (dosage)	Controls	Region of interest	Synaptic transmission	Enzyme activity	Neurotr. release	Neurotr. uptake	Extracellular levels	Method	Statistics
Shen and Thayer (1999)	✓ rat	✓ ED 17, then cultures grown for at least 12 days	×	×	NA	✓ 1. 100 nM or 1 μM	✓ 2. WIN55,212-2 (100 nM) 3. SR141716A (300 nM)	×	✓ Hippocampus	✓ **1. 40%↓Ca2+ spiking, independently of dosage, prevented by SR; 57%↓EPSCa 2. 100%↓Ca2+ spiking, partially reversed by THC; 96%↓EPSCa** 3. NS **1+2(combined). 75%↓EPSCa**	NA	NA	NA	NA	✓ Electrophysiology	✓ ANOVA
Monnet-Tschudi et al (2008)	✓ rat	✓ ED 16, then cultures grown for 24 days	×	×	NA	✓ 1. 2 and 5 μM at day 24, then 1 and 2 μM at day 25 and every 2nd day for 10 days (up to day 35)	✓ 2. JWH-015 (as for Δ9-THC) 3. ACEA (as for Δ9-THC)	✓	✓ Mixed brain cell aggregates	NA	✓ **1.ST and RT↓GAD; only RT↓GS 2.ST and RT↓GAD; only RT↓GS** 3. NS	NA	NA	NA	✓ Radiometric assay	✓ ANOVA
Tomasini et al (2002)	✓ rat (Sprague Dawley)	✓ PND 1	×	×	NA	✓ 1. 0.01, 0.3, 3, 300, and 1000 nM	✓ 2. SR141716A (10 nM)	✓	✓ Cerebral Cortex	NA	NA	✓ **1.↑KCl-evoked at minimum dosage**	✓ 1. NS	✓ **1.↑ concentration dependent, prevented by SR**	✓ fluorimetric HPLC, LSC	✓ ANOVA
Brown et al (2003)	✓ rat (Sprague Dawley)	×	✓ male	✓ 250-300 gm	NA	✓ 1. 10 μM	✓ 2. SR141716A (100 nM) 3. AM251 (500 nM)	✓	✓ Striatum	✓ **1. ↓spiking, prevented by SR and AM, partially reversed by AM after THC washout**	×	✓ **1.↓K2+-evoked dose dependent, prevented by SR;** NS basal 2. NS	✓ **1.↓, dose dependent, prevented by SR** 2. NS	NA	✓ Radiometric assay, Electrophysiology	✓ ANOVA

Straiker and Mackie (2005)	✓ mouse	✓ PND 0-2	×	×	✓ CB1R WT (+/+) and KO (-/-)	✓ 1. increasing up to 10 μM, then exceeding this dosage	✓ 2. WIN55,212-2 (100 nM) 3. 2-AG (1-10 μM) 4. 2-AGE (10 μM) 5. AEA (4.5 μM) 6. HU-210 (100 nM) 7. SR141716A (100 nM)	×	✓ Hippocampus (neurones isolated from the CA1-CA3 region)	✓ 1. NS **2. ↓EPSC, absent in CB1R KO, prevented by THC** **3. ↓EPSC, absent in CB1R KO, recovered on washout** **4. ↓EPSC, absent in CB1R KO, not recovered on washout** **5. ↓EPSC, absent in CB1R KO, not recovered on washout, reversed by SR** **6. ↓EPSC, absent in CB1R KO, prevented only by THC overnight (~18 h)**	NA	NA	NA	NA	✓ Electrophysiology	✓ t-test
Hoffman et al (2010)	✓ a. rat b. mouse (C57BL/6 J)	✓ a. PNW 4-6 b. PNW 4-12	✓ male	×	✓ b. CB1R WT (+/+) and KO (-/-)	✓ 1. 10 μM	✓ 2. WIN55,212-2 (500 nM) 3. AM251 (1 μM)	×	✓ Hippocampus	✓ 1. **↓fEPSP in rats, prevented by AM,** NS in mice, **absent in CB1R KO** 2. **↓fEPSP in rats**, NS in mice	NA	NA	NA	NA	✓ Electrophysiology	✓ ANOVA

Irie (2015)	✓ mouse (ICR)	✓ PND 20-57/PND 14-20	✓ either	×	NA	✓ 1. increasing 0.1-100 μM	✓ 2. MAM-2201 (0.1-100 μM) 3. WIN55,212-2 (0.1-100 μM) 4. JWH-018 (0.1-100 μM) 5. AM251 (5 μM)	✓	✓ Cerebellum (AtT-20 cells)	✓ **1.↓PF-PC EPSCa, not concentration dependent; ↑PPR; ↑CV; 2. ↓PF-PC EPSCa, concentration dependent, prevented by AM; ↑PPR; ↑CV; ↓ PF-PC qEPSCf** (NS qEPSCa)**; ↓CF-PC EPSC 3. ↓PF-PC EPSCa, ↑PPR, ↑CV 4. ↓PF-PC EPSCa, concentration dependent**	NA	NA	NA	NA	✓ Electrophysiology	✓ t-test

Δ9-THC, delta-9-tetrahydrocannabinol; ICR, imprinting control region; ED, embryonal day; PND, postnatal day; PNW, postnatal week; ×, no information provided; CB1R, cannabinoid receptor 1; WT, wildtype; KO, knockout; SR141716A, rimonabant; ACEA, arachidonyl-2'-chloroethylamide; 2-AG, 2-arachidonoylglycerol; 2-AGE, 2-arachidonyl glyceryl ether or noladin; AEA, anandamide; MAM-2201, 4'-methyl-AM-2201, 5"-fluoro-JWH-122; JWH-018, 1-pentyl-3-(1-naphthoyl)indole) or AM-678; AtT-20 cells, murine tumor line; NS, not significant; NA, not applicable; EPSCa, excitatory postsynaptic current amplitude; fEPSP, field excitatory postsynaptic potentials; PF-PC, parallel fiber-Purkinje cell ; PPR, paired-pulse ratio; CV, coefficient of variation; q-EPSC, quantal EPSC; CF-PC, climbing fiber-Purkinje cell; Neuotr., neurotransmitter; ST, single treatment; RT, repeated treatment; GAD, glutamic acid decarboxylase; GS, glutamine synthetase; HPLC, high-performance liquid chromatography; LSC, liquid scintillation counting; ANOVA, analysis of variance

Table 4. Summary of animal studies of effect of embryonal in vivo Δ9-THC exposure on glutamate

Study	Animal model	Stage of development (when studied)	Sex	Weight	Δ9-THC way of admin., period, dosage	Other used cannabinoids (way of admin., period, dosage)	Controls	Region of interest	Enzyme activity	Neurotr. release	Neurotr. uptake	Extracellular levels	Neurotr. transporters	Neurotr. receptors	Method	Statistics
Castaldo et al (2007)	✓ rat	✓ PND 40	✓ male	×	✓ 1. PO in dams, daily, from GD 5 to GD 20, 5 mg/kg	✓ 2. WIN55,212-2 (SC, daily, from GD 5 to GD 20, 0.5 mg/kg)	✓	✓ Right frontal cortex	NA	NA	✓ **2.↑Vmax in synaptosomes, time dependent** (NS Km)	✓ **1. ↓ basal** **2. ↓ basal**	✓ **2. ↑GLT1; ↑EAAC1;** NS GLAST (NS GLT1, EAAC1, and GLAST transcripts)	NA	✓ in vivo MD, fluorimetric HPLC, LSC, WB analysis, ICC, RT-PCR analysis	✓ t-test, ANOVA, regression analysis
Garcia-Gil et al (1999)	✓ rat	✓ > PNW 10	✓ either	✓ recorded in dams from GD 0 to GD 20 (200-250 gm), and in litter	✓ PO in dams, daily, from GD 5 to PND 24, 5 mg/kg	NA	✓	✓substantia nigra/ ventral tegmental area, globus pallidus, caudate-putamen/ nucleus accumbens	✓ NS GAD	NA	NA	NA	NA	NA	✓ Radiometric assay	✓ ANOVA
Suárez et al (2002)	✓ rat	✓ PND 20, PND 30, PND 70	✓ either	×	✓ PO in dams, daily, from GD 5 to PND 20, 5 mg/kg	NA	✓	✓ Cerebellum (cortex)	✓ **↓ GS, not reversed at PND 70 in M, completely reversed at PND 70 in F**	NA	NA	NA	NA	NA	✓ IHC for microscopy and morphometry, WB analysis	✓ ANOVA

Suárez et al (2004a)	✓ rat	✓ PND 20, PND 30, PND 70	✓ either	×	✓ PO in dams, daily, from GD 5 to PND 20, 5 mg/kg	NA	✓	✓ Cerebellum	NA	NA	NA	NA	✓ **↓GLAST, not reversed at PND70, M<F at PND20 (WB) and 70 (IHC); ↓EAAC1, not reversed in IHC at PND70, but reversed in WB at PND70 for M, and PND30 and 70 for F**	NA	✓ IHC for microscopy and morphometry, WB analysis	✓ t-test
Suárez et al (2004b)	✓ rat	✓ PND 20, PND 30, PND 70	✓ either	×	✓ PO in dams, daily, from GD 5 to PND 20, 5 mg/kg	NA	✓	✓ Cerebellum	NA	NA	NA	NA	NA	✓ **continuous ↓ GluR1, not reversed at PND70 (↑ from PND20 to 30 in CON); continuous ↓ GluR2/3, not reversed at PND70 (↑ from PND20 to 70 in CON)**	✓ IHC for microscopy and morphometry, WB analysis	✓ ANOVA
Campolongo et al (2007)	✓ rat	✓ PND 80	✓ male	✓ recorded in dams from GD 15 to PND 9, and in litter	✓ PO in dams, daily, from GD 15 to PND 9, 5 mg/kg	NA	✓	✓ Prefrontal cortex	✓ **↓GCLM; ↓ GLUD1; ↑GOT1**	NA	NA	✓ **↓ basal**	NA	✓ **↓GRIP2; ↓ GRID2; ↓GRIK1; ↑GRIK3**	✓ microarray analysis, in vivo MD, fluorimetric HPLC	✓ t-test, ANOVA
Castaldo et al (2010)	✓ rat	✓ PND 40	✓ male	✓ recorded in dams (250-280 gm), and in litter	✓ 1. PO in dams, daily, from GD 15 to PND 9, 5 mg/kg 2. in vitro application of 0.1 µM at PND 40	✓ 3. SR141716A (in vitro application of 100 nM at PND 40)	✓	✓ Hippocampus	NA	✓ **1. ↓basal, ↓K+-evoked** **2. ↓ in THC rats** **3. reversed K+-evoked ↑ in CON**	✓ **1. ↓**	NA	✓ **1. ↓GLT1; ↓GLAST;** NS EAAC1	NA	✓ fluorimetric HPLC, LSC, WB analysis	✓ t-test, ANOVA

Δ9-THC, delta-9-tetrahydrocannabinol; PND, postnatal day; PNW, postnatal week; ×, no information provided; admin., administration; SR141716A, rimonabant; GD, gestational day; PO, per os; SC, subcutaneously; NA, not applicable; GAD, glutamic acid decarboxylase; GS, glutamine synthetase; neurotr., neurotransmitter; M, male; F, female; GCLM, glutamate cysteine ligase, modifier subunit; GLUD1, glutamate dehydrogenase 1; GOT1, glutamate oxaloacetate transaminase 1; GLT1, glutamate transporter 1; EACC1, excitatory amino acid carrier 1; GLAST, L-glutamate/L-aspartate transporter; GluR1, glutamate receptor 1; CON, controls; GluR2/3, glutamate receptor 2/3; GRIP2, glutamate receptor-interacting protein 2; GRID2, glutamate receptor, ionotropic, delta 2; GRIK1, glutamate receptor, ionotropic, kainate 1; GRIK3, glutamate receptor, ionotropic, kainate 3; MD, microdialysis; HPLC, high-performance liquid chromatography; LSC, liquid scintillation counting; WB, western blot; ICC, immunocytochemistry, RT-PCR, real-time polymerase chain reaction; IHC, immunohistochemistry; ANOVA, analysis of variance

Table 5. Summary of animal studies of effect of postnatal in vivo Δ9-THC exposure on glutamate

Study	Animal model	Stage of development	Sex	Weight	Genetics	Δ9-THC way of admin., period, dosage	Other used cannabinoids (way of admin., period, dosage)	Controls	Region of interest	Synaptic transmission	Enzyme activity	Neurotr. release	Intracerebral levels	Neurotr. receptors	Method	Statistics
Edery and Gottesfeld (1975)	✓ rat (albino)	×	✓ either	✓ 170-200 gm	NA	✓ IP, daily (repeated/single), for 14 consecutive days, 20 mg/kg	NA	✓	✓ Cerebellum, striatum, motor cortex	NA	✓ **↓ GAD in cerebellum and motor cortex**, NS in striatum	NA	NA	NA	✓ Radiometric assay	✓ t-test
Ames et al (1979)	✓ adult chacma baboon	×	✓ either	×	NA	✓ PO, daily, for 15 consecutive days, or 25 days with a 3-day interval, 10% cannabis sativa	NA	✓	✓ Right temporal lobe	NA	NA	NA	✓ NS Glutamine NS Glutamate	NA	✓ Brain biopsy	×
Ghoneim et al (1980)	✓ rabbit	×	×	×	NA	✓ PO, every other day, over 1 month, hashish	NA	×	✓ Peripheral	NA	✓ **↑ GLDH compared to baseline levels**	NA	NA	NA	×	×
Hikal et al (1988)	✓ rat	×	✓ male	×	NA	✓ IP, single administration, 20 or 50 mg/kg	NA	✓	✓ Hippocampus	NA	NA	NA	✓ NS Glutamine NS Glutamic acid	NA	✓ HPLC	×

Romero et al (1998)	✓ rat	✓ > PNW 8	✓ male	×	NA	✓ 1. IP, single administration, 5 mg/kg	✓ 2. HU210 (IP, single administration, 50 µg/mg) 3. SR141716A (IP, single administration, 3mg/kg)	✓	✓ Striatum, ventral midbrain	NA	✓ 1. NS GAD, either after SR or vehicle 2. NS GAD, either after SR or vehicle	NA	NA	NA	✓ Radiometric assay	✓ t-test, ANOVA
Pistis et al (2002)	✓ rat (albino)	×	✓ male	✓ 250-280 gm	NA	✓ 1. IV, single administration, 1 mg/kg	✓ 2. SR141716A (IV, single administration, 1 mg/kg)	×	✓ Prefrontal cortex	NA	NA	NA	✓ **1.↑ basal EGL, prevented by SR** 2. NS	NA	✓ in vivo MD	✓ ANOVA
Sano et al (2008)	✓ rat (Wistar)	✓ PNW 7	✓ male	✓ 200-250 gm	NA	✓ 1. IP, single administration, 10 mg/kg	✓ 2. SR141716A (IP, single administration, 10 mg/kg)	✓	✓ Nucleus accumbens	NA	NA	✓ **1. ↓, reversed by SR**	NA	NA	✓ in vivo MD	✓ ANOVA
Fan et al (2010)	✓ mouse (C57BL /6J)	✓ PNW 6-9	✓ either	×	✓ CB1R WT (+/+) and KO (-/-)	✓ 1. IP, daily, for 7 consecutive days, 10 mg/kg	✓ 2. SR141716A (IP, for 7 consecutive days, 5 mg/kg)	✓	✓ Hippocampus	✓ **1. ↓fEPSP (↓of LTP), prevented by SR, absent in CB1R KO; ↓PPR; ↑EPSCf**	NA	✓ **1. ↑ basal**	✓ **1.↑ IGL**	✓ 1. **↓GluR1, GluN2A, and GluN2B total, surface , and mRNA, prevented by SR, absent in CB1R KO** (NS GluN1 and GluR2)	✓ WB analysis, RT-PCR analysis	✓ t-test, ANOVA
Good and Lupica (2010)	✓ rat	✓ PND 14-19	×	×	NA	✓ 1. IP, single administration, 10 mg/kg	✓ 2. AM251 (IP, single administration, 2 mg/kg	✓	✓ PPN, ventral tegmental area	✓ **1. ↓EPSC (LTD), ↑RI of evoked EPSC, and ↓post LTD RI in PPN, prevented by AM;** NS intra-VTA stimulation	NA	NA	NA	NA	✓ Electrophysiology	✓ ANOVA

Liu et al (2010)	✓ rat	✓ PND 16-92	✓ male	×	✓ CB1R WT (+/+) and KO (-/-, using CB1R shRNA)	✓ 1. IP, daily (single), for 5 consecutive days, 5 mg/kg	✓ 2. HU210 (IP, daily, single, for 5 consecutive days, 100 µg/kg) 3.AM281 (IP, daily, single, for 5 consecutive days, 3 mg/kg)	✓	✓ ventral tegmental area	✓ **1. ↓fEPSP (LTD) 2. ↓fEPSP (LTD), prevented by AM and absent in CB1R shRNA**	NA	NA	NA	✓ **2. LTD via activation of GluN2B and postsynaptic endocytosis of GluR1/GluR2**	✓ Electrophysiology, WB analysis	✓ t-test, ANOVA
Galanopoulos et al (2011)	✓ rat	✓ PND 80-90	✓ male	✓ 250-300 gm	NA	✓ 1. IP, single administration, 0.75 or 3 mg/kg	✓ 2. WIN55,212-2 (IP, single administration, 0.1 or 1 mg/kg)	✓	✓ Prefrontal cortex, DS, NAc, Hippocampus, AMYG, HTH	NA	NA	NA	**1.↑tissue levels in PFC, DS, NAc, HPC**; NS AMYG and HTH **2. ↑ tissue levels in PFC, NAc, and HPC; ↓ in AMYG and HTH**; NS in DS	NA	✓ HPLC	✓ ANOVA
Orrú et al (2011)	✓ rat	×	✓ male	✓ 300-350 gm	NA	✓ 1. IP, single administration, 3 mg/kg	✓ 2. SR141716A (IP, single administration, 1 mg/kg)	✓	✓ Striatum	NA	NA	✓ **1. ↓, reversed by SR** 2. NS	NA	NA	✓ in vivo MD	✓ ANOVA
Han et al (2012)	✓ mouse (C57BL/6JOlaHsd)	✓ a. PNW 6-8 b. PNW 10-16	✓ male	×	✓ CB1R WT (+/+) and KO (-/-, also using CB1R shRNA)	✓ 1. IP, single administration, 5 mg/kg	✓ 2. HU210 (IP, single administration, 0.1 or 0.5 mg/kg; intra-CA1 infusion) 3. AM281 (IP, single administration, dosage of 3 mg/kg)	✓	✓ Hippocampus	✓ **1. ↓fEPSPa (LTD) 2. ↓fEPSPa >24h (LTD), prevented** (but not reversed) **by AM, present in CB1R shRNA but absent in CB1R KO**	NA	NA	NA	✓ **2. LTD via activation of GluN2B and postsynaptic endocytosis of GluR1/GluR2; ↓ GluR1 and GluR2**	✓ Electrophysiology, WB analysis	✓ t-test, ANOVA
Long et al (2013)	✓ mouse (C57BL/6J)	✓ PND 31	✓ male	×	✓ NRG1 WT (+/+) and HET (+/-)	✓ IP, daily, for 21 consecutive days from PND 31, 10 mg/kg	NA	✓	✓ Hippocampus, Cg, ACo, PMCo	NA	NA	NA	NA	✓ NS, ↑ **NMDA binding in ACo, Cg, and Hippocampus only in NRG1 HET** (NS PMCo)	✓ Autoradiography	✓ ANOVA

Spencer et al (2013)	✓ mouse (C57BL/6J)	✓ PND 32±2	✓ male	×	✓ NRG1 WT (+/+) and HET (+/-)	✓ IP, daily, for 21 consecutive days from PND 31, 10 mg/kg	NA	✓	✓ Hippocampus	NA	NA	NA	NA	✓ NS, ↑ **FLOT1 and APOA1,** ↓ **GPSM2 only in NRG1 HET**	✓ Mass spectrometry, WB analysis	✓ t-test
Zamberletti et al (2014)	✓ rat	✓ PND 28	✓ female	×	NA	✓ IP, twice a day, from PND 35 to PND 45, increasing doses (2.5 mg/kg, PND 35–37; 5 mg/kg, PND 38–41; 10 mg/kg, PND 42–45)	NA	✓	✓ Prefrontal cortex, Hippocampus	NA	✓ ↓ **GAD only at PND75 in Prefrontal cortex** (NS PND 46 and 60; NS hippocampus)	NA	NA	NA	✓ WB analysis, IHC for microscopy, In vivo MD	✓ t-test, ANOVA
Rubino et al (2015)	✓ rat	✓ PND 28	✓ female	×	NA	✓ 1. IP, twice a day, from PND 35 to PND 45, increasing doses (2.5 mg/kg, PND 35–37; 5 mg/kg, PND 38–41; 10 mg/kg, PND 42–45)	✓ 2. AM251 (IP, from PND 35 to PND 45, or from PND 46 to PND 60, or from PND 60 to 75, 0.5 mg/kg)	✓	✓ Prefrontal cortex	NA	NA	NA	NA	✓ 1. ↑ **PSD-95 at PND 46 and 60** (NS PND 75), ↑ **GluN2A at PND 60** (NS PND 46 and 75), ↑ **GluN2B at PND 75** (NS PND 46 and 60), ↑ **GluR1 at PND 75** (NS PND 46 and 60), NS GluR2 2. ↑**PSD-95 at PND 46 and 60, and** ↓**at PND 75.** ↑ **GluN2A at PND 46, 60, and 75,** ↑ **GluR2 at PND 46, 60** (NS PND 75), NS GluR1 and GluN2B	✓ WB analysis	✓ ANOVA

Δ9-THC, delta-9-tetrahydrocannabinol; SD, sprague dawley; PND, postnatal day; PNW, postnatal week; ✕, no information provided; NA, not applicable; CB1R, cannabinoid receptor 1; NRG1, neuregulin 1; WT, wildtype; KO, knockout; HET, heterogeneous; shRNA, short hairpin RNA; admin., administration; SR141716A, rimonabant; IP, intraperitoneally; PO, per os; IV, intravenously; PPN, pedunculopontine nucleus; DS, dorsal striatum; NAc, nucleus accumbens; AMYG, amygdala; HTH, hypothalamus; Cg. cingulate cortex; Aco. auditory cortex; PMCo, posteromedial cortical amygdaloid nucleus; fEPSPa, field excitatory postsynaptic potentials amplitude; LTP, long term potentiation; EPSCa(f), excitatory postsynaptic current amplitude (frequency); PPR, paired-pulse ratio; LTD, long term depression; RI, rectification index; GAD, glutamic acid decarboxylase; GLDH, glutamate dehydrogenase; neurotr., neurotransmitter; EGL, extracellular glutamate levels; IGL, intracellular glutamate levels; PFC, prefrontal cortex; HPC, hippocampus; GluR1, glutamate receptor 1; GluR2, glutamate receptor 2; NMDA, N-methyl-d-aspartate; FLOT-1, flotillin-1; APOA1, apolipoprotein A1; GPSM2, G-protein-signaling modulator 2; PSD-95, postsynaptic density protein 95; HPLC, high-performance liquid chromatography; MD, microdialysis; RT-PCR, real-time polymerase chain reaction; WB, western blot; IHC, immunohistochemistry; ANOVA, analysis of variance

Table 6. Summary of animal studies of effect of postnatal in vivo Δ9-THC exposure on glutamate, followed by additional in vitro Δ9-THC exposure

Study	Animal model	Stage of development	Sex	Weight	Genetics	Δ9-THC way of administration, period, dosage	Other used cannabinoids (way of administration, period, dosage)	Controls	Region of interest	Synaptic transmission	Neurotr. transporters	Neurotr. receptors	Method	Statistics
Hoffman et al (2003)	✓ rat	✓ PNW 2-4	✓ male	×	NA	✓ 1. IP, daily, for 7 consecutive days, 10 mg/kg	✓ 2a. WIN55,212-2 (IP, daily, for 7 consecutive days, 10 mg/kg) 2b. WIN55,212-2 (in vitro application, 1 μM) 3. SR141716A (in vitro application, 1 μM)	✓	✓ Nucleus accumbens	✓ 1. NS LTD (PSs amplitude) after 5 min at 10 Hz stimulation 2a. NS LTD (PSs amplitude) after 5 min at 10 Hz stimulation **3. prevented LTD after 5 min at 10 Hz stimulation** 2b. ↓ **N2 component** (NS N1 component), **concentration-dependent, less effective in chronic Δ9-THC treated rats**	NA	NA	✓ Electrophysiology	✓ ANOVA
Mato et al (2005)	✓ mouse (C57BL/6 J)	✓ PNW 4	✓ male	×	NA	✓ 1a. IP, daily, for 7 consecutive days, 3 mg/kg 1b. IP, single administration, 3 mg/kg	✓ 2. CP55,940 (in vitro application, 0.1-100 μM) 3. SR141716A (in vitro application, 1 μM)	✓	✓ Nucleus accumbens	✓ 1a. NS LTD (fEPSP) after 10 min at 13 Hz stimulation; NS eEPSC; NS PPR; NS Miniature sEPSC; NS basic electrophysiology 1b. ↓ **fEPSP (LTD) after 10 min at 13 Hz stimulation**, but NS LTD (fEPSP) after 5 min at 10 Hz stimulation 2. ↓ **fEPSP, dose response dependent, less effective in chronic Δ9-THC treated rats** 3. NS fEPSP; **prevented LTD after 10 min at 13 Hz stimulation only in controls** (NS in chronic Δ9-THC treated rats) **and animals taken 1 week after the last THC injection**	NA	NA	✓ Electrophysiology	✓ t-test

Tonini et al (2006)	✓ mouse (C57BL/6 J)	✓ PND 40-50	×	×	✓ RasGRF1 WT (+/+) and KO (-/-)	✓ 1. SC, twice per day, for 4.5 consecutive days, 10 mg/kg	✓ 2a. SR141716A (IP, 20 min before THC, 3 mg/kg) 2b. SR141716A (in vitro application, 10 μM) 3. CP55,940 (in vitro application, 0.1-20 μM)	✓	✓ Cerebellum	✓ 1. **↑PF-PC EPSCa;↓ PPR, prevented by SR in vivo, but not reversed by SR in vitro, absent in RasGRF1 KO**; NS PF-PC LTD, **but ↓ presynaptic PF-PC LTP, absent in RasGRF1 KO** 3. **↓ EPSC, dose response dependent, reversed by SR, less effective in chronic Δ9-THC treated rats, but equally effective in RasGRF1 KO**	NA	NA	✓ Electrophysiology	✓ t-test
Hoffman et al (2007)	✓ rat (Sprague Dawley)	✓ PNW 2-4	✓ male	×	NA	✓ 1. IP, daily, for 7 consecutive days, 10 mg/kg	✓ 2a. AM251 (IP, either alone or 30 min before THC, 2 mg/kg) 2b. AM251 (in vitro application, 1 μM) 3. SR141716A (in vitro application, 1 μM) 4. WIN55,212-2	✓	✓ Hippocampus	✓ 1. **↓fEPSP (↓of HFS and TBS LTP), time dependent (effect from day 3), partially reversed 3-14 days after the last THC injection; ↓ PPR; ↑ sEPSCa but not sEPSCf** 2a. NS 2b. NS 3. NS **4. ↓fEPSP (↓ LTP), dose-response dependent, equally effective in chronic Δ9-THC treated rats**	NA	✓ 1. NS NMDA synaptic currents	✓ Electrophysiology	✓ ANOVA
Chen et al (2013)	✓ mouse (C57BL/6 J)	✓ PNW 7-14	×	×	NA	✓ 1a. IP, daily, for 7 consecutive days, 10 mg/kg 1b. In vitro application, 3 μM	NA	✓	✓ Hippocampus	✓ 1a. **↓fEPSP (↓of LTP)** 1b. **↑ Miniature sEPSC**	✓ 1a. **↓ GLAST and GLT1,** (NS EAAC1)	✓ 1a. **↓GluR1, GluN2A, and GluN2B total, surface, synaptic, extrasynaptic**	✓ Electrophysiology , IHC for microscopy and morphometry, WB analysis	✓ t-test, ANOVA

Δ9-THC, delta-9-tetrahydrocannabinol; PND, postnatal day; PNW, postnatal week; ×, no information provided; NA, not applicable; RasGRF1, Ras protein-specific guanine nucleotide-releasing factor; WT, wildtype; KO, knockout; SR141716A, rimonabant; IP, intraperitoneally; SC, subcutaneously; LTD, long term depression; PSs, glutamate-driven population spikes; fEPSP, field excitatory postsynaptic potentials; e(s)EPSCa, evoked (spontaneous) excitatory postsynaptic current amplitude; PPR, paired-pulse ratio; PF-PC, parallel fiber-Purkinje cell; LTP, long term potentiation; HFS, high-frequency stimulation; TBS, θ burst stimulation; neurotr, neurotransmitter; GLT1, glutamate transporter 1; EAAC1, excitatory amino acid carrier 1; GLAST, L-glutamate/L-aspartate transporter; GluR1, glutamate receptor 1; GluR2, glutamate receptor 2; NMDA, N-methyl-d-aspartate; WB, western blot; IHC, immunohistochemistry; ANOVA, analysis of variance