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Cannabinoids alter spontaneous firing, bursting and cell synchrony of hippocampal principal cells

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Abstract

Both natural and synthetic cannabinoid receptor (e.g. CB1) agonists such as Δ^9 -THC, WIN 55,212-2 (WIN-2) and HU-210 disrupt spatial cognition presumably through the inhibition of synchrony of hippocampal ensemble firing to task-related events (Hampson and Deadwyler, J. of Neuroscience, 2000; 20:8932-8942; Robinson et al., Brit. J. Pharmacology, 2007; 151:688-700). And although the CB1 receptor agonist CP 55,940 also disrupts the synchronous firing of hippocampal neurons, it does not seem to affect the average firing rate (Robbe et al., Nature Neuroscience, 2006; 9:1526-1533). This difference is not readily explained by the chemical structure and pharmacology of the different compounds thus warranting a more detailed examination into (i) how other cannabinoids affect the spontaneous firing, bursting and cell synchrony of hippocampal principal cells located in CA3 and CA1 sub-fields and (ii) whether these effects are indeed mediated through CB1 receptors, which will be explored by the selective antagonist AM-251. Adult, Long-Evans rats surgically implanted with multi-electrode arrays to hippocampal CA3 and CA1 were anesthetized and principal cells discharging at 0.25-6 Hz isolated and 'tracked' following the systemic administration of Tween-80, Δ^9 -THC (1 or 3 mg/kg), or WIN-2 (1 mg/kg), or HU-210 (100 μ g/kg), and 1.5 mg/kg AM-281 respectively. All cannabinoids except for 1 mg/kg Δ^9 -THC reliably reduced average firing rates and altered 'burst' characteristics, which were reversible with AM-281 for Δ^9 -THC and WIN-2, but not for HU-210. In addition, all cannabinoids disrupted intra-subfield and inter-subfield ensemble synchrony of pyramidal cells, an effect insensitive to AM281 and thus unlikely to be CB1 mediated. We consider these cannabinoid effects on spike timing and firing/bursting of principal hippocampal neurons carried by CB1 and non-CB1 receptors to be physiological underpinnings of the cognitive impairments inherent to cannabinoid exposure.

Keywords

hippocampus; cannabinoid; single unit; firing rate; bursting; synchrony; rat

Introduction

The role of cannabinoids in memory processes can be traced back to early observations in humans reporting severe impairments in short-term recall as the most consistent disturbance of marijuana intoxication (Miller and Branconner, 1983). They are due to Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the psychoactive constituent of cannabis (Gaoni &

Mechoulam, 1964), acting through specific cannabinoid receptors (e.g. CB1) located abundantly in memory-relevant brain regions such as the hippocampus and pre-frontal cortex (Herkenham et al., 1991). These observations were supported by recent human studies, in which Δ^9 -THC impaired working and episodic memory (Ilan et al., 2004; Squire, 2004; Curran et al., 2002). In rats, both systemic and intra-hippocampal injection of Δ^9 -THC and synthetic CB1 agonists including WIN 55,212-2 (WIN-2), HU-210 and CP 55,940 impairs hippocampal-dependent (i.e. short-term) memory across a variety of learning tasks (Barna et al., 2007; Hampson et al., 2003; Hampson and Deadwyler, 2000; Ferrari et al., 1999; Lichtman and Martin, 1996; Lichtman et al., 1995).

Numerous electroencephalogram (EEG) recordings provided compelling evidence that Δ^9 -THC reduced the overall power spectra (i.e. power of various frequency bands including alpha, theta, beta and gamma) in both hippocampus and neocortex in humans, rabbits and rats (Ilan et al., 2004; Buonamici et al., 1982; Constoe et al., 1975; Willinsky et al., 1975) by *hitherto* unknown mechanisms. Recently, Robbe and co-workers (2006) using local field potential (LFP) recordings confirmed that both Δ^9 -THC (5 mg/kg) and CP,55940 (0.1-0.3 mg/kg) can decrease the power of theta (4–10 Hz), gamma (30-80 Hz) and fast ripple (100-200 Hz) oscillations in the hippocampus of head-restrained and freely moving rats. Hippocampal theta and gamma oscillations play a fundamental role in the coordination of synchronous firing between hippocampal and cortex and may be critical to memory formation (Siapas et al., 2005). The ripple oscillations are believed to be important in memory consolidation as they enable transfer of hippocampal memory traces to neocortical circuits for long-term storage (Karlsson and Frank, 2009; Davidson et al., 2009; Buzaki, 2002; Kudrimoti et al., 1999). In fact, Robbe and coworkers (2006) did show that these cannabinoid-induced decreases in theta power were i) correlated with memory deficits of a hippocampal-dependent task, and ii) brought about by a CB1 receptor mediated mechanism, as these changes in power spectra were prevented by the pre-treatment of SR141716A (SR), a CB1 receptor antagonist/inverse agonist.

Congruent to these data on global brain activity, *in vivo* single-unit recordings performed in our laboratory yielded cannabinoid-induced suppression of hippocampal ensemble firing during task-relevant events in rats trained in a delayed-non-match to sample (DNMS) paradigm (Hampson et al., 2003; Hampson and Deadwyler, 2000; 1999; 1998). Both Δ^9 -THC and WIN-2 reduced firing rates of hippocampal principal cells during *encoding* (sample) but not during the *retrieval* (nonmatch) of this information. Encoding of information by hippocampal neurons is a prerequisite to perform the DNMS task successfully (Hampson et al., 2003; Hampson and Deadwyler, 2000). Moreover, reductions in firing frequency and bursting of CA3 and CA1 principal cells by HU-210 was proposed as mechanistic underpinning of spatial learning deficits observed with the same drug (Robinson et al., 2007), an observation in line with cannabinoid actions on cell firing in both the basolateral amygdala and the globus pallidus in rats (Pistis et al., 2004, Miller and Walker, 1998). Thus, it appears that cannabinoid-induced deficits in learning are most likely brought about by alterations in hippocampal activity related to encoding.

Delta-9-THC ($K_i = 50$ nM) is known to act as a partial agonist at the CB1 receptor, whereby it activates these receptors but not to their full extent. On the other hand, WIN-2 ($K_i = 3.3$ nM) and HU-210 ($K_i < 1$ nM) act as full CB1 receptor agonist. Both WIN-2 and HU-210 not only have greater affinities than Δ^9 -THC but they are also more efficacious than Δ^9 -THC. However, no comparative study is available which comprehensively evaluated the effects of pharmacologically distinct cannabinoids (i.e. Δ^9 -THC, WIN-2 & HU-210) on physiological discharge characteristics of hippocampal principal cells. Here we undertook such a comparison thereby assessing the effects of these cannabinoids on: (i) baseline firing activity, (ii) patterns of spontaneously emitted bursts, and (iii) cell synchrony of principal

neurons located in CA3 and CA1 in anesthetized rats. To confirm whether cannabinoid-induced alterations are mediated by CB1 receptors, we also evaluated their reversibility by testing AM281, a CB1 receptor antagonist/inverse agonist. AM281 was selected since it is also a well characterized CB1 receptor antagonist/inverse agonist that is more potent ($K_i = 12$ nM) than SR141716A in the hippocampus (Gifford et al., 1997) and more specific to CB1 receptors as opposed to AM251, another CB1 receptor antagonist/inverse agonist that is also known to act as an agonist on GPR55, a novel cannabinoid receptor. Our data reveal that the drugs produced differential activation of mechanisms that could be accounted for by both CB1 and non-CB1 receptor-dependence.

Materials and Methods

Subjects

Twenty-four male, Long-Evans rats (Harlan, USA) were used for electrophysiological recordings *in vivo*. All animals were 4-5 months old, weighing 300-350 g, on a 12:12h day/night cycle, and individually housed with free access to food and water. All animal protocols were approved by the Wake Forest University Institutional Animal Care and Use Committee and were in concordance with the National Institute of Health (NIH) guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Drug preparation

Stock solutions of Δ^9 -THC (20 mg/ml; Research Triangle Inst., Raleigh, NC), WIN-2 (20 mg/ml; Sigma, St Louis, USA), HU-210 (5 mg/ml; Tocris Cookson, Bristol, UK), and AM-281 (20 mg/ml; Sigma, St Louis, USA) in ethanol were used. Drugs were prepared fresh each day using these stock solutions in a vehicle of Tween-80. The drug-detergent (1:1 drug/ethanol: Tween-80 by volume) solution was stirred rapidly and placed under a steady stream of nitrogen gas to evaporate the ethanol (approx. 10 min) whilst adding saline (0.9%) drop-wise to make up the final doses of Δ^9 -THC (1.0 and 3.0 mg/kg), WIN-2 (1.0 mg/kg), HU-210 (100 μ g/kg) and AM-281 (1.5 mg/kg), respectively. Drug doses were selected based on published efficacy of the respective doses in behaving rats. All injections were administered intra-peritoneally (i.p.) at a volume of 0.1 ml/100 g of the body weight.

Drug treatment and schedule

Subjects were divided into four treatment groups ($n = 6$ each) with the following successive administration regimes: Group 1 - Tween-80, Δ^9 -THC (3 mg/kg), AM-281 (1.5 mg/kg); Group 2 - Tween-80, WIN-2 (1 mg/kg), AM-281 (1.5 mg/kg); Group 3 - Tween-80, HU-210 (100 μ g/kg), AM-281 (1.5 mg/kg); and Group 4 - Tween-80, Δ^9 -THC (1 mg/kg), AM-281 (1.5 mg/kg). All animals in groups 1-3 were used in *experiment 1* and those in group 4 were used in *experiment 2*. Hippocampal principal cells (i.e. single-units) isolated from subjects in each group were recorded for 20 minutes following respective treatments and 15 minutes were allowed for drugs to take effect prior to the start of each recording period. Figure 1A illustrates the general timeline for all recordings.

Surgery

Rats were anesthetized with a cocktail of ketamine (100 mg/kg) and xylazine (10 mg/kg) and positioned in a stereotaxic frame for unilateral implantation with a multi-electrode recording array (Neuroline, NY, USA) consisting of 16 stainless steel electrodes (40 μ m) arranged in two rows (800 μ m between rows), such that each row consisted of eight electrodes with 200 μ m center-to-center spacing. The array was constructed with asymmetric lengths such that CA3 electrode tips would be automatically positioned 1.2 mm latero-ventral to the CA1 electrode tips. The scalp was incised along the midline, pulled

back to the lateral cranial ridges and single holes drilled for insertion of a 100 μ m silver ground wire into the parietal cortex. The centre of the array was positioned 3.4 mm posteriorly and ± 2.7 mm medio-laterally from Bregma (Paxinos and Watson, 1998), depending on whether the array targeted the left or right hippocampus. The posterior end of the array was angled laterally such that the longitudinal axis was 30° from midline of the skull. An oval-shaped craniotomy was made approximately 2 mm larger than the array, the dura resected and the long electrode tips placed in contact with the surface of the brain. The electrode was then driven in 50 μ m steps to a depth of 2.8 mm for the CA1 leads and 4.0 mm for the CA3 leads, then lowered to a final depth of approximately 3.0 mm for CA1 and 4.2 mm for CA3, using continuous recording of neural activity to confirm optimum depth of the electrode tips in pyramidal cell layers. The electrodes were allowed to stabilize in place for approximately 15-20 minutes prior to sealing the craniotomy with dental cement.

Electrophysiological recording

Following stabilization of the electrode array, extra-cellular action potentials of identified principal cells with firing rates of 0.25 – 6.0 Hz were isolated using a Multi-unit Acquisition Processor (MAP, Plexon Inc., Dallas, TX, USA). The neuronal ensemble activities of CA3 and CA1 hippocampal principal cells were then ‘tracked’ and recorded following the aforementioned treatments.

Recording parameters and analysis of neuronal data

Single neuron spike trains were analyzed utilizing the Neuroexplorer software (Nex Technologies, MA, USA). The mean frequencies of firing (FR, Hz) for selected principal cells across treatments were determined for each experiment. Furthermore, ‘bursts’ of spikes were characterized using the ‘Surprise’ method that identified sequences of at least three consecutive spikes with inter-spike intervals (ISIs) less than one-half of the mean ISI over all spike occurrences. Bursts were identified by: (i) calculating mean FR and ISI for all spikes, (ii) identifying sequences of three or more consecutive spikes, (iii) computing surprise (S) such that $S = -\log_{10}(P)$ where, P is the probability that the same sequence of spikes could occur in a random Poisson distribution with the same mean frequency as FR, (iv) maximizing S by either adding consecutive spikes to the end of the burst, or removing spikes from the beginning of the burst. Bursts with S -values > 10 were used to calculate the *mean number of bursts* in the spike train, *mean frequency within bursts*, *mean inter-burst interval (IBI)* and *mean number of spikes in bursts*. These burst parameters are summarized in an example spike train in figure 1B. Statistical significance between treatments of interest was assessed using student’s t -tests (paired) for all parameters described above. In addition, cross-correlograms between selected CA1 - CA1; CA3 - CA3 and CA1 - CA3 cell pairs were carried out for all animals. Each cross-correlogram represents the mean firing frequency (bin size = 5 ms) of a single cell referenced to the firing of another cell on a separate electrode (using a common ground) across a ± 0.2 s time window. Peak z-scores were calculated for each cross-correlogram as follows: (i) The peak value (i.e. histogram maximum) is found; (ii) The mean (M) and standard deviation (S) of the background bin values are calculated. The background parameters are calculated from bins outside peak (i.e. bins that are less than peak value / 2 away from the peak bin); (iii) peak z-score = (peak value – M) / S . All peak z-scores were averaged across the selected CA1 - CA1; CA3 - CA3 and CA1 - CA3 cell pairs respectively. Statistical reliability was confirmed using student’s t -tests (paired) to determine differences in mean peak z-scores between treatments and the null-hypothesis accepted for p ’s < 0.05 .

Results

Experiment 1 – Cannabinoids suppress spontaneous firing and bursting of principal cells in CA3 and CA1

Since 3 mg/kg Δ^9 -THC, 1 mg/kg WIN-2 and 100 μ g/kg HU-210 are doses, which have previously yielded impairments in hippocampal-dependent learning and memory tasks (Robinson et al., 2007; Pamplona & Takahashi, 2006; Hampson et al., 2003; Hampson & Deadwyler, 2000; Ferrari et al., 1999), these doses were administered to determine their effects on spontaneous firing and bursting of hippocampal principal cells located in CA3 and CA1.

Initially, a total of 53 principal cells were isolated from rats in group 1 ($n = 6$). They discharged at an average firing frequency of 1.79 ± 0.15 Hz following the treatment of Tween-80 (figure 2A) and this was significantly reduced by the partial cannabinoid receptor agonist, Δ^9 -THC ($t = 3.03$, $p < 0.01$) in an AM-281 (1.5 mg/kg) sensitive manner ($t = 2.80$, $p < 0.01$ relative to THC) fully reversing the Δ^9 -THC induced suppressions in hippocampal neuronal ensemble activity. Similarly, the 32 CA3 and CA1 principal cells isolated from animals in group 2 ($n = 6$) with an average firing frequency of 1.57 ± 0.27 Hz were significantly reduced following WIN-2 treatment ($t = 3.89$, $p < 0.01$; figure 2B); again, this action was fully reversed by AM-281 ($t = 3.30$, $p < 0.01$). These results provide evidence that both phytocannabinoids (Δ^9 -THC) and synthetic cannabinoids (WIN-2) have similar pharmacological activity. However, the potent cannabinoid receptor agonist HU-210 also reduced the average firing frequency from 2.22 ± 0.27 Hz across an additional 29 cells isolated from rats in group 3 ($t = 7.51$, $p < 0.001$; $n = 6$; figure 2C), but AM-281 failed to reverse this suppression ($t < 1$). Figures 2D, 2E and 2F further represent the rate histograms (*bin size* = 3 ms) of three representative CA3 principal neurons that were ‘tracked’ following the treatment of vehicle, cannabinoid and AM-281 respectively. Note the decrease in firing activity following agonist treatment and subsequent reversal by AM-281 for Δ^9 -THC and WIN-2, but not HU-210 treated animals.

Recordings were also utilized to establish burst characteristics of hippocampal principal neurons. Administration of Δ^9 -THC produced significant reductions in the average number of bursts ($t = 3.64$, $p < 0.001$), frequency within bursts ($t = 3.70$, $p < 0.001$) and spikes per burst ($t = 3.25$, $p < 0.01$), alongside a reliable increase in the average inter-burst interval ($t = 3.91$, $p < 0.001$) compared to vehicle (figure 3A). Subsequent administration of AM-281 fully reversed all of these changes (t 's > 2.1 , p 's < 0.05). Similar results in principal cell bursting were seen in animals treated with WIN-2 (see figure 3B for details and statistics). As for the average firing rate, HU-210 significantly reduced the mean number of bursts ($t = 5.88$, $p < 0.001$), frequency within bursts ($t = 4.75$, $p < 0.001$), spikes per burst ($t = 2.09$, $p < 0.05$) and increased inter-burst intervals ($t = 6.08$, $p < 0.001$) relative to Tween-80 (figure 3C). However, subsequent injection of AM-281 failed to reverse these alterations (all t 's < 1.96 , p 's > 0.05).

Cannabinoids disrupt synchronous activity within and between CA1 and CA3

The overall reduction in firing properties and burst characteristics seem to support the hypothesis that cannabinoids desynchronize neuronal assemblies in hippocampus (Robbe et al., 2006). This was more directly explored next. Temporal coupling between hippocampal principal cells determined as peak z-scores from cross-correlograms of pairs of principal cells located within and between CA3 and CA1 in the presence of Δ^9 -THC, yielded significant reductions in peak z-scores (CA3 - CA1 $n = 6$: $t = 4.58$, $p < 0.01$; CA3 - CA3 $n = 7$: $t = 3.80$, $p < 0.01$; CA1 - CA1 $n = 8$: $t = 3.80$, $p < 0.01$) relative to Tween-80 (see Table 1). Similar reductions in peak z-scores were observed for WIN-2 (CA3 - CA1 $n = 6$: $t =$

4.40, $p < 0.01$; CA3 - CA3 $n = 7$: $t = 3.74$, $p < 0.01$; CA1 - CA1 $n = 6$: $t = 3.30$, $p < 0.05$) and HU-210 (CA3 - CA1 $n = 5$: $t = 3.13$, $p < 0.05$); CA3 - CA3 $n = 5$: $t = 9.74$, $p < 0.001$; CA1 - CA1 $n = 6$: $t = 7.87$, $p < 0.001$). Subsequent treatment of AM-281 failed to reverse these reductions in peak z-scores (i.e. recover synchrony) induced by Δ^9 -THC (all t 's < 1.5 , p 's > 0.05), WIN-2 (all t 's < 1 , p 's > 0.05) and HU-210 (all t 's < 1.9 , p 's > 0.09) respectively. These observations are further illustrated in figures 4, 5 and 6, depicting representative cross-correlograms obtained from selected intra- and inter-subfield pairs of principal cells. Collectively, these results confirm that Δ^9 -THC, WIN-2 and HU-210 were capable of disrupting the temporal synchrony of ensemble firing within and between CA3 and CA1. They further imply mechanistic differences in cannabinoid effects on hippocampal cell physiology and the role of CB1 receptors in these actions.

Experiment 2 - Δ^9 -THC at 1 mg/kg also disrupts the temporal synchrony within CA3 and CA1

We noted that Δ^9 -THC at 3 mg/kg was a relatively high dose which may carry un-specific side effects possibly intractable to AM-281 injection. Moreover, since another full CB1 receptor agonist, CP 55,940, at a low dose of 0.3 mg/kg de-synchronized hippocampal neural activity without affecting the overall firing rate (Robbe et al., 2006), we proceeded by testing whether a lower dose of Δ^9 -THC (1 mg/kg) would replicate the reported CP 55,940 effect. As before, temporal co-ordination of hippocampal principal cells was significantly reduced by 1 mg/kg Δ^9 -THC (CA3 - CA3 $n = 7$: $t = 5.38$, $p < 0.01$; CA1 - CA1 $n = 8$: $t = 8.70$, $p < 0.001$) in comparison to Tween-80 (see Table 2) and AM-281 failed to reverse these reductions (all t 's < 1). Contrasting with the results of the higher dose of Δ^9 -THC (i.e. 3 mg/kg), however, no significant reductions in peak z-scores were observed across five pairs of CA3 - CA1 ($t = 1.80$, $p = 0.15$) principal cells. Subsequent AM-281 treatment also did not produce any effects ($t < 1$). These observations are further illustrated in figure 7.

Hence, these results demonstrate that Δ^9 -THC disrupts temporal synchrony between ensembles of cells located within the same sub-field (i.e. CA3 - CA3 or CA1 - CA1) at a much lower dose than it disrupts the synchrony of ensembles of cells located in different sub-fields (i.e. CA3 -CA1). Furthermore, these effects seem to be CB1 receptor independent.

1 mg/kg Δ^9 -THC does not alter firing and bursting of hippocampal principal cells

A total of 70 CA3 and CA1 principal cells (incl. those used to generate cross-correlograms) isolated from all animals in group 4 ($n = 6$) were subjected to a dose of 1 mg/kg of Δ^9 -THC. At this dose, Δ^9 -THC did not alter average firing frequency ($t < 1$) in comparison to Tween-80 (figure 8A). By contrast, AM-281 produced a reliable increase in firing frequency ($t = 5.55$, $p < 0.001$) as is clearly visible in the example depicted in figure 8B. While 1 mg/kg Δ^9 -THC did not affect burst characteristics (figure 8, all t 's < 1.75 , p 's > 0.09), subsequent CB1 blockade by AM-281 significantly increased the mean number of bursts ($t = 2.87$, $p < 0.01$; figure 8C), frequency within bursts ($t = 6.06$, $p < 0.001$; figure 8D), spikes per burst ($t = 2.98$, $p < 0.01$; figure 8E), and shortened the average inter-burst interval ($t = 3.79$, $p < 0.001$; figure 8F) compared with Δ^9 -THC. These results confirm the dose-dependence of Δ^9 -THC effects on synchrony and spike/burst firing and suggest that local network synchrony is more readily disrupted by cannabinoid treatment than single cell firing properties.

Discussion

The present *in vivo* multiple single-unit study reports three major findings: 1) both partial (Δ^9 -THC) and full (WIN-2 and HU-210) cannabinoid agonists suppressed overall firing and burst characteristics of hippocampal principal cells located in CA3 and CA1 sub-fields at

doses, which have produced memory deficits in rats (Robinson et al., 2007; Hampson and Deadwyler, 2000; Ferrari et al., 1999); 2) all three cannabinoids disrupted the synchronous firing of principal cells both within and between CA3 and CA1 regions; 3) effects were dissociable by drug dose as exemplified for Δ^9 -THC in which local network synchrony was more sensitive to low doses whilst alterations in spike/burst firing were evident at relatively higher doses, most likely caused by more severe network interferences in the hippocampus.

Cannabinoid-induced suppression of hippocampal cell firing is mediated by CB1 and non-CB1 receptors

Given that a considerable dose of 3 mg/kg Δ^9 -THC was required to markedly suppress firing and bursting confirms a dose-dependent effect on hippocampal principal cell activity. This is in line with our results of the more potent (Δ^9 -THC: $K_i = 50$ nM; WIN-2: $K_i = 3.3$ nM) cannabinoid receptor agonist WIN-2, which conferred similar effects already at a lower dose of 1 mg/kg. Reversal of suppression (i.e. reduced excitability of principal neurons), by AM-281 further implicates CB1 receptors as possible mediators of this effect.

It is clear to date that high-frequency bursts have a special importance in brain function, not only in hippocampus but also in other brain regions such as the amygdala, pre-frontal and entorhinal cortices (Peyrache et al., 2009; Viskontas et al., 2007; Funahashi et al., 2000; Stewart, 1999; Chrobak and Buzsaki, 1996). Hippocampal pyramidal cells, however, can encode information pertinent to memory formation by brief episodes of bursts or increased firing rates (Hampson and Deadwyler, 2000; Hampson et al., 2003; Robinson et al., 2007), while consolidation of this information occurs over extended periods of time (Riedel et al., 1999) and consist of replaying the ensemble codes during sleep and quite awake vigilance stages (Karlsson and Frank, 2009; Siapas and Wilson, 1998; Wilson and McNaughton, 1994). Thus, bursts of ensembles appear to have a specific role in synaptic plasticity and information processing (Lisman, 1997), especially in the hippocampus, where a single burst can evoke long-term synaptic modifications (Huerta and Lisman, 1993; 1995).

Hippocampal bursting is subject to modulation. Kainate-induced burst firing was reduced by cannabinoids such as HU-210 (Mason and Cheer, 2009) or after elevation of endocannabinoid levels by fatty acid amide hydrolase (FAAH) inhibition through URB-597 (Coomber et al., 2008). We here add to this a similar reduction in spontaneous burst firing due to Δ^9 -THC and WIN-2 administration and corroborate that shunting of bursts can be reversed by CB1 receptor blockade (Coomber et al., 2008; Mason and Cheers, 2009; this report). However, suppression of spontaneous bursting by HU-210 was not reversed by AM-281. This may be due to 1.5 mg/kg AM-281 being too low a dose to reverse HU-210 effect, which may also explain the failure to reverse HU-210 dependent de-synchronizations. More likely, however, is the assumption that HU-210 also acts through a CB1 independent mechanism.

There is ample evidence for non-CB1 receptor(s) in the rodent brain that may well be sensitive to HU-210. Di Marzo and co-workers (2000) were the first to demonstrate the existence of a novel $G_{i/o}$ -protein coupled, non-CB1 receptor in the hippocampus, which may be located pre-synaptically on glutamatergic terminals (Breivogel et al. 2001). Yet another non-CB1 receptor distinct from the one reported by Breivogel was suggested by Kofalvi and colleagues in 2003, also located at glutamatergic synapses in hippocampus. These non-CB1 receptors along with the newly discovered orphan receptor, GPR55 (Brown, 2007; Pertwee, 2007), which is also present in hippocampus (Sawzdargo *et al.*, 1999), are conceivable targets for HU-210 to suppress hippocampal principal cell firing and bursting. It is also in line with recent work by Pistis and colleagues (2004) demonstrating that the non-CB1 receptor described by Breivogel et al. (2001) was involved in suppressing principal cell firing in the basolateral amygdala.

Together, these findings suggest that cannabinoid induced alterations in firing and bursting rates occur through a combination of CB1 and non-CB1 receptor mediated mechanisms. Given the evidence to suggest that these receptors are located on pre-synaptic terminals of GABAergic inter-neurons (Freund et al., 2003; Freund and Hajos, 2003; Katona et al., 1999; Tsou et al., 1999, 1998), glutamatergic pyramidal cells (Nemeth et al., 2008; Kawamura et al., 2006; Domenici et al., 2006; Takahashi and Castillo, 2006; Shen et al., 1996) and cholinergic neurons (Gessa et al., 1998; Gifford et al., 1997a,b; Gifford and Ashby, 1996) in the hippocampus, this leaves one with the view that cannabinoid induced suppressions of principal cell firing/bursting are modulated through mixed actions of inhibitory and excitatory neurotransmission and the most parsimonious interpretation would favour cannabinoid induced reductions in excitatory drive (e.g. glutamatergic/cholinergic) over decreases in inhibitory (e.g. GABAergic) neurotransmission. However, due to the systemic dosing regime employed here, drug effects on extra-hippocampal CB1 and non-CB1 receptors cannot be excluded, which in turn may impinge on hippocampal principal cell firing/bursting.

Cannabinoids disrupt ensemble synchrony

All three cannabinoids under scrutiny also disrupted the temporo-spatial cohesion of principal cell firing within and between CA3 and CA1. Each cross-correlogram presents the degree of coupling between a selected pair of neurons and tight coupling or closer temporal coordination between two cells is implicated by a stronger peak z-score. Our results contrast with a recent study on CP55940, which disturbed the organization of cell assemblies devoid of considerable shunting of average firing rates in hippocampal neurons (Robbe et al., 2006). At the single cell level, this was reflected by a reduction in pyramidal cells firing bursts and a decreased probability of short-interval spikes in interneurons. The impairment of temporal coordination was even more striking at the population level as network oscillation patterns diminished. Since CP55940 was delivered at a low dose of 0.3 mg/kg (Robbe et al., 2006), we also lowered the dose of Δ^9 -THC to 1 mg/kg and determined its effects on synchronous firing of hippocampal principal cells. A clear de-synchronization of principal cell firing was confirmed within CA3 and CA1, but not for inter-CA3→CA1 ensembles. This selective and dose-dependent effect of Δ^9 -THC on synchronous firing is particularly interesting, as it suggests that temporal binding within local networks of CA3 and CA1 principal cells involving GABAergic feed-forward and feedback inhibition (Sargsyan et al., 2001; Nitsch et al., 1990) is more vulnerable to cannabinoids, while inter-regional synchrony (here possibly issued by the Schaffer collaterals) is more resilient to cannabinoid stimulation. A possible mechanistic underpinning may be the presence of CB1 and non-CB1 receptors at cholecystokinin containing GABAergic interneurons (Freund et al., 2003; Katona et al., 1999) with a seemingly higher sensitivity to exogenous cannabinoid than the glutamatergic neurons of Schaffer collaterals.

Furthermore, our results provide mechanistic insight as to how different doses of cannabinoids may differentially affect the neurophysiology of the hippocampus. The fact that a low dose of Δ^9 -THC selectively de-synchronized principal neurons without having an effect on firing/bursting rates suggests that functional decoupling of principal neurons precedes alterations in their firing/bursting patterns. Interestingly, single cell burst characteristics appear intact as long as CA3→CA1 synchrony was maintained (Harris et al., 2001; Lisman, 1997). This hypothesis can now be assessed for the first time, and if related to learning may enable the dissection of the functional involvement of local versus global hippocampal networks in memory processing to reveal the underlying mechanisms of cannabinoid-induced impairments in different forms and phases of memory (Robinson *et al.*, 2007; Robbe *et al.*, 2006; Hampson *et al.*, 2003; Hampson and Deadwyler, 2000; Sullivan, 2000; Ferrari *et al.*, 1999; Lichtman and Martin, 1996).

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Figure 1.

Illustrates the temporal sequence of drug administration and single-unit recordings (**A**). The vertical arrows indicate the time points at which vehicle (Tween-80); cannabinoid agonists (Δ^9 -THC, WIN-2 & HU-210) and the CB1 receptor antagonist/ inverse agonist, AM-281 were injected (i.p.). The black rectangles indicate when the respective recordings were carried out. Note that all recordings were initiated 15 min following each treatment. (**B**) represent different burst parameters that were quantified from individually recorded spike trains for further analysis. These included calculating the: (i) number of bursts; (ii) spikes in burst; (iii) inter-burst interval and (iv) frequency in bursts (burst duration/ spike in burst) respectively.

Figure 2.

Means $\pm$ s.e.m. of overall firing frequency (Hz) of CA3 and CA1 hippocampal principal cells following 3 mg/kg of Δ^9 -THC ($n=70$; **A**) or WIN-2 ($n=32$; **B**) or HU-210 ($n=29$; **C**) respectively. All subjects were given AM-281 following the cannabinoid treatment. Δ^9 -THC, WIN-2 and HU-210 were all able to attenuate spike firing. AM-281 was able to fully reverse the Δ^9 -THC & WIN-2 induced suppressions in firing rate but not the HU-210 induced inhibitions in firing. Rate histograms (mean frequency/3s bin, 20min epochs) depicting firing activity of individual hippocampal principal cells following Δ^9 -THC (**D**), WIN-2 (**E**) and HU-210 (**F**) with subsequent AM-281 treatments respectively. Asterisks indicate ** $p < 0.01$; *** $p < 0.001$.

Figure 3.

Means $\pm$ s.e.m. of number of bursts; frequency in bursts (Hz); spikes per burst and inter-burst interval (s) of CA3 and CA1 hippocampal principal cells following 3 mg/kg of Δ^9 -THC ($n = 53$; **A**) or WIN-2 ($n = 32$; **B**) or HU-210 ($n = 29$; **C**) respectively. All subjects were given AM-281 following the respective cannabinoid treatment. AM-281 reversed most Δ^9 -THC and WIN-2 induced alterations in burst characteristics, but failed to reverse HU-210 induced changes across all burst parameters. Asterisks indicate * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 4.

Cross-correlograms (bin size = 5ms; smoothed to 3 bins) performed on selected cell pairs between CA3 – CA1; CA3 – CA3 and CA1 – CA1 sub-fields following Tween-80, 3 mg/kg of Δ^9 -THC and AM-281 treatments respectively. Δ^9 -THC was able to de-synchronize principal cell activity both within and between CA3 and CA1 hippocampal sub-fields. AM-281 failed to reverse this cannabinoid-induced disruption in cell synchrony. Note the loss in peak ness across all cross-correlograms following Δ^9 -THC treatment. The horizontal lines in each cross-correlogram represent the 99% confidence limits.

Figure 5.

Cross-correlograms (bin size = 5ms; smoothed to 3 bins) performed on selected cell pairs between CA3 – CA1; CA3 – CA3 and CA1 – CA1 sub-fields following Tween-80, WIN-2 and AM-281 treatments respectively. Similar to Δ^9 -THC, WIN-2 de-synchronized principal cell activity both within and between CA3 and CA1 hippocampal sub-fields. Subsequent AM-281 treatment was unable to reverse these disruptions in cell synchrony. The horizontal lines in each cross-correlogram represent the 99% confidence limits.

Figure 6.

Cross-correlograms (bin size = 5ms; smoothed to 3 bins) performed on selected cell pairs between CA3 – CA1; CA3 – CA3 and CA1 – CA1 sub-fields following Tween-80, HU-210 and AM-281 treatments respectively. Similar to Δ^9 -THC and WIN-2, HU-210 was also able to disrupt the synchronous firing of principal cells within and between CA3 and CA1 hippocampal sub-fields that were not reversed by subsequent AM-281 treatment. The horizontal lines in each cross-correlogram represent the 99% confidence limits.

Figure 7.

Cross-correlograms (bin size = 5ms; smoothed to 3 bins) performed on selected cell pairs between CA3 – CA1; CA3 – CA3 and CA1 – CA1 sub-fields following Tween-80, 1 mg/kg of Δ^9 -THC and AM-281 treatments respectively. Unlike the higher dose of 3 mg/kg, this low dose of Δ^9 -THC was able to disrupt the principal cell synchrony only within CA3 and CA1 sub-fields but not between CA3 and CA1 sub-fields of the hippocampus. Subsequent AM-281 failed to reverse these disruptions in cell synchrony. The horizontal lines in each cross-correlogram represent the 99% confidence limits.

Figure 8.

(A) Means $\pm$ s.e.m. of overall firing frequency (Hz) of hippocampal principal cells ($n = 70$) following Tween-80, 1.0 mg/kg of Δ^9 -THC and AM-251 respectively. (B) Rate histograms (mean frequency/3s bin, 20 min epochs) depicting the firing activity of a CA3 principal cell following Tween-80; Δ^9 -THC (1 mg/kg) and AM-281 treatment. Means $\pm$ s.e.m. of Number of bursts (C); Frequency in bursts (D); Spikes per burst (E) and Inter-burst interval (F) of the same principal cells following the aforementioned treatments. The given low dose of Δ^9 -THC (1 mg/kg) failed to suppress the overall firing frequency and alter burst activity unlike that observed with the higher dose of 3 mg/kg. Subsequent AM-281 treatment significantly increased the firing frequency and bursting of these principal cells. Asterisks indicate ** $p < 0.01$; *** $p < 0.001$.

Table 1

Mean peak z-scores derived from cross-correlograms of principal cell pairs located in CA3 and CA1 hippocampal sub-fields following Tween-80, Δ^9 -THC (3 mg/kg) or WIN-2 (1 mg/kg) or HU-210 (100 μ g/kg) and AM-281 (1.5 mg/kg) respectively.

Cell pair	Treatment	peak z-score (Mean $\pm$ s.e.m.)	<i>p</i>
CA3 - CA1 (<i>n</i> = 6)	Tween-80	4.72 $\pm$ 0.45	
	3 mg/kg Δ^9 -THC	2.60 $\pm$ 0.25	< 0.01 (**)
	1.5 mg/kg AM-281	3.12 $\pm$ 0.57	> 0.05
CA3 - CA3 (<i>n</i> = 7)	Tween-80	3.86 $\pm$ 0.46	
	3 mg/kg Δ^9 -THC	2.81 $\pm$ 0.45	< 0.01 (**)
	1.5 mg/kg AM-281	3.38 $\pm$ 0.23	> 0.05
CA1 - CA1 (<i>n</i> = 8)	Tween-80	5.67 $\pm$ 0.72	
	3 mg/kg Δ^9 -THC	4.15 $\pm$ 0.86	< 0.01 (**)
	1.5 mg/kg AM-281	3.87 $\pm$ 0.51	> 0.05
CA3 - CA1 (<i>n</i> = 6)	Tween-80	5.03 $\pm$ 0.63	
	1.0 mg/kg WIN-2	3.32 $\pm$ 0.28	< 0.01 (**)
	1.5 mg/kg AM-281	3.28 $\pm$ 0.29	> 0.05
CA3 - CA3 (<i>n</i> = 7)	Tween-80	6.14 $\pm$ 0.92	
	1.0 mg/kg WIN-2	3.87 $\pm$ 0.42	< 0.01 (**)
	1.5 mg/kg AM-281	3.97 $\pm$ 0.30	> 0.05
CA1 - CA1 (<i>n</i> = 6)	Tween-80	6.03 $\pm$ 0.52	
	1.0 mg/kg WIN-2	4.70 $\pm$ 0.32	< 0.05 (*)
	1.5 mg/kg AM-281	4.41 $\pm$ 0.76	> 0.05
CA3 - CA1 (<i>n</i> = 5)	Tween-80	6.99 $\pm$ 0.35	
	100 μ g/kg HU-210	4.10 $\pm$ 0.65	< 0.05 (*)
	1.5 mg/kg AM-281	3.09 $\pm$ 0.37	> 0.05
CA3 - CA3 (<i>n</i> = 5)	Tween-80	8.82 $\pm$ 0.75	
	100 μ g/kg HU-210	6.62 $\pm$ 0.79	< 0.001 (***)
	1.5 mg/kg AM-281	7.40 $\pm$ 0.58	> 0.05
CA1 - CA1 (<i>n</i> = 6)	Tween-80	5.98 $\pm$ 0.36	
	100 μ g/kg HU-210	3.21 $\pm$ 0.29	< 0.001 (***)
	1.5 mg/kg AM-281	3.45 $\pm$ 0.27	> 0.05

Table 2

Mean peak z-scores derived from cross-correlograms of principal cell pairs located in CA3 and CA1 hippocampal sub-fields following Tween-80, Δ^9 -THC (1 mg/kg) and AM-281 (1.5 mg/kg) respectively.

Cell pair	Treatment	peak z-score (Mean $\pm$ s.e.m.)	<i>p</i>
CA3 - CA1 (<i>n</i> = 5)	Tween-80	5.63 $\pm$ 0.79	
	1.0 mg/kg Δ^9 -THC	5.00 $\pm$ 0.46	> 0.05
	1.5 mg/kg AM-281	5.31 $\pm$ 0.69	> 0.05
CA3 - CA3 (<i>n</i> = 7)	Tween-80	4.95 $\pm$ 0.40	
	1.0 mg/kg Δ^9 -THC	3.41 $\pm$ 0.16	< 0.01 (**)
	1.5 mg/kg AM-281	3.48 $\pm$ 0.54	> 0.05
CA1 - CA1 (<i>n</i> = 8)	Tween-80	4.95 $\pm$ 0.39	
	1.0 mg/kg Δ^9 -THC	3.18 $\pm$ 0.48	< 0.001 (***)
	1.5 mg/kg AM-281	3.37 $\pm$ 0.37	> 0.05