EFFECTS OF ENHANCING ANANDAMIDE LEVELS ON HIPPOCAMPAL

NEUROPHYSIOLOGY AND SHORT-TERM MEMORY PROCESSING IN RATS

By

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ABBREVIATIONS

2-AG	2-arachidonoyl glycerol
5-HT	5-hydroxytryptamine
Δ9-THC	Delta-9-tetrahydrocannabinol
ACEA	arachidonyl-2′-chloroethylamide
AD	Alzheimer's disease
AM251	N-(piperidin-1-yl)-1-(2, 4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide
AM281	N-(morpholin-4-yl)-1-(2, 4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide
AM404	N-(4-hydroxyphenyl)-5Z, 8Z, 11Z, 14Z-eicosatetraenamide
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
CA	cornu ammonis
CB	Cannabinoid receptor 1
CBD	Cannabidiol
CBN	Cannabinol
CCK	cholecystokinin
CHO	Chinese hamster ovary
CNS	central nervous system
COX	cyclooxygenase
CP55940	(1R, 3R, 4R)-3-[2-hydroxy-4-(1, 1-dimethylheptyl) phenyl]-4-(3-hydroxypropyl) cyclohexan-1-ol
CS	conditional stimulus

DAG   diacyglycerol

DNMS   delay nonmatch to sample

DRG   dorsal root ganglion

EA   ethanolamide

EC   endocannabinoid

ERK   extracellular-signal regulated kinase

FAAH   fatty acid amide hydrolase

FAK   focal adhesion kinase

GABA γ-amino butyric acid

GPCR   G protein-coupled receptor

GTPγS   guanosine 5′-O-(3-thio) triphosphate

HD   Huntington's disease

HEK   human embryonic kidney

HPA   hypothalamic-pituitary-adrenal

HU210   6aR, 10aR analog of 11-hydroxy-Δ8-THC-dimethylheptyl

ICAM-1   intercellular adhesion molecule-1

IL   interleukin

iNOS   inducible NOS

IP3   inositol-1, 4, 5-triphosphate

JNK   c-Jun N-terminal kinase

JWH-015   (2-methyl- 1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone

JWH-051   1-deoxy-11-OH-Δ8-THC-dimethylheptyl

JWH-1333   (1, 1-dimethylbutyl)-6, 6, 9-trimethyl-6a, 7, 10,10a-tetrahydro-

PEA   palmitoylethanolamide

PF-3845   4-(3-(5-(trifluoromethyl) pyridin-2-yloxy) benzyl)-N-(pyridin-

                            3-yl) piperidine-1-carboxamidePL   phospholipase

PLD   phospholipase D

PPAR   peroxisome proliferator-activated receptor

RhoA   Ras homolog gene family, member A

RT-PCR   reverse transcription-polymerase chain reaction

SN   substantia nigra

SR141716A   N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2, 4-

                            dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide

                            hydrochloride

TM   transmembrane

TRP   transient receptor potential

TRPV1 or VR1   transient receptor potential vanilloid 1 or vanilloid 1

URB597   cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl ester

URB602   biphenyl-3-ylcarbamic acid cyclohexyl ester

VDM11   N-(4-hydroxy-2-methylphenyl) arachidonoyl amide

WIN55,212-2   (R)-(+)-[2, 3-dihydro-5-methyl-3-(4-morpholinylmethyl)

                            pyrrolo-[1, 2, 3-de]-1, 4-benzoxazin-6-yl]-1-naphthalenyl-

                            methanonemesylate

ABSTRACT

The endocannabinoids affect spatial-working memory by altering neuronal activity of pyramidal neurons present in the hippocampus. In this study extracellular action potentials were recorded using multi-neuron electrodes implanted in the CA1 and CA3 subfields of the hippocampus. The acute effects of *R*-Methanandamide; VMD11 and URB597 were measured on firing rates, bursting characteristics and synchrony of hippocampal cells and performance of the DNMS task. *R*-methanandamide and URB597 but not VDM11 showed a consistent decrease in firing rates, burst duration and interspike intervals, in behaving animals and anesthetized animals. Pretreatment with the cannabinoid receptor antagonist, SR141716 (Rimonabant), was able to block some of these effects of the R-methanandamide, VDM11 and URB597. In addition, *R*-methanandamide but not, VDM11 and URB597, caused a significant decrease in performance of the DNMS task and reduction during sample but not during recall phases of the task and desynchronized CA3 pyramidal neurons. These results suggest that, in rats, enhancing anandamide may be related to alteration in hippocampal neuronal activities and performance in the short term spatial memory task. This work demonstrates that modulation of the anandamide system might be responsible for producing the observed deficits in spatial learning and memory that develop after acute cannabis (Δ^9-THC) use.

CHAPTER 1: BACKGROUND AND SIGNIFICANCE

1.1. Memory and the hippocampus

1.1.1. Memory-Basic concepts, definitions and classification of memory

The human brain is constantly acquiring and learning new information that provides a way to model the external world and produce the capacity to support behavior. In recent times, researchers have shown that neurons in the brain are responsible for the retention of learned information by a process known as memory (Squire and Zola-Morgan, 1991). Remembering is the ability to retrieve information from memory stores while forgetting is the inability to retrieve information (Nicoll et al., 1988). In humans and most animals, memory can be laid down in stages based on the nature of the task, the capacity to hold the information, and the amount of time that elapses between presentation of the task and recall of information (Schacter, 1992). One form of memory is known as short-term memory, which is maintained up to minutes but is lost or replaced by new information when not sustained by active rehearsal. Short term memory has limited capacity and holds few pieces of information at one time. In contrast, long term memory lasts days to years, and has much greater capacity with the potential of holding memory information indefinitely. The process involved in converting short term memory to long term memory is termed consolidation (Squire and Davis, 1981;White and McDonald, 2002). Encoding, retention, and retrieval refers respectively to the process by which information is acquired and transformed into a stored mental representation, maintained over time without active rehearsals, and brought back into consciousness from storage (Nicoll et al., 1988). Failure to remember encoded information may indicate a problem with either retention or retrieval. Nowadays, there are well-known associations of memory and learning (Zola-Morgan et al., 1986;Eichenbaum et al., 1996a).

Studies of memory demonstrate that memory involves different kinds of information systems and the mechanisms. The capacity or process of remembering new facts and events and making this information subsequently available for conscious recollection is "declarative" memory, while memory for habitual motor or skill behaviors is "non-declarative" memory (Squire and Davis, 1981). In most animals the capacity of declarative memory extends to both remembering specific context, that is spatial and temporal context (episodic memory) and learning new facts or general knowledge about the world (semantic memory). Examples of declarative memory include remembering places visited or learning a formula (Tulving, 1987;Tulving, 1992). It has been proposed that the medial temporal lobe is involved, as damage to this area produces anterograde amnesia. Anterograde amnesia is a memory deficit characterized by an inability to make lasting memories of one's daily experiences (Nicoll et al., 1988). Within the medial temporal lobe, the important structures are the hippocampus and the entorhinal cortices (Manns et al., 2003) Therefore, the hippocampus ordinarily supports memory systems for remembering new facts and events and makes this information subsequently available for conscious recollection. The non-declarative form of memory is expressed through performance rather than recollection. There are two kinds of non-declarative memory: associative learning such as classical conditioning and operant conditioning, and non-associative learning such as reflex pathways (Nicoll et al., 1988). Associative learning, in which there is an association between two stimuli (example food and sound) is called classical conditioning, and when there is an association between a response and a stimulus it is known as operant or instrumental conditioning (Staddon and Cerutti, 2003). Classical conditioning is induced by an association between two stimuli. Three key steps are involved: (1) an unconditional stimulus (US) such as food, (2) a conditional stimulus (CS) such as a sound and (3) a conditioning

response (CR), salivation. The association between conditioning and US is necessary, and the lost association produces a process known as extinction. On the other hand, operant conditioning includes motivational state such as hunger and thirst and mostly depends on brain systems outside the medial temporal lobe (Nicoll et al., 1988).

1.1.2. Learning and memory: Dysfunction and neurobiology

Although there are still some unknown issues related to human memory, it has become increasingly clear that bilateral damage to the medial temporal lobe disrupts declarative memory processes, especially episodic memory function (Squire and Zola-Morgan, 1991). This form of memory disruption is known as global declarative and retrograde memory deficit. The most common form of this type of brain dysfunction is amnesia. These memory problems are usually associated with trauma, stroke and exposure to drug of abuse (Skovronsky et al., 2006). Alzheimer's disease is characterized by progressive degenerative neuro-pathological changes associated with memory loss and the inability to process new memories. It displays degeneration and tangle formation (Skovronsky et al., 2006). Interestingly, hippocampal neuronal loss in aging and Alzheimer's disease seems to be consistent with the well described disruptive effects of cannabinoids. Disruptions of the cannabinoids system may alter normal physiological state and might play a role in the Alzheimer's disease. These may be due to the effects of cannabinoids on neurotransmitter release and selective vulnerability of certain hippocampal subregions where cannabinoid receptor are present (Simic et al., 1997).

Most intriguing findings are shown by changes in the brains of animals performing memory related tasks (Bunsey and Eichenbaum, 1996;Eichenbaum et al., 1996b;Squire, 1993).There are a wide range of well-developed models that are available for study and analysis memory. Of the many models, the most commonly used is the Morris water maze in which an

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animal's capacity to remember spatial cues is required to locate a hidden platform underwater (Morris et al., 1982). In this task, hippocampal lesioned and non-lesioned rats perform in a comparable manner when the platform is visible; however, hippocampal lesioned rats perform poorly when the platform is not visible (Teng and Squire, 1999). Another model used for memory related tasks are in studies of conditioning and delayed non-matching to sample tasks. The delayed non-matching to sample task requires an animal to compare a novel sample object with a previously presented familiar object and the selection of a novel object over the familiar object (Diamond et al., 1989;Zola et al., 2000). Surgical removal of the hippocampal caused impairments in the ability to acquire the cognitive strategy needed for performing explicit aspects of the DNMS task. The hippocampus and related brain regions are needed for successful completion of the DNMS task. Furthermore, neuroimaging findings show the hippocampus play a role during DNMS training's phase (Monk et al., 2002).and the overall scores of rats' performances declined dramatically when the hippocampus was removed and in the presence of drugs like Δ^9-THC. Furthermore, the performances of the rats were shown to decline in correlation to changes in firing characteristics of hippocampal pyramidal cells (Deadwyler et al., 1996).

Several years ago, it was thought that learning and memory occur by the growth of new neurons much like muscle strength that occurs through growth of new muscle tissue. It is now clear that existing neurons possess the ability to form new connections necessary for learning and memory. Researchers have shown that when animals are trained to perform specific tasks or exposed to enriched environments, new synapses grow and pre-existing synaptic connections develop better (Martin et al., 2000). Neuronal function can be modified by intense activity such as high–frequency stimulation of presynaptic neurons that tends to increase responsiveness and

efficiency of postsynaptic membranes. This process, known as potentiation, can be associated with both short and long term memory. The ability to encode information into more permanent long-term storage is believed to be a function of long term potentiation (LTP) (Bear and Malenka, 1994;Daniel et al., 1998). LTP is now regarded as a neuronal model for learning (Nicoll et al., 1988) and has been extensively studied in the CA1 field of the hippocampus (Martin et al., 2000). Unlike LTP, long term depression (LTD) is the inhibition of an event following strong stimulation. LTD has been studied in the Purkinje cells of the cerebellum, and has some functional characteristics which parallel LTP (Bear and Malenka, 1994;Daniel et al., 1998). The cellular basis for LTP is complex and has been shown to involve release of glutamate, an excitatory neurotransmitter, from the presynaptic cell. Glutamate acts on both the N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) receptors. Glutamate acting on NMDA receptors eventually leads to elevation of calcium ion levels in the post-synaptic cell. These elevations in intracellular calcium ions cause the phosphorylation of the sodium-gated AMPA receptors which then leads to an increased protein synthesis and release of diffusible factors. LTP can last for hours to days and may be important for clearing old memory traces (Bear and Malenka, 1994;Nicoll and Malenka, 1995). For many years researchers have been looking for factors that can diffuse back to the presynaptic cell to enhance or reduce the release of additional glutamate. At present, cannabinoid receptor activation by exogenous cannabinoids and endocannabinoids has been implicated in modulating both LTP and LTD induction in the hippocampus (Stella et al., 1997;Misner and Sullivan, 1999). During memory processing, many areas of the brain cortex serve as indices where the information converges then later passes through the entorhinal cortex and hippocampus (Eichenbaum, 2004) before entering in the prefrontal cortex (Squire and Zola-Morgan, 1991).

1.2. The hippocampus

1.2.1. General features and anatomy

The basic arrangement of the hippocampus is known from the abundant body of work found in the literature (Eichenbaum et al., 1996b;Amaral and Witter, 1989;Swanson et al., 1978). The general arrangement of the hippocampus is shown in Figure1. The hippocampus is located bilaterally in the temporal lobe of the cerebral cortex, forming a ridge that extends along the temporal horn of the lateral ventricle. It functions as part of the limbic system and plays important roles in the formation, consolidation and retrieval of episodic memories. One striking feature of the hippocampus is its convoluted pattern and shape that vaguely resembles a sea horse. The hippocampus can simply be divided into three main segments: a body; or middle segment, a head; or anterior segment and a tail, or posterior segment (Squire and Zola-Morgan, 1991). In transverse section, it consists of three distinct layers, the Cornu Ammonis (CA1, CA2 and CA3) or hippocampus proper, the dentate gyrus (DG) and the subiculum. One layer, or lamina, is rolled up inside the other resembling two interlocking, U-shaped laminas. The CA and the DG structures are separated from each other by the hippocampal sulcus Anatomically, in rodents, the superior region contains the CA1, and the inferior region the CA3. However, in humans, the hippocampus is more ventral and the relation of CA1 to CA3 is opposite to that found in rodents. The structure of the CA2 is often ignored because it represents a small portion of the hippocampus function. The subiculum wraps around the dentate gyrus and has a loosely packed pyramidal cell layer.(Nicoll et al., 1988;Amaral and Witter, 1989).

An intriguing feature of the hippocampus is the intrinsic neuronal path that flows from the dentate gyrus through the CA3 to CA1 to the subiculum. The major afferents or inputs to the hippocampus originate from the entorhinal cortex by way of a fiber system called the perforant

path. The perforant path fibers terminate in the dentate gyrus (Squire and Zola-Morgan, 1991). The major efferent fibers originate from the subiculum and project to the thalamic nuclei, the hypothalamus, and the amygdala (Aggleton et al., 2005). Anatomically, the neurons of the hippocampus can be classified as pyramidal or non-pyramidal cells according to the (1) morphological characteristics and location, (2) types of postsynaptic target structures, (3) content of neuropeptides and (4) physiological characteristics (Freund and Buzsaki, 1996;Somogyi et al., 1985). Pyramidal neurons are regarded as the principal units of the mammalian hippocampus and typically contain two types of dendrites: a triangular base facing the upper layer know as the alveus, and an apex facing the deeper level (Nicoll et al., 1988). The apical dendrite is a single projection that gives rise to other branches while the basal axon sends out collaterals, which curve back to reach other pyramidal neurons. The soma of the pyramidal cell is usually surrounded by a dense plexus of non-pyramidal interneurons known as basket cells. Anatomical studies of the CA have demonstrated hippocampal pyramidal neuron receiving tens of thousands of excitatory and inhibitory synaptic inputs onto their dendrites (Megias et al., 2001;Gulyas et al., 1999). There are many reports of pyramidal neurons activity switching from single action potentials to burst firing in the form of complex spikes (Hoffman et al., 1997;Magee et al., 1998). These firing modes have been demonstrated both in vivo and in vitro and have been shown as critical for learning and memory. These firing modes are representative of dramatic changes in the way in which signals are processed by neurons (Buzsaki, 1986).

Non-pyramidal cells have been demonstrated to contain peptides (cholecystokinin, somatostatin) and calcium-binding proteins (parvalbumin and calbindin), which give them a characteristic fine structure and unique synaptic input (Freund and Buzsaki, 1996;Acsady et al., 1996). Most non-pyramidal cells contain gamma-aminobutyric acid (GABA) as the principal

neurotransmitter (Freund and Buzsaki, 1996;Acsady et al., 1998), which is the major inhibitory transmitter of adult mammals. A considerable amount of experimental evidence has also shown that non-pyramidal GABA containing neurons mediate most of the synaptic activities around the cell bodies and proximal dendrites of pyramidal neurons. GABA containing neurons are demonstrated to be highly present in the hippocampus mossy fibers (Farrant and Kaila, 2007). Given that GABA containing neurons are widely expressed around most pyramidal neurons throughout the hippocampus, it is reasonable that varying the numbers of GABA input synapses from non-pyramidal cells could differently regulate synaptic processes in pyramidal neurons. Therefore, activation of these types of non-pyramidal cells will result in inhibition of pyramidal neurons (Ottersen and Storm-Mathisen, 1986).

In coronal sections, the DG of the hippocampus is a narrow structure, located dorsally. Structurally, DG is much simpler than the CA and consists of three visible regions: the molecular, granular, and polymorphic layers. Of these regions, the middle granular layer is the most prominent feature and contains densely packed granule cells. These granule cells are small, round and possess dendrites which project perpendicular to the granule cell layer. Also, mossy fibers terminate onto apical dendrites of the pyramidal neurons (Raisman and Ebner, 1983;Nicoll et al., 1988). Mossy fibers are mostly glutamatergic but some GABA and opiate peptides like dynorphin and enkephalin can be present (Frotscher et al., 1988). The CA is a hook-shaped process divided into six main layers or strata. The layers range from the most superficial layer, the alveus to the deepest layer known as the stratum molecular. In between these two layers are the stratum oriens, pyramidale, lucidum, radiatum and lacunosum. One of these six layers of the CA3 sends extensive connections to regions of the CA1 through a set of fibers called the

Schaffer collaterals. In the stratum radiatum of the CA1, apical dendrites then send fibers to the septal nuclei, and commissural fibers (Olbrich and Braak, 1985).

1.2.2. Function as a cognitive map

Animal studies have indicated that the hippocampus plays a significant role in storing and processing spatial information. One intriguing finding in rodents is that firing rates of hippocampal cells were found to correlate with the location of the animal in an environment. Neurons known as place cells (O'Keefe and Recce, 1993) exhibit a high rate of firing whenever an animal is in a different location. The outstanding discovery of place cells by O'Keefe and others (O'Keefe and Dostrovsky, 1971) has led to the idea that the hippocampus acts as a cognitive map. That is, the hippocampus depicts a neural representation of the layout of the environment. This implies that specific hippocampal pyramidal cells are involved in encoding information about the location of a particular space relative to spatial cues. Also, hippocampal cells can also respond to many properties of the stimulus environment besides spatial location (Berger and Thompson, 1978;Eichenbaum et al., 1999). Prominent forms of alteration of synaptic function had been seen in GABA containing neurons. Endocannabinoid signaling can selectively regulate different subtypes of GABA containing neuronal inputs onto hippocampal CA1 pyramidal neurons (Freund et al., 2003). Neuronal firing on CA3 and CA1 sub-regions can cause adaptive changes in the brain function by altering synaptic terminals leading spatial memory deficit (Khazipov et al., 1995). Altogether it can be stated that the hippocampal system is (a) principally concerned with memory, (b) operates with associated cortical areas to establish and maintain memory and (c) involved in certain aspects of motor control. (Squire and Davis, 1981) (The Human Hippocampus, 2005, 39-72).

Figure 1.1.The structure of the human Hippocampus

Pyramidal cell

Cross section of hippocampus- The perforant path (pp) carries sensory information from neurons in the entorhinal cortex to the granule cells in the dentate gyrus (DG). Mossy fibers (mf) run from the dentate gyrus to CA3 pyramidal neurons, and Schaffer collateral fibers (Sch) run from CA3 pyramidal neurons to CA1 pyramidal neurons. The CA1 pyramidal neurons project back to the entorhinal cortex. The fimbria and commisural fiber are collaterals project to area CA1 of the hippocampus   [Figure adapted from Kandel et al, Principles of Neural Science 4th Ed, 2000.]

1.3. Cannabinoid receptors

1.3.1. Cannabinoid receptors: Structure and functions

The active ingredient of marijuana and related cannabinoids binds to specific receptors in the brain. In the nineteen-eighties, an important finding by Howlett and colleagues (Howlett et al., 1989) showed that cannabinoids inhibit the activity of Adenylyl Cyclase in neuroblastoma cell membranes, which spurn a lot of interest in studies of the actions of marijuana and related cannabinoids in the brain (Devane et al., 1988;Howlett, 1985;Howlett et al., 1986). This finding was followed by studies demonstrating specific binding sites and stereoselectivity of various Δ^9-THC analogs in the cerebral cortex of rodents (Devane et al., 1988). Further insight also came as the idea that specific proteins in the membrane, now known as cannabinoid receptors are responsible for these actions. These receptors were de-orphanized, isolated and cloned from the rat (Matsuda, 1997). Today, the DNA coding sequences of the cannabinoid receptors have been reported for mice (Chakrabarti et al., 1995) and humans (Glass et al., 1997). A very interesting observation from the cloning of the receptor was that the structures of the cannabinoid receptor in the human, rat and mouse are very similar (Howlett et al., 2002). Also, the location and distribution of the cannabinoid receptors in the body was extensively characterized by Herkenham and colleagues (Herkenham et al., 1990). It was observed that the density of the cannabinoid receptors present throughout the nervous system was high compared with neuropeptides, cortical GABA receptors, and dopamine receptors in the striatum, and ion channel gated glutamatergic receptors.

Currently, two types of receptors (CB_1 and CB_2) have been identified on the basis of their structure, distribution and ligand binding properties. The CB_1 receptor is found throughout the body including the brain, the pituitary gland, immune cells, gastrointestinal tissues, heart, and

lungs, but is largely confined to the central nervous system. The CB_2 receptor is mainly present in peripheral tissues (Howlett et al., 1990). There is ongoing evidence for the existence of another receptor subtype in the brain. However, its cloning, expression and characterization is not yet defined (Wiley and Martin, 2002). In particular, high densities of CB_1 receptors are found in certain brain regions where they may account for many biological actions of cannabinoids. These actions include processes of cognition and memory, control of locomotor activity, pain sensation, neuroendocrine function and thermoregulatory states (Howlett et al., 2002). Brain regions primarily concerned with these actions include the cortex, hippocampus, cerebellum, spinal cord, basal ganglia and substantia nigra and hypothalamus. The lack of receptors has been shown to have serious adverse effects in mice including changes in cognition (Ledent et al., 1999). CB_1 receptor dysfunction in humans has been linked to various aspect of Alzheimer's disease, Parkinson's disease, schizophrenia and depression (Eubanks et al., 2006). In most of these disorders, the distribution and densities of CB_1 has been shown to change as the disorders progress. This suggests a role of CB_1 receptors in various aspects of neuropsychiatric disorders (Glass et al., 1997).

The amino acid sequence of the cannabinoid receptor was determined as 472 residues in human and 473 residues in rats with a molecular weight of 64kDA (Howlett et al., 1998;Song and Howlett, 1995). The receptor is located on human chromosome 6, rat chromosome 5 and mouse chromosome 4 (Ryberg et al., 2005;Stubbs et al., 1996). The human CB_1 receptors and rat CB_1 receptors show a strong sequence homology, about 95–97% identity at both nucleic and amino acid levels(Gerard et al., 1990) . The CB_1 and CB_2 receptors have been shown to belong to a large family of rhodopsin-like G-protein coupled receptors (GPCR)(Howlett et al., 2002). Like other GPCR, the CB receptor structure consists of seven large hydrophobic transmembrane-

spanning domains with a third transmembrane domains, possessing a cysteine amino acid residue which might be important for interaction with Δ^9-THC and related analogs (Howlett et al., 2002). Site-directed mutagenesis studies have begun to shed light on some of the functions of the binding domains of cannabinoids and the role they play in determining the activation of receptor coupling and desensitization. Tolerance is a process that develops rapidly during chronic administration of cannabinoids(Childers et al., 1993). Receptor desensitization or uncoupling has been implicated as one of the molecular events that underlie the development of tolerance to cannabinoids in many brains systems (Freedman and Lefkowitz, 1996;Hsieh et al., 1999).

With proper techniques for labeling of receptor levels, high densities have been detected in the cortex and other brain regions. In the rat brain, the main region where cannabinoids receptors are present in significant amount is the hippocampus. Within the hippocampus, higher levels of the CB_1 receptors are in the molecular layer, pyramidal and lacunosum layers of the CA1 and CA3 sub-region, dentate gyrus and subiculum (Matsuda et al., 1993). An interesting observation using specific antibodies to the C- terminal of the CB_1 receptor, found that CB_1 receptors are primarily distributed in axon terminals of cholecystokinin (CCK)-8 positive (Tsou et al., 1999;Katona et al., 2000;Mackie, 2005) subset of GABA interneurons (Doherty and Dingledine, 2003;Irving et al., 2000). The CB_1 receptor is typically a presynaptic receptor, and there are accounts of the involvement of the cannabinoid function in modulation of GABA activities (Matsuda et al 1993). Moreover, the actions of cannabinoids in the central nervous system have been linked to mediating the synthesis and release of other neurotransmitters (Alger and Pitler, 1995;Yoshida et al., 2002;Piomelli, 2003). In many structures of the brain, such as the cerebellum and nucleus accumbens, CB_1 receptors shows similar distribution pattern to that of the hippocampus and in the nucleus accumbens. Cannabinoid receptors have been associated

with the mediation of the rewarding affects of cannabinoids on the brain (Howlett et al., 2002;Elphick and Egertova, 2001). Moreover, low levels of the CB_1 receptor have also been detected in structures such as the brain stem, medulla oblongata and hypothalamus. This low levels of cannabinoid receptor in these structures has been proposed as the reason for the low acute toxicity and the lack of mortality of marijuana use (Howlett et al., 1990).

At present, there is new evidence suggesting that CB_1 receptors may also exist in parts of central nervous system as dimers, formed by combination with other classes of GPCR. These dimers can exist as homodimers or heterodimers and may account for the synergistic interaction between CB_1 receptors and receptors such as alpha-2 adrenergic receptors (Reyes et al., 2009;Oropeza et al., 2007). Despite 44% overall homology between the CB_1 and the CB_2, CB_2 is more diffusely distributed mainly in the immune cells such as leukocytes and spleen (Onaivi et al., 1996) However, there are substantial differences in the pharmacological and biological characteristics.

1.3.2. Cannabinoids receptor ligands: Agonists and antagonist

Since the discovery of Δ^9-THC in the *cannabis Sativa* plant and the cannabinoid receptor system, a large number of compounds have been synthesized as agonists of cannabinoid receptors (Evans et al., 1994;Martin et al., 1991;Mechoulam et al., 1995;Howlett et al., 2002). These compounds act on the cannabinoid receptors and possess varying degrees of selectivity and specificity. The three main groups are the natural cannabinoids derived the *cannabis Sativa* plant, the endogenous cannabinoids or endocannabinoids, and the synthetic cannabinoids. Amongst these three main groups are the classical, non-classical and aminoalkylindole ligands. Classical cannabinoids are a class of chemically related compounds derived from natural compounds synthesized by the *cannabis Sativa* plant (Howlett et al., 2002;Palmer et al., 2002). Among the sixty or more natural cannabinoids, Δ^9-THC is the main psychoactive compound,

considered as more abundant in the plant and produces more potent effects (Rhee et al., 1997). Several selective ligands for each of the cannabinoid receptor proteins have been designed and synthesized. The dibenzopyran derivatives are less specific, less active and have provided a framework for the development of a series of similar compounds as binding ligands and molecular probes (Martin et al., 1991;Howlett et al., 2002;Wiley et al., 1998;Razdan, 1986). Other groups of compounds which are large components of the plant are the phytocannabinoids, cannabinol (CBN), cannabidiol (CBD) and cannabigerol. The CBN, CBD and cannabigerol are behaviorally inactive and have been shown to have negligible affinity for CB_1 and CB_2 receptors and may act in a different manner from Δ^9-THC (Mechoulam et al., 2002;Pertwee, 2006).

The discovery of specific receptors for cannabinoids also boosted interest in the search for the brain's own endogenous ligands. This led to the isolation of endogenous fatty acid compounds from the porcine brain and the intestine of canines. These compounds, known as endocannabinoids, showed typical pharmacological activities of psychotropic cannabinoids. Anandamide (N-arachidonylethanolamide) (AEA) was the first compound found (Mechoulam et al., 1995;Zygmunt et al., 2000b) and shortly after Mechoulam's lab and colleagues showed that 2AG is also a potent CB_1 receptor (Figure 1.2). Other endocannabinoids include 2AG ether, virodhamine and endogenous analogs of anandamide (eicosatrienoylethanamide and docosatetraenoylamide) (Freund et al., 2003). Despite their isolation and structural elucidation, their biochemical role in the brain is not completely understood. Some interesting findings over the years are that endocannabinoids have a much shorter duration of action *in vitro* and *in vivo,* are rapidly inactivated by reuptake into neurons through a cellular transport system and are degraded by metabolic enzymes in the cell. In addition to activating CB_1 and CB_2 receptors, they

can also interact with other GPCRs and ion channels like TRPV, 5HT3, α7 nicotinic and GPR55 receptors (De et al., 2004;Pertwee et al., 2010;Pacher et al., 2006).

Certain derivatives developed from Δ^9–THC turn out to possessed high selectivity and increased biological activities. They are quite heterogeneous in structure and most of them have been widely used for characterization purposes. These include the non-classical agonist CP55940 (Howlett et al., 1990;Devane et al., 1988;Hillard et al., 1999). It shows a high potency and approximately equal affinity for both CB_1 and CB_2 receptors (Wiley et al., 1998). Other, classical CB_1-agonists used for characterization purposes include JHWU, L-759633, JWH-133 and the potent HU210 (Howlett et al., 2002;Pertwee and Ross, 2002). On the basis of binding properties, HU210 can also act on peroxisome proliferator activated receptor γ (PPAR-γ), a group of pharmacologically important nuclear receptors (Liu et al., 2003). The aminoalkylindole, WIN55212-2 has very high potency, relative intrinsic activity and a higher affinity for CB_1 receptor (Hillard et al., 1999;Felder et al., 1995). HU210 and JWH-133, readily cross the blood-brain barrier, and can inhibit TRPV1 and regulate glutamate transmission through a direct blockade of voltage-gated calcium channels (Ferraro et al., 2001).

An important feature in cannabinoid pharmacological research was the development of compounds that interfere with the normal physiological actions of agonist and generally block the receptor or elicit responses in some tissues that are opposite in direction from that produced by agonists (Rinaldi-Carmona et al., 1994).The cannabinoid receptors antagonists are very heterogeneous in structure. Most of them have been widely used for characterization purposes. Some antagonists developed at present include SR141716A (now called rimonabant) (Huffman et al., 1996;Rinaldi-Carmona et al., 1994;Showalter et al., 1996), AM251 (Gatley et al., 1997), AM281 (Gatley et al., 1997;Lan et al., 1999) and LY320135 (Felder et al., 1995). Among these

compounds, rimonabant and AM251 are the most potent and can extensively block the effects of CB_1 receptor activation *in vitro* and *in vivo*. (Collins et al., 1995;Rinaldi-Carmona et al., 1994) Rimonabant have been shown to improve memory in rodents(Terranova et al., 1996). However, the mechanism by which rimonabant exerts these effects both *in vitro* and *in vivo* remains unknown. They may act as a partial agonist in some tissues. So far, a very useful tool for distinguishing between the CB_1 and CB_2 receptor mediating effects is by using SR144528, a selective CB_2 receptor inverse agonist (Rinaldi-Carmona et al., 1998;Hurst et al., 2002)**.** Based on several data, more potent and selective antagonists are being investigated as potential candidates for treatment of brain reward system disorders (alcoholism and nicotine abuse) and certain disorders of memory (Cota et al., 2003;Cohen et al., 2002;Gardner, 2005;Wolff and Leander, 2003). However, their use might be limited by potential central nervous system side effects (Jagerovic et al., 2008).

Figure 1.2. Cannabinoid receptor location in the rat brain
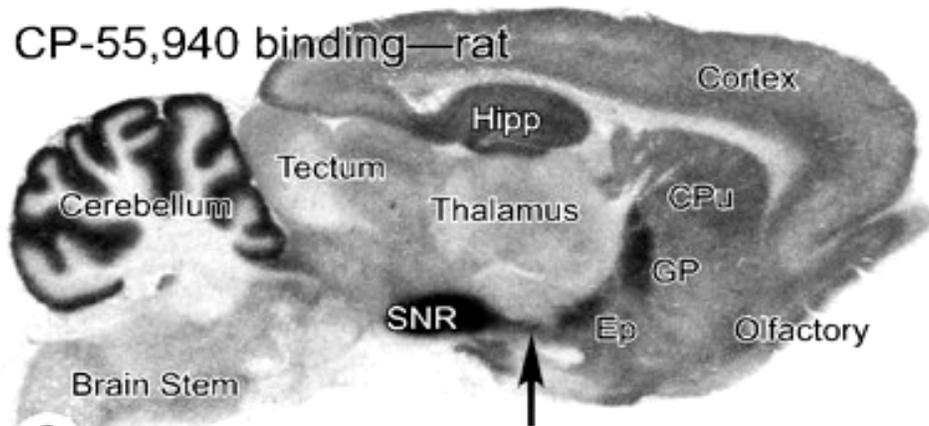
CB$_1$ receptor is highly expressed in the following regions: the basal ganglia (entopeduncular nucleus (Ep), globus pallidus (GP), caudate putamen (CPu), the cerebellum; the cortex, the hippocampus (Hipp), substantia nigra (SNR), tectum and less dense in the brain stem and thalamus. This image was obtained by the use of CP-55,940 in vitro binding in rat brain. Figure extracted from (Herkenham et al., 1990).

1.4. The endocannabinoid system: Metabolism

1.4.1. The biosynthesis of endocannabinoids: Anandamide and 2-AG

Endocannabinoids have been implicated in the function of many brain regions including the hippocampus, cerebral cortex, brainstem, basal ganglia, cerebellum, and olfactory bulb. However, there is little correlation between the distribution of the CB_1 receptor and the levels of endocannabinoids in different brain regions (Elphick and Egertova, 2001). These compounds, despite their similar structures differ in their binding affinity for the cannabinoid receptor, distribution and biological functions. Moreover, the release of endocannabinoids from neurons is through the activation of postsynaptic neurons (Piomelli, 2003). Unlike classical neurotransmitters, endocannabinoids are not stored within synaptic vesicles but are released based on the activity of neurons. Like many classical neurotransmitters, endocannabinoids are synthesized and released from postsynaptic neurons and degraded by intracellular enzymes. As lipophilic compounds, endocannabinoids diffuse freely across membranes. Precursors for endocannabinoid synthesis reside in the membrane as phospholipids and are released after the activation of a phopholipase or related enzymes. Endocannabinoids are broken down into arachidonic acid, glycerol and related products. It is reasonable that inhibitors of both the transport and metabolism could be used to increase synaptic levels of endocannabinoids locally. In addition, this mechanism is useful for understanding this system since the synthesis, cellular transport and degradation of endocannabinoids are tightly regulated processes (Howlett et al., 2002;Wilson and Nicoll, 2001;Kreitzer and Regehr, 2002).

Anadamide (AEA) is an amide of arachidonic acid and ethanolamine, which is widely distributed in the brain and periphery of rats and humans (Felder et al., 1996). Anandamide is released upon the arrival of action potentials on postsynaptic neurons (Ohno-Shosaku et al.,

2001). The level, composition and activities of AEA vary in different tissues in humans, mice and rats. In the mouse and rat brain, AEA levels reach 10-15 pmol/g tissue and 11-90 pmol/g tissue, respectively(Di Marzo, 1999) Most measurements in humans have reported significant levels (50 nmol/g tissue) in the hippocampus (Cadas et al., 1997;Maccarrone et al., 2001;Koga et al., 1995). Neuronal biosynthesis of AEA has been reported to occur through the conversion of N-acyl-phosphatidylethanolamine (NAPE) by an enzyme called Phospholipase D. N-acyl-phosphatidylethanolamine (NAPE) formed by the conversion of phosphatidylethanolamine by the N-acyltransferese (NAT). Phospholipase D is found in many species and highly conserved throughout the animal kingdom from insect, to rodent, to human (Elphick and Egertova, 2001;Liu et al., 2006). Once released into the extracellular space, AEA is taken up into the cell and degraded by fatty acid amide hydrolase (FAAH) in the endoplasmic reticulum

2-AG is a monoacylglycerol lipid consisting of arachidonic acid and a glycerol backbone. It is considered as a degradation product of inositol phospholipid and as a possible source for arachidonic acid in cells (Sugiura et al., 2002). It was found that 2-AG has high binding activity for the cannabinoid receptors and behaves as full agonist at both CB_1 and CB_2 receptor. Although the distribution of 2-AG is not well characterized, its concentration in the rat brain is approximately thought to be in the nanomolar range, a value hundred times higher than that of AEA. It is however twenty times less potent than AEA (Mechoulam et al., 1995;Stella et al., 1997). In human and rat brains, levels of 2-AG have been reported to be as high as 35 and 65 nmol/g tissue (Maccarrone et al., 2001), with significant amounts in hippocampus, the cerebellum and cortex (Di Marzo and Deutsch, 1998) Neuronal 2-AG is synthesized from plasma membrane by many pathways. The main pathway for the synthesis of 2-AG is through hydrolysis of arachidonic- acid containing membrane phospholipids and phosphatidylinositol to

produce diacyglycerol. This hydrolysis is catalyzed by phopholipase C. Then 2-AG is produced from diacyglycerol by the action of diacyglycerol lipase (DAGL) (Bisogno et al., 1997;Bisogno et al., 2005).

1.4.2. Transport and degradation of endocannabinoids

In the cell, AEA can be biotransformed by two different pathways: hydrolysis by FAAH and oxidation by cyclooxygenase (COX2) (Vandevoorde and Lambert, 2007). FAAH has been characterized, isolated, and cloned (Cravatt et al., 1996) from rat liver and has been found mainly in the intracellular compartments of presynaptic neurons but emerging evidence suggests that FAAH can be expressed in postsynaptic neuron (Cravatt and Lichtman, 2002;Hillard et al., 1995). COX-2 can also metabolize anandamide into prostaglandin-ethanolamides and esters (Cravatt and Lichtman, 2002;Maccarrone et al., 2002;Deutsch et al., 2001). Also, the diffusion and reuptake of anandamide into the cytoplasm is coupled to the degradation by intracellular enzymes (Cravatt et al., 1996). Many selective and nonselective inhibitors of the FAAH enzyme have been developed (Deutsch et al., 1997;Deutsch et al., 1997;Deutsch et al., 2001). Using some of these inhibitors, AEA levels were shown to increased both *in vitro* and *in vivo* conditions (Lichtman et al., 2002;Piomelli et al., 2006). FAAH activity can be irreversibly inhibited by URB597 and phenylmethylsulfonylfluoride, leading to a marked increase in the levels of anandamide. These effects of URB597 can be reliably blocked by CB_1 receptor antagonists (Lichtman et al., 2004;Tarzia et al., 2003;Tarzia et al., 2006;Piomelli et al., 2006;Cravatt and Lichtman, 2002). Like AEA, 2-AG is metabolized rapidly to yield arachidonic acid and glycerol. This can be achieved by multiple pathways: hydrolysis by monoacylglycerol lipase (MAGL), ABH6 and COX2 (Vandevoorde and Lambert, 2007)**.** MAGL has been shown to account for over eighty–five percent of 2AG hydrolysis in the brain. Selective blockade of 2-

AG hydrolysis by JZL184 and URB602 significantly increases 2AG levels in the brain (Lopez-Rodriguez et al., 2003;Makara et al., 2005).

Several studies have suggested that 2-AG and AEA are transported into the cell by the same transport system (Piomelli et al., 1999;Giuffrida et al., 2001;Beltramo and Piomelli, 2000). In neurons and many other tissues, uptake of endocannabinoids has been shown to be selective and occur in an energy-independent mechanism (Sugiura et al., 1996;Sugiura et al., 1995;Deutsch and Chin, 1993;Deutsch et al., 2001). Specific compounds now known as endocannabinoid transport inhibitors can inhibit endocannabinoid reuptake and have shown to induced an elevation of plasma anandamide level (Giuffrida et al., 2001). Among many inhibitors, the most widely used are AM404 and VDM11 (Giuffrida et al., 2000;Beltramo et al., 1997;Hogestatt et al., 2005).

Figure 1.3. Anandamide metabolism (synthesis, transport and inactivation)
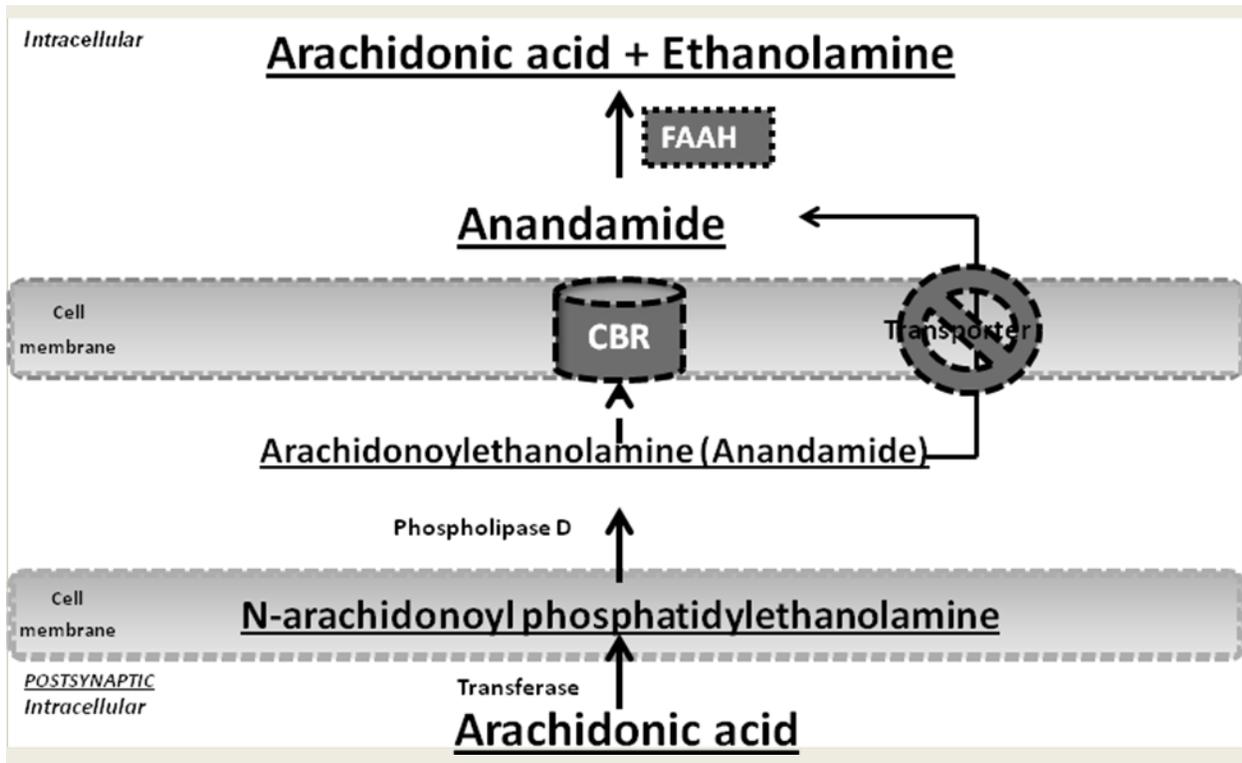
The anandamide biosynthetic enzymes are N-acyltransferase and N-acylphosphatidyl-ethanolamine-specific phopholipase D. The inactivating enzyme is FAAH located on intracellular membranes mostly on neurons postsynaptic to CB_1 receptors neurons. Also, an as yet uncharacterized endocannabinoid membrane transport mechanism may facilitate both endocannabinoid release and re-uptake, and might be localized on both pre- and postsynaptic neurons.

1.5. Interaction of cannabinoids with other neurotransmitter systems

1.5.1. Amino acids neurotransmitters: GABA and Glutamate neurotransmitters

Immunhistochemistry studies have indicated that CB_1 receptors are localized on axon terminals of CCK containing GABAergic interneurons(Herkenham et al., 1991). These interneurons are believed to be responsible for synchronous firing of neurons associated with regulation of pyramidal cell organization and a number of cognitive processes, such as memory or attention (Wilson et al., 2001;Buzsaki et al., 2002;Buzsaki et al., 2002). CB_1 receptor activation has been shown to reduce release of GABA (Irving et al., 2000), inhibit reuptake of GABA, and decrease local field potential (Misner and Sullivan, 1999). Also, the inhibition of GABA release from neurons has been shown to occur in a SR141617 (rimonabant) dependent manner (Katona et al., 2000). The consequences of such action causes alterations of many neuronal processes including shortening of the duration of action potentials, leading to postsynaptic depolarization and a net excitation. The excitable glutamate receptors are responsible for modulation of neuronal excitability and neurotransmitter release. Activation of NMDA receptors may be responsible for spontaneous excitatory postsynaptic potentials or miniature excitatory postsynaptic currents (mEPSCs) and for calcium ion permeability of pyramidal neurons (Lenz and Alger, 1999;Doherty and Dingledine, 2003). CB_1 receptors have also been detected on postsynaptic neurons but the significance is still elusive (Shen et al., 1996). In the CA1 hippocampus, the activation of postsynaptic mGluR5 receptors causes the release of endocannabinoids. Cannabinoids also alter the glutamate concentration in the synapses, modulate NMDA receptors, decrease calcium ion permeability of pyramidal neurons, and alter timing patterns of synaptic signals. Key factors that might contribute to the mechanism by which cannabinoids curtail the release of GABA and glutamate neurotransmitters include blocking of

voltage dependent N- and P/Q-type calcium current (Mackie and Hille, 1992) and the modulation of potassium channels (Twitchell et al., 1997). These mechanisms lead to DSI, since endocannabinoids serve as retrograde messengers and inhibit IPSCs, suggesting further the role of cannabinoids and GABA in memory and other related cognitive processes (Wilson et al., 2001).

1.5.2. The biogenic amines: Acetylcholine and serotonin (5-HT) receptors

Cholinergic receptors are also present on pyramidal cells. Presently, there are conflicting reports about the interaction between the cannabinoid system and the cholinergic system (Robinson et al., 2010;Acquas et al., 2000;Goonawardena et al., 2010b). CB_1 receptors are not likely directly involved in synaptic transmission via cholinergic neurotransmitters, but may serve as sensors for the actions of nicotine. Cannabinoids have been shown to inhibit the release of acetylcholine from neurons through CB_1 receptors activation (Carta et al., 1998;Gifford and Ashby, Jr., 1996). The particular subtype of receptor has not been detected but could include pharmacological modulation of cannabinoid release from cholinergic neurons.

Noradrenergic cell groups also send projections to the hippocampus from nuclei including the locus coeruleus; however cannabinoids are not directly involved in noradrenergic transmission but may effectively controls GABAergic input through adrenoreceptors (Kathmann et al., 2001). Moreover, activation of adrenoreceptors has been shown to enhance the release of endocannabinoids in the hippocampus (Kim et al., 2002).

Cannabinoids also excite dopamine neurons through activation of the D2 dopamine receptors. These receptors are responsible for evoking fast non-desensitizing inward currents in neurons and might release endocannabinoids through activation of Na^+/Ca^{2+} exchange channels (Cheer et

al., 2004). There is ongoing evidence for the role of the endocannabinoid system on serotonergic neurons (Bambico et al., 2007;Kelai et al., 2006).

1.5.3. Peptides and Opioid receptors and other hormones

Cannabis has been observed to be involved in appetite and feeding behavior, and the endocannabinoid system may functions as signaling molecules involved in endocrine and homeostatic functions (Avraham et al., 2005). AEA and 2-AG, also have potential involvement in appetite and energy metabolism by increasing food intake and appetite (Kirkham and Williams, 2004;Cota et al., 2003). Conversely, SR141716 and AM251 reduce food intake. Also, CB_1 receptor knockout mice have increased energy expenditure (Jbilo et al., 2005). Emerging studies have implicated both the endogenous cannabinoid and opioid systems as playing roles in eating behavior. Presently, cannabinoid and opioid receptor antagonists are promising anti-obesity drugs, since they are targeting homeostatic components of energy balance control (Cota et al., 2006). Leptin and orexins are hormones that that control food intake and body weight and are also targets for endocannabinoid action in the brain (Kirkham and Williams, 2004). Other studies suggest an interaction of endocannabinoids with corticotropin releasing hormone, neuropeptide Y and ghrelin which functions to regulate feeding behaviors (Salamone et al., 2007;Hao et al., 2000;Cani et al., 2004;Tucci et al., 2004).

1. 6. The Cannabinoid receptor and other brain systems

1. 6. 1. Cannabinoids and cognitive system-learning and memory

Considerable numbers of studies have ascertained the idea that chronic (heavy) cannabis use interferes with the processes of learning in humans. These effects are associated with impairments in performance on tasks that measure memory and executive functioning (Ranganathan and D'Souza, 2006;Solowij and Battisti, 2008). Studies in rodents and nonhuman primates have further provided useful insight into deficits in learning and memory processes caused by both natural and synthetic cannabinoids impairment (Hampson and Deadwyler, 1999;Hampson and Deadwyler, 2000;Lichtman and Martin, 1996). These deficits have been seen mainly on short term-working memory, which is dependent on the hippocampus and prefrontal cortex. These observations are in keeping with findings of high densities of cannabinoid receptors together with high levels of endocannabinoids in brain regions such as the hippocampus, prefrontal cortex, amygdala and striatum (Matsuda et al., 1993;Herkenham et al., 1990). Models such as the eight arm radial maze (Lichtman et al., 1995;Lichtman, 2000;Silva de Melo et al., 2005), water maze (Robinson et al., 2003) or DNMS task (Hampson et al., 1999;Hampson and Deadwyler, 2003), are usually used to characterize the role of cannabinoids in spatial learning and memory processes. Some of the most profound findings were shown with cannabinoids such as WIN55, 212-2, Δ^9-THC and HU210 on rodents performing the DNMS task which cause a decrease in performance of DNMS task, increase the number of errors at longer delay intervals, and increase the latency to response and disrupt hippocampal activity at a specific phase in the task (Hampson and Deadwyler, 1998;Deadwyler et al., 1996). Similarly, in other spatial tasks such as the radial arm maze and Morris water maze, cannabinoids at specific doses produced impairment performance together with a decreased accuracy of responding

(Lichtman and Martin, 1996;Hernandez-Tristan et al., 2000;Robinson et al., 2003). These deficits induced by cannabinoids were shown to be associated a with specific decrease in firing rates of hippocampal CA3 and CA1 neurons during the sample phase (encoding phase) but not the nonmatch phase (retrieval phase) of the task (Deadwyler and Hampson, 2008;Hampson et al., 2001).

An interesting finding was that, unlike hippocampal lesions or damage which is irreversible, the deficit in impairment caused by cannabinoid receptor agonist were mainly temporal and reversible (Hampson et al., 1993;Heyser et al., 1993). Also, direct intra-hippocampal administration of CB_1 receptor antagonists, SR141716 (rimonabant) into the dentate gyrus and CA sub-regions of the hippocampus was shown to enhance memory as seen in radial arm maze tasks that lasted over several minutes (Lichtman, 2000;Terranova et al., 1996). Altogether, these findings suggest the activation of cannabinoid receptors present in the hippocampus impair short-term working memory, while inactivation shows opposite effects (Deadwyler and Hampson, 2008;Hampson and Deadwyler, 1999;Davies et al., 2002).

Studies have also demonstrated that cannabinoid agonist interfere with suppression of previously learned responses in spatial learning (Marsicano et al., 2002). Disruption of the cannabinoid signaling in the brain modulates extinction of spatial memory in mice, and elevated endocannabinoid levels seem to attenuate the forgetting process in the tasks that involve extinction of learned behavior (Marsicano et al., 2002). These effects have also been suggested as the basis for which the increase in errors in performance of spatial and short-term memory tasks, and the suppression or forgetting of information already stored (Terranova et al., 1995a). The process of active forgetting of irrelevant information is an important component of behavior, but the mechanisms by which it occur is still unclear (Wilson and Nicoll, 2002). It seems

possible that indirectly potentiating the levels of endocannabinoids by pharmacological inhibition of the metabolic enzymes may affect CB_1 receptor transmission and memory related processes (Marsicano and Lutz, 2006;Suzuki et al., 2004). The cellular mechanisms that underlying memory related changes to cannabinoids are still unclear. Memories are believed to be formed by process involving long term potentiation (LTP) and long term depression (LTD) at glutamatergic synapses. These processes have been shown to alter postsynaptic neuronal responses associated with decreases in neurotransmitters released from presynaptic neurons (Chevaleyre and Castillo, 2003;Chevaleyre et al., 2006;Sullivan, 2000).

Further insight into cannabinoid action came when cannabinoid was shown to induced suppression of inhibitory and excitable neurons in the hippocampus and cerebellum (Wilson and Nicoll, 2001). These effects in the forms of depolarization suppression of inhibitory and excitable neurons (DSI and DSE) could contribute to forms of synaptic plasticity in the hippocampus. DSI and DSE have been associated with neural correlates of learning and memory. Endocannabinoid mediate signaling in a retrograde manner by reducing synaptic transmission in GABA interneurons. The subcellular localization of CB_1 receptor as well as its function has been further showed the role of endocannabinoid in controlling the activity of pyramidal cells in the hippocampal. The modulation of neurotransmitter release and the disruption of pyramidal cell ensemble firing have been suggested to function in encoding information necessary for the formation of memory (Chevaleyre et al., 2006;Deadwyler and Hampson, 2008;Alger, 2002). Cannabinoid agents modulate inhibitory and excitatory in presynaptic and postsynaptic hippocampal neurons and neural circuitry has been shown in animals using various electrophysiological and behavioral models (Hajos et al., 2000;Moore et al., 2010;Rodriguez de et al., 2005;Hampson and Deadwyler, 1998).

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1.6.2. Cannabinoids in cognition, mood and related disorders

Δ^9-Tetrahydrocannabinol and other CB_1 agonists produce a number of cognitive effects in mammals that range from the alteration in perception to abuse potential. Heavy, long-term use of cannabis caused subtle impact on cognition and memory, largely associated with cannabinoid agonist suppression of GABAergic, glutamatergic and cholinergic neurons in cortical areas (Marsicano and Lutz, 2006;Lupica et al., 2004). Cannabinoids appear to disrupt memory and cognition by interfering with the filtering of information that reaches consciousness and interfering with the ability to store relevant information. Animals lacking CB_1 receptors or treated with CB_1 receptor antagonists show altered memory processing, attention and arousal (Marsicano et al., 2002;Marsicano and Lutz, 2006). For now, the mechanism of action leading to altered cognition is largely complex. Pharmacological and genetic studies in animals have indicated that cannabinoid reduce anxiety. This is in keeping with location of the receptors in brain targets responsible for emotion including midbrain septum, hippocampus (Marsicano et al., 2002;Kathuria et al., 2003) and the amygdale, a structure associated with storage and extinction of learning memories (Davis and Whalen, 2001). Likewise, CB_1R-knockout mice are less anxious than wild-type mice, but show improved amygdala and hippocampus-dependent contextual fear acquisition (Kathuria et al., 2003;Valverde et al., 2000;Kathmann et al., 2001).
The effects of brain endocannabinoids on primary reward areas is thought to be mainly due to activation of dopaminergic neurons in the ventral tegmentum area, resulting in release of dopamine in the nucleus accumbens. The endocannabinoid system is widely distributed in areas associated dopaminergic and striatal reward systems (Maldonado et al., 2006;Gardner, 2005).

Endocannabinoids have been shown to increase dopamine activity by interacting with other drugs of abuse including the opioid system (Balerio et al., 2004), ethanol (Ferrer et al.,

2007) and nicotine (Valjent et al., 2002). In particular, studies have shown that cannabinoids increase alcohol sensitivity and increase withdrawal symptoms in animals (Ferrer et al., 2007). These findings are in keeping with reinforcing and addictive properties of cannabinoids. The endocannabinoids act in concert with and complementary to both primary reward and punishment systems to influence aversive behaviors (Giuffrida et al., 1999;Rodriguez de et al., 2005;Marsicano et al., 2002)

Schizophrenia is a chronic, severe, debilitating mental illness characterized by changes in personality. Basic science and clinical studies observed elevation in anandamide and major cannabinoid metabolites in blood and cerebrospinal fluid in schizophrenia patients (De et al., 2003;Hall et al., 2004). CB_1 receptor binding sites were increased in the prefrontal and cingulate cortex, basal ganglia and hippocampus in postmortem brain samples (Marsicano et al., 2002;De et al., 2003;Sundram et al., 2005). Also, CB_1 antagonists reduced negative symptoms in schizophrenics (Biegon and Kerman, 2001). These findings have implicated the endocannabinoid system may serve as a major factor in disease susceptibility and pathogenesis (Giuffrida et al., 1999). However, the use of cannabis has been associated with an increased risk of developing depression and anxiety. CB_1R activation activated serotonergic neurons in the medial prefrontal cortex, which is the major transmitter system involved in mood control, but the mechanism of action remains poorly understood (Gobbi et al., 2005). The modulation of cannabinoid transmission may therefore prove to be useful in the treatment of depression and related mood disorders (Gobbi et al., 2005;Haj-Dahmane and Shen, 2009).

1.7. Electrophysiology and short term memory in rats

1.7.1 Neuronal recordings in the rat hippocampus

In the body, the most excitable tissues are neurons and muscle cells. Like muscles, neurons are excitable and generate action potentials in response to various stimuli. Since there are billions of neurons, it is possible to measure the electrical activities of groups of neurons with distinct firing characteristics. The action potentials or field potentials generated are featured as shapes of waveforms which can then be isolated, sorted and measured (Robbe et al., 2006;Buzsaki, 1986). Implantation of electrode wires by means of stereotaxic instruments in conjunction with precision brain coordinates, allows placement of electrodes in specific brains regions. There is a close relationship between firing characteristics and frequency of extracellular and intracellular action potentials (Buzsaki, 1986;Buzsaki et al., 2002). The population of spikes or waveforms detected can be isolated based upon spatial and temporal firing patterns and further differentiated by size, shape and spatial orientation using certain variable and computational tools (Lee et al., 2006). Also, this method allows the identification of shapes of the waveforms and captures segments of these in real time. In many cases, a plot of each neuronal population as a function of time creates spike trains over the course of an experiment. Action potentials generated by a neuronal population are considered as serving as a code for the information transmission (Buzsaki et al., 2002;Henze and Buzsaki, 2001). In the brain, the neurons in the hippocampus and in particular certain subfields, has a unique spike waveforms, and firing characteristics and recordings made in dentate, CA1, CA3 and subiculum subfields may reflect short-term storage of a stimulus trace (Robbe et al., 2006). The electrophysiological recording technique is similar to that used in studying the firing properties of neurons in rat brain slices.

Multiple microelectrode extracellular recordings can be performed *in vivo*. However, the advantages of using this method in animals are numerous and include simultaneous recordings of neuronal activities in animals performing a behavioral task. This technique offers many other advantages. Recordings can be made continuously with high consistency and neuronal activities can be manipulated using tools such as drugs (Robbe et al., 2006;Buzsaki, 1986). The immense advantages are tempered by limitations inherent to this approach. These limitations include background noise and interference, potential lesioning of brain regions by projected electrode probe(Lee et al., 2006)*.* Neural responses can also be analyzed in terms of firing rates and bursting of neurons. It is known that information in the brain can be represented and predicted through the temporal relationships between spike-trains of many neurons (Deadwyler and Hampson, 2008). The modes of firing exist in the forms of spiking and high frequency bursting, which can occur in phases as a sudden onset of clusters of spiking action potentials emitted at high frequency. The changes in firing rates and frequencies also depend on a combination of different properties such as neuromodulation, signaling inputs and synaptic connections. In addition, the neural signals that occur around particular events of neuronal activity are associated with certain animal behaviors (Eichenbaum et al., 2007;Squire, 1993). These neuronal activities or spiking patterns observed in many areas of brains can be used to gain knowledge about changes in cognitive and certain brain processes underlying memory formation, learning and plasticity (Maguire and Hassabis, 2011;Kirby et al., 2000;Hassabis et al., 2009).

1.7.2. Animal models of short-term memory-The DNMS task

One of the most common models for testing the role of the hippocampus in learning and short term memory is the DNMS task (Otto and Eichenbaum, 1992). DNMS task is an operant conditioning task basically consists of three main phases: a stimuli phase, a delay phase and a

response phase. In the stimuli or sample phase, an animal is presented with a sample which can be a novel object or lever (Heyser et al., 1993;Hampson et al., 1999;Pontecorvo et al., 1996). The sample is followed by a delay phase that requires the subject to hold the information in the brain and later recall it in the response phase. A delay-dependent performance decrement has been observed with increasing separation of the delay response. This type of memory formed, known as working memory, involves forming relationships between different events: sample (encoding) and non-match (recall). In this test, an animal's performance becomes remarkably impaired as the delay is extended. This task have demonstrates that the hippocampus, including CA, dentate gyrus, and subiculum, are critical to hold memory during the encoding and retrieval phases of memory (Monk et al., 2002). These effects have been exploited further with the effects of ibotenate-mediated hippocampal and extra-hippocampal destruction in rats which causes significant decrease performance in rats (Hampson et al., 1999). Consistent findings have shown that performance decreases following increasing length of interval during the delay phase (Deadwyler et al., 1990). Drugs that specifically act on working memory have been shown to have larger effects on long delays than trials with short delays Drugs that do not act on short term working memory will likely show effects that occur independently of delay. (Herremans and Hijzen, 1997) Many studies have brought into light the role of the hippocampus not only for short term memory but for spatial cues and long memory intervals (Squire and Zola-Morgan, 1991). Some investigators have suggested hippocampus and parahippocampal cortical structures are necessary to support memory across delays and maintain a persistent trace for recent stimuli. Therefore, these studies can help in generation of a wealth of information about the memory process (Otto and Eichenbaum, 1992;Eichenbaum, 2000).

1.8 Aims and Objectives

    The primary goal of this study is to determine the effects of anandamide on the neurophysiological responses of hippocampal neurons in anesthetized and neurophysiological responses and behavioral effects on animals performing a DNMS task. Many studies have shown an association between cannabinoids, the endocannabinoid system and the functions of the hippocampus and it role in memory processing. The impairment in effects of Δ^9-THC in memory are well characterized and has been investigated in disease such as Alzheimer's disease which is characterized by dysfunction in neurons present in the cortex and the hippocampus. Cannabinoids has being shown to induce memory impairment in humans and in many animal models of memory (Breivogel et al., 1999) al., 1999; Robinson et al., 2008; Varvel et al., 2005). It is believed that these effects are the result of cannabinoids receptors found in subpopulations of neuron in the hippocampus (Herkenham et al., 1991). Acute exposure to the natural cannabinoid Δ^9-THC and the synthetic compound WIN 55,212-2 has been shown to alter performance of a spatial memory task through the modulation of neuronal firing of hippocampal pyramidal neurons. Also, many studies have demonstrated that endocannabinoids, such as anandamide, acts as partial agonists at the cannabinoid receptor level, are present at low concentration in the hippocampus and have a short metabolic half-life. However, determining the involvement of anandamide on the firing rates and burst characteristics of hippocampal neurons is still elusive In this study, the pharmacological actions of anandamide was looked act in relation to other cannabinoid receptor agonists like Δ^9-THC and WIN55212-2 (Fride and Mechoulam, 1993), which have been shown to impair memory in rats (Mallet and Beninger, 1998). In the brain, anandamide biosynthesis and degradation are highly regulated by enzymatic in vivo. FAAH is the predominant enzyme responsible for the catabolism of endocannabinoid,

anandamide. In addition, the termination of the activity of anandamide by cells has been shown to take place through one of many still uncharacterized transporters mechanisms. Therefore, the inhibition of FAAH and the inhibition of anandamide uptake specifically enhance anandamide levels in the brain in multiple regions of the brain (Kathuria et al., 2003;Gobbi et al., 2005;Cravatt et al., 1996)

This study assessed the neurophysiological changes that occur in hippocampal pyramidal neuron spike train activity when anandamide levels are enhanced with R-Methanandamide, the stable analog of anandamide, VDM11, an anandamide membrane transporter inhibitor, and URB597, a FAAH inhibitor. Extracellular action potentials were recorded using multi-electrodes in the CA1 and CA3 subfields of the hippocampus in anesthetized animals and in animals performing a DNMS task. In this study, pyramidal neurons from hippocampal units were tracked and recorded by multi-electrode arrays in rats. Different groups of animals were assessed under these two conditions; under anesthetized condition and another during performance of DNMS. The approach taken was that, same units of neurons were tracked and analyzed throughout drug administration. Modulating the endocannabinoid system induced alterations in basal firing rates, number of bursts and burst duration of hippocampal neurons which might be responsible for producing the observed deficits in spatial learning and memory in rodents. Experiments in this study were carried out on Long-Evans rats, to investigate the role of endocannabinoids on ensembles of hippocampal neurons under anesthetized condition and on task-relevant ensembles of hippocampal neurons during performance of a DNMS in rats. Acutely, enhancing endocannabinoid activity in the brain might alter ensembles of hippocampal neurons under anesthetized condition and alter short-term memory processing by modulating the encoding of task-relevant information of hippocampal pyramidal neurons. Understanding these effects might

be impotent as it might provide might further evidence of the role of cannabinoid, the endocannabinoid system in synaptic transmission in vivo and the relevant neuro-physiological activities that occur in learning, memory and related cognitive processes. This study is might be important because, enhancing anandamide levels by blocker its reuptake and inhibiting FAAH enzymes could provide insight of the role of anandamide and the FAAH inhibitors as mediators for treatment of disease that as associated with cannabinoid neuropathology

The main objective of these studies was to further our knowledge on the effects of endogenous cannabinoids on various aspects of learning and memory and hippocampal neurophysiology, given its importance in spatial learning and memory. In order to do this, a series of experiments were carried out to:

(i)Assess the effects of R-Methanandamide, VDM11, and URB597 on baseline firing of hippocampal neurons in animals under anesthetized conditions.

(ii)Assess how the R-Methanandamide, VDM11, and URB597 alter spontaneous 'burst' activity of hippocampal neurons in animals under anesthetized conditions and gain a better insight as to how these compounds alter neuronal responses of hippocampal neurons.

(ii)Assess how the R-Methanandamide, VDM11, and URB597 affect baseline firing and on spontaneous 'burst' activity of hippocampal neurons in animals under anesthetized conditions and examine whether these effects on are mediated by cannabinoid receptor. These effects will be determined by the use of the cannabinoid receptor antagonist, rimonabant. These are important questions that need to be addressed as previous studies have demonstrated that short term-spatial memory is sensitive to endocannabinoid-induced deficits.

(iv) Assess how acute dose of the R-Methanandamide, VDM11, and URB597 affect short-term memory in animals during performance DNMS task.

(vi)Examine the *R*-Methanandamide, VDM11, and URB597 effects on locomotion during performance of the DNMS task.

(vii)Examine *R*-Methanandamide, VDM11, and URB597 effects on working/short-term memory processes and hippocampal neurophysiology during sample (encoding) and non-match (retrieval) phases of the DNMS task.

(viii)Assess t*h*e effects of *R*-Methanandamide, VDM11, and URB597 on baseline firing of hippocampal neurons in animals during performance of DNMS task.

(ix)Assess the effects of *R*-Methanandamide, VDM11, and URB597 on spontaneous 'bur*s*t' activity of hippocampal neurons in animals during performance DNMS task.

CHAPTER 2: EFFECTS OF ANANDAMIDE ON NEURONAL FIRING CHARACTERISTICS IN ANAESTHETIZED ANIMALS

2.1 Rationale

Despite its well known cognitive effects, the recreational cannabis use is very common and widespread in most part of the western world. Among many cognitive effects of acute and chronic cannabis use in humans, impairment of cognition and attention is the most commonly reported (Elphick and Egertova, 2001;Ranganathan and D'Souza, 2006;Battisti et al., 2010). In addition, many studies in humans have shown Δ^9-THC, the active ingredient of *cannabis* causes substantial reduction in short term memory processing of encoding and recall of information (Robinson et al., 2008;Hampson and Deadwyler, 1999). In the hippocampus, the endocannabinoid systems is believed to play a role in processing of short term memory function in operant conditioning tasks such as DNMS through the CB_1 mediated mechanism (Hampson et al., 2003;Deadwyler and Hampson, 2008). These finding have been replicated in laboratory studies in animals where, WIN,55212-2, a potent agonist, acts on the pyramidal neuron population by changing the basal firing rates (Nemeth et al., 2008) However, the mechanisms that account for the change in firing rate of neurons is unclear. Anandamide is one of the endogenous ligands that bind to CB_1 receptors, but there are limited studies about how potentiating the level of endocannabinoids affects pyramidal cell neurophysiology. In rat hippocampus, firing activity was correlated with the response of subjects during working memory tasks (Deadwyler et al., 2007).

Several studies have shown changes in neuronal activity when studied in awake animals during treatment with cannabinoids. First, it is now well recognized that the hippocampus possesses cannabinoid receptors that are important for expression of short-term memory

(Breivogel et al., 1999;Hampson and Deadwyler, 2000). Second, rats have impairments of short-term memory associated with reduced firing rates of hippocampal neurons (Hampson and Deadwyler, 2003). Third, hippocampal pyramidal cells spontaneously evoke action potentials by endocannabinoids (Deadwyler and Hampson, 2008). Enhanced levels anandamide, in particular in the hippocampus, can be obtained by using inhibitors that blocked the transporter and metabolic enzymes. One target by which to enhance anandamide levels in the brain is by inhibition of fatty acid amide hydrolase (FAAH) and inhibition of anandamide transport (Piomelli et al., 2006). Studying the role of anandamide on the firing rates and burst characteristics of hippocampal principal neurons is interesting as it is still not fully understood. Therefore, this study examined the effects of drugs on the pyramidal neurons by recoding and analyzing neurophysiological changes of populations of pyramidal neurons. The acute effects of *R*-methanandamide, the stable analog of anandamide, URB597, a FAAH inhibitor, and VDM11, an anandamide membrane transport inhibitor, were investigated. Multi-unit electrodes implanted in the CA1 and CA3 subfields of the hippocampus were utilized to measure baseline firing rates of pyramidal neurons followed by drug treatment. Bursting or spontaneous spike train firing was also measured. Overall firing rates, burst duration, number of burst, spikes in burst and interspike intervals were recorded and analyzed to reveal substantial changes in firing characteristics.

2.2 Objectives

(i) To determine the effects of the endocannabinoid agonist, *R*-methanandamide, on firing rates, on bursting, on spike train of hippocampal pyramidal cells located in CA3 and CA1 hippocampal sub-fields;

(ii) To examine how the cannabinoid agonist, *R*-methanandamide, affect synchronous firing hippocampal pyramidal cells pairs located in CA1 – CA1; CA3 – CA3 and CA3 - CA1 hippocampal sub-fields;

(iii) To investigate whether the of cannabinoid agonist *R*-methanandamide on firing rates, bursting, spiking of principal cells are CB_1 receptor-mediated by administering the CB1 receptor antagonist, rimonabant.

(iv) To determine the effects of the endocannabinoid metabolic inhibitors, VDM11, on firing, on bursting, on spiking of hippocampal pyramidal cells located in CA3 and CA1 hippocampal sub-fields;

(v) To examine how the endocannabinoid metabolic inhibitor,VDM11, affect synchronous firing of hippocampal pyramidal cells pairs located in CA1 – CA1; CA3 – CA3 and CA3 - CA1 hippocampal sub-fields;

(vi) To investigate whether the effects of endocannabinoid metabolic inhibitor, VDM11, on firing rates, bursting, spiking of hippocampal pyramidal cells are CB_1 receptor-mediated by pretreatment with the antagonist, rimonabant.

(vii) To determine the effects of endocannabinoid metabolic inhibitors, URB597, on baseline firing, on bursting, on spiking of hippocampal pyramidal cells located in CA3 and CA1 hippocampal sub-fields;

(viii) To examine how the endocannabinoid metabolic inhibitors, URB597, affect synchronous firing of hippocampal pyramidal cells pairs located in CA1 – CA1; CA3 – CA3 and CA3 - CA1 hippocampal sub-fields;

(ix) To investigate whether these effects endocannabinoid metabolic inhibitor, URB597, on firing rates, bursting, spiking of principal cell are CB_1 receptor-mediated by pretreatment with the antagonist, rimonabant.

2.3. Methods

2.3.1. Subjects and drug treatment and schedule:

Subjects: (N=6) Male, Long-Evans rats (Harlan, Indianapolis, Indiana, USA), approximately 120-180 days old and weighing 280-350 grams were used as subjects. Animals were housed individually in plastic cages, in approved animal facilities on a 12h: 12h day/night cycle. *Ad libitum* food was always available, but prior to assessing behavior, animals were water regulated to eighty- three percent of their *ad libitum* body weight.

Drug preparation and administration**:** *R*-Methanandamide, URB597, and VDM11 were from Cayman Chemical, Ann Arbor, Michigan, U.S.A. Stock solutions of cannabinoid agents were freshly prepared each day in a suspension of: 95% saline, 5% cremophor®(sigma, Steinheim, Germany). Solutions containing alcohol were stirred rapidly and placed under a stream of nitrogen gas to evaporate the alcohol. Control solutions consisted of saline and cremophor® with no cannabinoid agents.

Drug treatment and Schedule: A schematic of the experimental protocol is presented in table 2.1 Animals were injected with the drug suspension intraperitoneally. In all, the antagonists were administered fifteen minutes before cannabinoid agonists.

2.3.2. Multiunit Recording Implantation of microelectrode arrays

Implantation of Microelectrode Arrays: The basic concept of electrode implantation was adopted from Hampson and Deadwyler lab (Hampson et al., 1993) at the department of Physiology and Pharmacology at Wake Forest School of Medicine. Each animal was anesthetized with ketamine hydrochloride (10 mg/kg) and xylazine hydrochloride (10 mg/kg). Once anesthetized, holes were drilled in the exposed skull to hold support screws. Sixteen stainless steel, insulated, electrodes were purchased as an array from a commercial source (Neurolinc, New York, New York, U.S.A). The arrays were built according to the following specifications: two rows of 40 micron diameter wires, spaced 200 microns on center and 800 microns between the rows. Arrangements were made with asymmetrical lengths such that the longer CA3 electrode tip was automatically placed ventral to the CA1 sub-field electrode tips (see Figure 1.5). Positioning of the center of the array was performed using a stereotaxic instrument were 3.4 mm posterior to bregma and 2.7 (lateral to midline) and a depth of 3.2 – 3.4 mm. (Deadwyler and Hampson, 1999;Paxinos et al., 1985).

The longitudinal axis of the array was set at 30 degrees to the midline. Sealing of the exposed skull and securing the arrays was done with dental cement. The animal's breathing was monitored continuously and the body temperature maintained at 37°C with the use of a heat pad. Once implanted, animals were allowed a minimum of one week postsurgical recovery period. The scalp wound was treated periodically with Neosporin antibiotic and systemic injection of penicillin G (300 000 U, intramuscular) to prevent infection. Intramuscularly administered buprenorphine (0.01–0.05 mg/kg) was used for analgesia immediately following and six to nine hours after surgery. The animals were evaluated daily using a species-specific quantitative behavioral score

Figure 2.1. Electrophysiological recording in the hippocampus
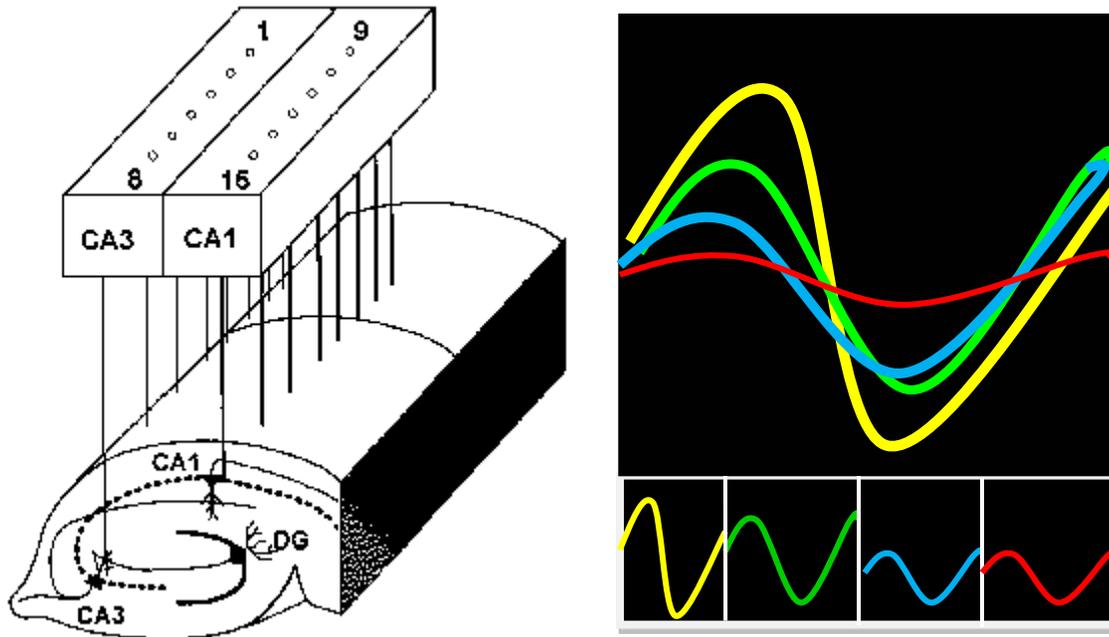
Right panel shows a 16 micro-wire electrode arrays positioned in CA3 and CA1 sub-fields for *in vivo* hippocampal recording (based on configuration devised by Hampson and Deadwyler (1993)). Left panel shows waveforms generated by using a multi-neuron acquisition processor (MAP) Waveforms are discriminated in real time on one electrode based on differences in size and shape. Hippocampal principal cells having a mean firing rate of 0.5 - 6Hz were pre-selected for recording.

During each recording session, rats were connected to a flexible recording cable, which allows unrestrained movement within the test apparatus. The head stage of each recording cable contained miniature unity-gain field effect transistors. After stabilization of the electrode array, selected principal cells with firing rates of 0.5–6 Hz were isolated and discriminated with a Multiunit Acquisition Processor (MAP) (Plexon Inc., Dallas, Texas, U.S.A). The parameters for isolation and discrimination of single unit activities were determined, recorded and saved from one session to another, using the MAP software package. The activities of these preselected CA3-CA1 principal cells were tracked and recorded following treatments. Waveform parameters were recalled on successive days and modified as necessary to discriminate unsorted new neurons or to identify previously recorded neurons.

Table 2.1. Drug treatment and Schedule order of drug administration and electrophysiological recording in anesthetized animals

15 min	20 min	15 min	20 min
Vehicle (Cremophor)	recording	*R*-methanandamide (3mg/kg)	recording
Vehicle (Cremophor)	recording	VDM11 (3mg/kg)	recording
Vehicle (Cremophor)	recording	URB597 (1mg/kg)	recording

All experiments consisted of 'tracking' hippocampal principal cell activities (section 2.32) 5.multi-neuronal recording technique) across 20 min recording sessions following respective treatments. The order of treatments were as follows: Vehicle (Cremophor); *R*-methanandamide (3mg/kg); Vehicle (Cremophor); VDM11 (3mg/kg); Vehicle (Cremophor) URB597 (1mg/kg) respectively. All treatments were administered approximately 15 minutes prior to the start of each recording session. A two day abstinence period was allowed between experiments.

Table1 represents the general sequence of the order of drug administration for all aforementioned experiments.

2.3.3 Recording parameters: Animal electrophysiology

Individual principal cell firing characteristics known as spike trains were analyzed using Neuroexplorer software (Nex Technologies, Massachusetts, U.S.A). The following parameters were calculated for each animal and for each selected cell throughout treatments: the average firing rate (FR, Hz), the average number and duration of bursts, the average number of spikes and inter-spike intervals (ISI). Furthermore, 'bursts' of the spikes were identified by: (1) calculating mean FR and ISI for all spikes, (2) identifying sequences of three or more consecutive spikes with ISIs, in which all ISIs were less than one-half the mean ISI, (3) computing 'Surprise' (S) such that $S=-\log10(_P)$ 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. Bursts with S-values >10 were used to assess the mean burst duration, mean number of spikes in a burst, mean firing frequency and mean ISI within bursts. In addition, cross-correlograms between selected CA1 - CA1, CA3 - CA3 and CA1 - CA3 cell pairs were performed for all animals. Each cross-correlogram represents the average firing frequency (bin size = 5 ms) from a single cell referenced to the firing of another cell through a window of ± 0.15 seconds. The peak z-scores were calculated for each cross-correlogram as follows: (1) The peak value (that is the histogram maximum), (2) the mean (M) and standard deviation (S) from the background bin values are calculated. Peak z-score is equal to [(peak firing rate – mean of baseline firing rate) ÷ Standard deviation (S.D.) of baseline firing rate]. The background parameter was calculated from bins outside peak (i.e. bins that are less than peak value divided by 2 away from the bin with the histogram maximum). All peak z scores were averaged across the selected CA1 - CA1; CA3 - CA3; and CA1 - CA3 cell pairs.

2.3.4. Anesthetized animal study set up

Anesthesia was induced with 5% vaporized isoflurane (Webster Veterinary Sterling, Massachusetts, USA) in oxygen (v/v) and maintained using 1.5% - 3% isoflurane in oxygen (v/v) throughout the recording period. The procedure was performed using an anesthesia machine (Bickford Inc, Wale Center, NY,U.S.A) with set-up that ensured minimal animal handling, ease of anesthetic control and rapid recovery times and was approved by Animal Care and Use Committee (ACUC) at Wake Forest University School of Medicine.

2.3.5. Data analysis-Data were analyzed using a Student's t-tests (paired) to compare the between drug treatment and vehicle across all electrophysiology parameters described above. The statistical significance level was set to $P<0.05$. All data were analyzed using the computer-based statistics package Graphpad Prism version 4.01 for Windows (Graphpad software, San Diego, California, USA).

2.4 Results: Effects of *R*-methanandamide on firing characteristics of hippocampal pyramidal cells.

Neural recordings were performed to determine whether endocannabinoids enhancement might activate cannabinoid receptor and alter neurophysiological change in neurons present in of the hippocampus in rats under anesthetized condition, was based on studies showing *R*-methanandamide produced AEA like effects in rats, *in-vivo*. Analysis was carried out on 40 CA3 and CA1 pyramidal cells following vehicle and *R*-methanandamide (1mg/kg) and 24 CA3 and CA1 cells following vehicle and *R*-methanandamide (1.0 mg/kg) after rimonabant (3.0 mg/kg) pretreatment. Animals were anaesthetized with and the drug injected intraperitoneally. Two representative example of firing rate of CA3 and CA1 pyramidal cells following vehicle and *R*-methanandamide treatment are presented in Fig. 2.41a, c and following vehicle, *R*-methanandamide after rimonabant pretreatment fig 2.41 b, d. For *R*-methanandamide at a dose of 3.0 mg/kg, there was no significant change in the firing frequency (t = 1.72, p > 0.05, Figure. 2.4.1e). There is no effect on mean firing rates on pretreatment with rimonabant (t = 0.11, p > 0.05, Figure. 2.4.1f). The main processes by which neurons process information is through action potentials, which can be measured in the form of bursts. To determine the effect of this compound on neuronal processing, the overall burst characteristics and spike activity of neuronal pyramidal neurons was further examined. *R*-methanandamide at a dose of 3.0 mg/kg produced no significant change in average number of bursts (t = 0.11, p > 0.05, Figure. 2.4.2a); burst duration (t = 0.71, p> 0.05, Figure. 2.4.2c), spike per burst (t = 0.37, p > 0.05, Figure. 2.4.3a) or inter-spike interval (ISI) within bursts (t = 0.87, p > 0.05, Figure. 2.4.3 c)
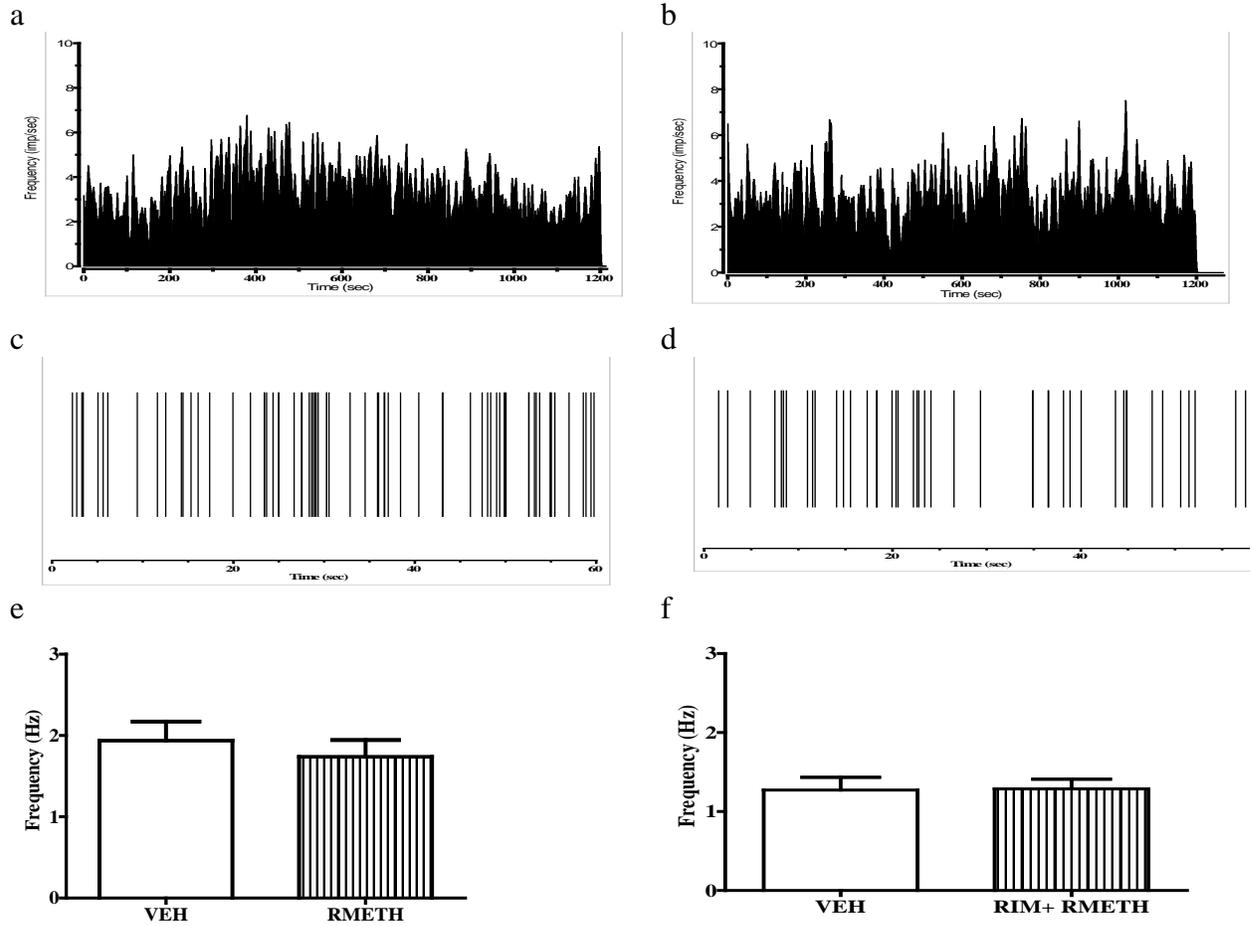
Figure 2.4.1. Effects of *R*-methanandamide on firing rate of hippocampal pyramidal cells

Representative firing rate of CA3 and CA1 pyramidal cells following vehicle and *R*-methanandamide treatment (a), and following vehicle, *R*-methanandamide after rimonabant pretreatment (b). Representative raster plots, each lasting sixty seconds, obtained from the same cell represented (c, d). Means ± S.E.M. of firing frequency following vehicle or *R*-methanandamide (e) and following vehicle, *R*-methanandamide after Rimonabant pretreatment (f). Analysis was carried out on 40 CA3 and CA1 pyramidal cells following vehicle and *R*-methanandamide (1mg/kg) and 24 CA3 and CA1 cells following vehicle and *R*-methanandamide (1.0 mg/kg) after rimonabant (3.0 mg/kg) pretreatment.

(a)

(b)

(c)

(d)
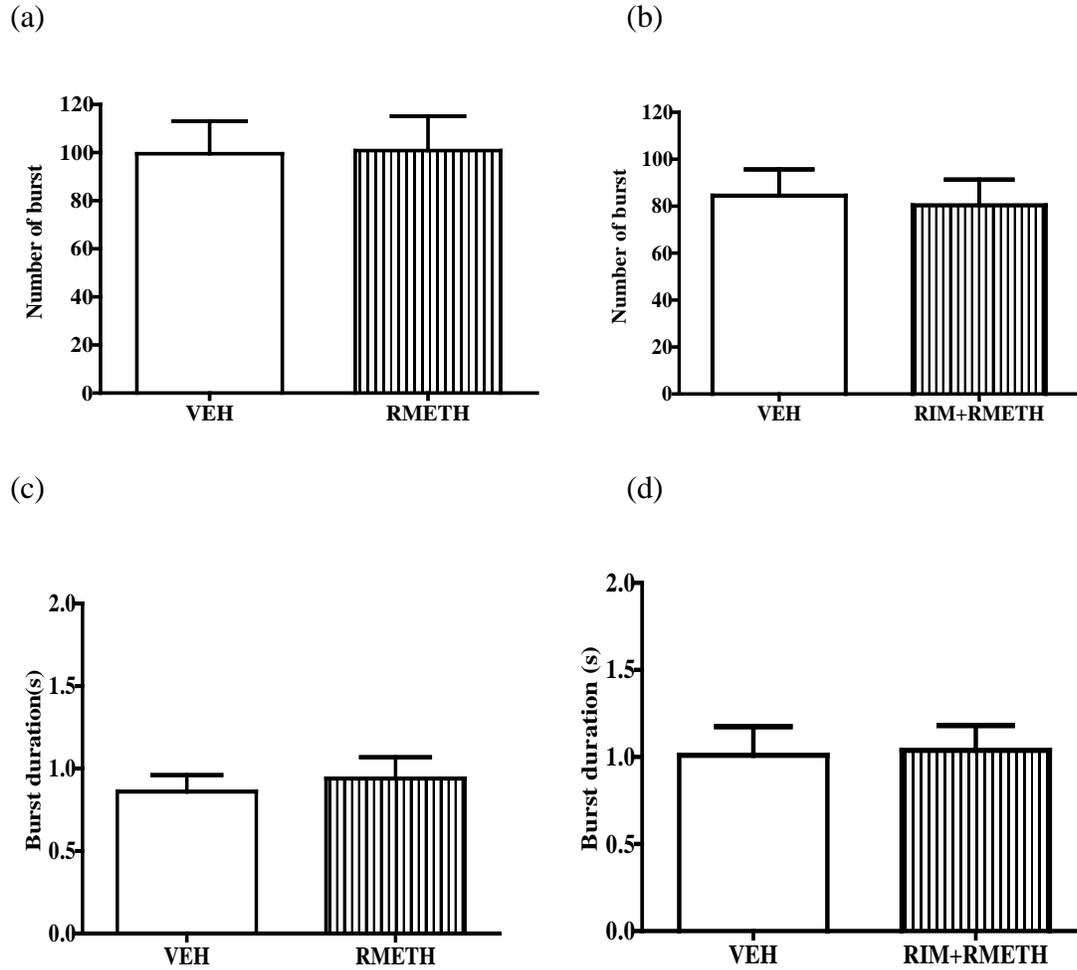
Figure 2.4.2. Effects of *R*-methanandamide on burst characteristics of hippocampal pyramidal cells

Means ± S.E.M. of hippocampal pyramidal cells number of burst and burst duration following vehicle and *R*-methanandamide (a, c) and following vehicle and *R*-methanandamide after rimonabant pretreatment (b, d). Analysis was carried out on 40 CA3 and CA1 pyramidal cells following vehicle and *R*-methanandamide (1mg/kg) and 24 CA3 and CA1 cells following vehicle and *R*-methanandamide (1.0 mg/kg) after rimonabant (3.0 mg/kg) pretreatment.

Figure 2.4.3. Effects of *R*-methanandamide on spike of hippocampal pyramidal cells.

Means ± S.E.M. of hippocampal pyramidal cells spikes per burst and inter-spike interval in bursts following vehicle and *R*-methanandamide (a, c) and following vehicle and *R*-methanandamide after rimonabant pretreatment (b, d) Analysis was carried out on 40 CA3 and CA1 pyramidal cells following vehicle and *R*-methanandamide (1mg/kg) and 24 CA3 and CA1 cells following vehicle and *R*-methanandamide (1.0 mg/kg) after rimonabant (3.0 mg/kg) pretreatment.
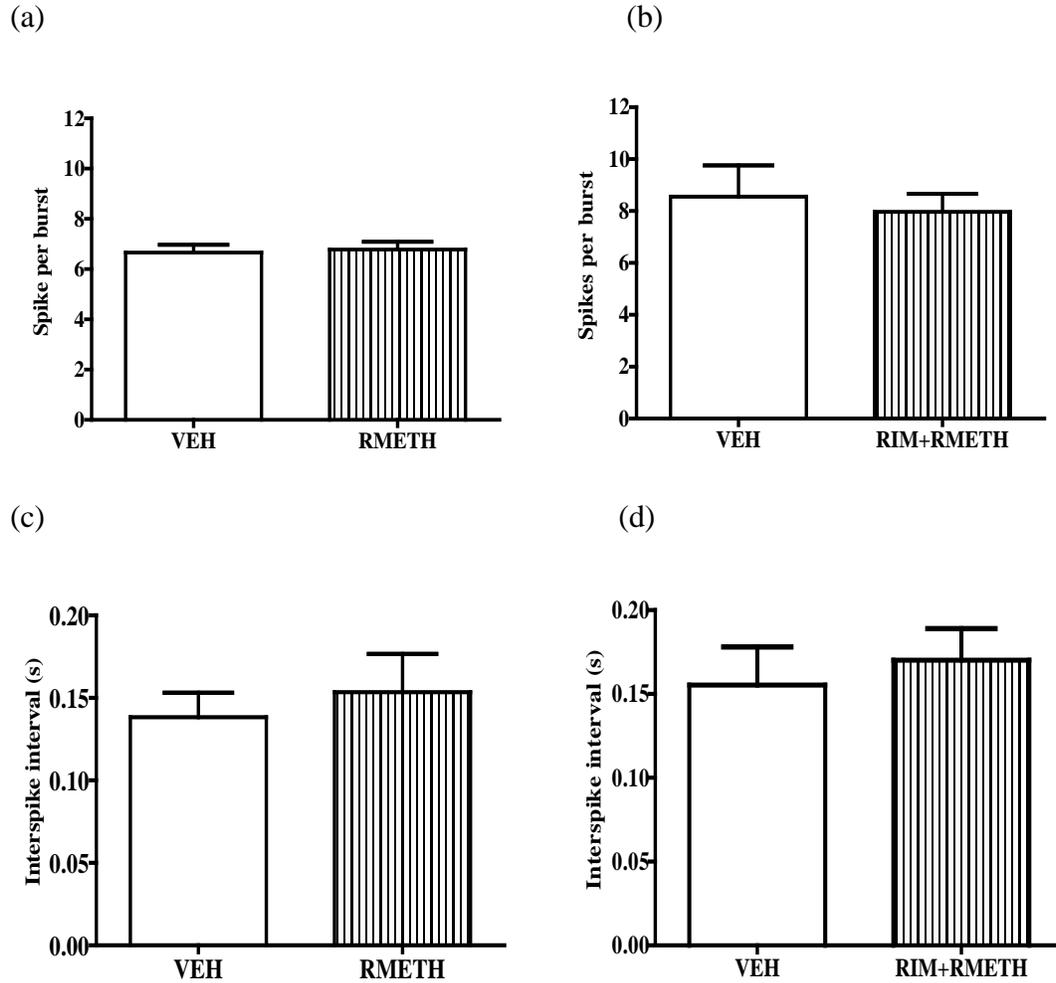
Since the activities of cannabinoids agents such as Δ^9-THC can be blocked by the CB1 receptor antagonist, rimonabant was administrated prior to administration of *R*-Methanandamide. Administration of rimonabant prior to administration of the drug did not produce any significant change in average number of bursts (t = 0.56 p > 0.05, Figure. 2.4.2b); burst duration (t = 0.76, p> 0.05, Figure. 2.4.2d), spike per burst (t = 0.38, p > 0.05, Figure. 2.4.3b) or inter-spike interval (ISI) within bursts (t = 0.62, p > 0.05, Figure. 2.4.4d) Since this compound did not produce any significant change in neuronal firing, we looked at the effect of neuronal across population of neurons. One of the main mechanisms by which neurons shared afferents input is through the process of short-term synchronization. Basically, this mechanism involves action potentials between pairs of cell that fires in phases. To assess the effect of *R*-methanandamide on synchrony, cross-correlogram analysis performed on eight CA1-CA1, four CA3-CA3, and six CA3- CA1 hippocampal pyramidal cell pairs. These observations are illustrated in Figure 2.4.4 that shows representative cross-correlograms for selected hippocampal pyramidal cells pairs between CA1 – CA1 (Figure. 2.4.4a); CA3 – CA3 (Figure. 2.4.4b) and CA3 – CA1 (Figure. 2.4.4c) following vehicle and *R*-methanandamide treatments. Results revealed that a 3.0 mg/kg *R*-methanandamide dose was not able to significantly reduce the average peak z-scores (CA1 - CA1: t=1.49, p> 0.05, Figure 2.4.4d, CA3 - CA3: t = 0.24, p > 0.05, Figure. 3.4.4b and CA3 - CA1: t = 1.54, p > 0.05, Figure 2.4.4f.) in comparison to vehicle treatment.
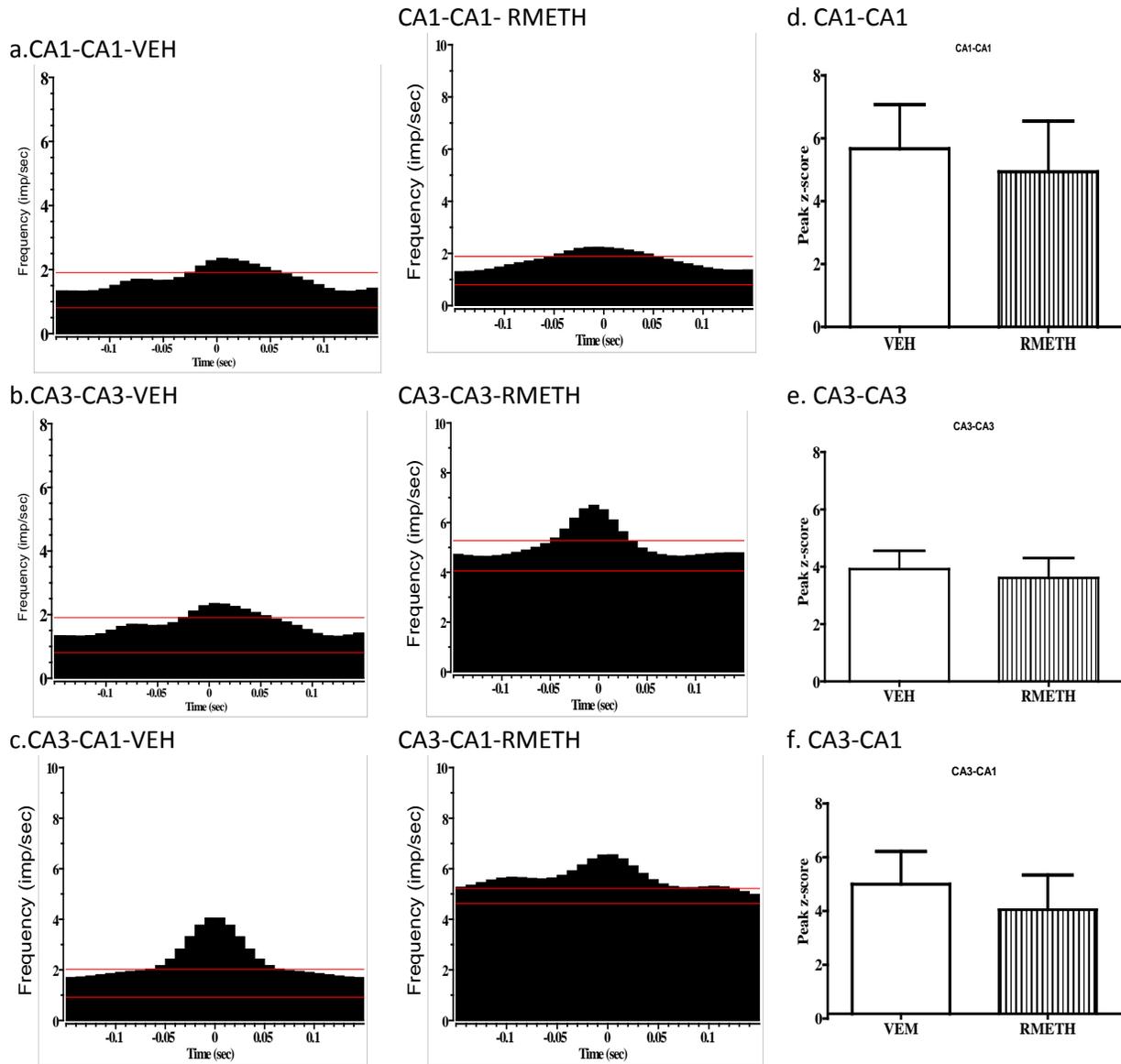
Figure 2.4.4. Effects of *R*-methanandamide on synchrony of hippocampal pyramidal cells pairs.

Representative cross-correlograms performed on single cell pairs between CA1 – CA1 (a); CA3 – CA3 (b) and CA3 – CA1 (c) sub-fields, following vehicle and *R*-methanandamide.

Note: Bin size = 3ms; smoothed to 4 bins. The horizontal red lines each cross-correlogram represent the 95% confidence limits.

Mean± S.E.M. of peak z-scores from CA1 – CA1 (d); CA3 – CA3 (e) and CA3 –CA1 (f) following vehicle and R-methanandamide (3.0 mg/kg)

2.5. Result: Effects of VDM11 on firing characteristics of hippocampal pyramidal cells

Experiments were performed to assess the effects of VDM11 on firing characteristics of hippocampal pyramidal cells. VDM11, a potent transporter inhibitor, increases AEA levels by inhibiting AEA reuptake. Analysis was carried out on 32 and 34 pyramidal neurons were tracked following vehicle and VDM11 (3.0 mg/kg) and following vehicle, VDM11 (3.0 mg/kg) after rimonabant (3.0 mg/kg) pretreatments. Representative firing rates of CA3 and CA1 pyramidal cells following vehicle and VDM11 and following vehicle and VDM11 after rimonabant pretreatment are presented in Fig. 2.5.1a, c and fig 2.5.1b, d respectively. VDM11 at 3.0 mg/kg dose produced significant increase in the firing frequency from baseline ($t = 2.28$, $p < 0.05$, Figure 2.5.1e). There appears to be an enhancement in effect in mean firing rates with rimonabant pretreatment (Figure. 2.5.1f). In addition, bursting and spike train analysis was carried out to determine the effect of this compound on neuronal processing of neuronal pyramidal neurons. VDM11 at a dose of 3.0 mg/kg dose produced no change in average number of bursts ($t = 1.93$, $p < 0.05$, Figure. 2.5.2a); produce significant change in burst duration ($t = 1.87$, $p > 0.05$, Figure. 2.5.2b), significant increase in spikes per burst ($t = 2.37.3a$, $p < 0.05$, Figure 2.5.3a) while having no effect in the inter-spike interval ISI ($t = 0.52$, $p > 0.05$, Figure 2.5.c). VDM11 at a dose of 3.0 mg/kg dose produced no change in average number of bursts ($t = 1.72$, $p < 0.05$, Figure. 2.5.2b); burst duration ($t = 0.59$, $p > 0.05$, Figure. 2.5.2d), no increase in spikes per burst ($t = 1.50$, $p < 0.05$, Figure 2.5.3b) and no effect in the inter-spike interval ISI ($t = 1.52$, $p > 0.05$, Figure 2.5.3d) after rimonabant pretreatment.
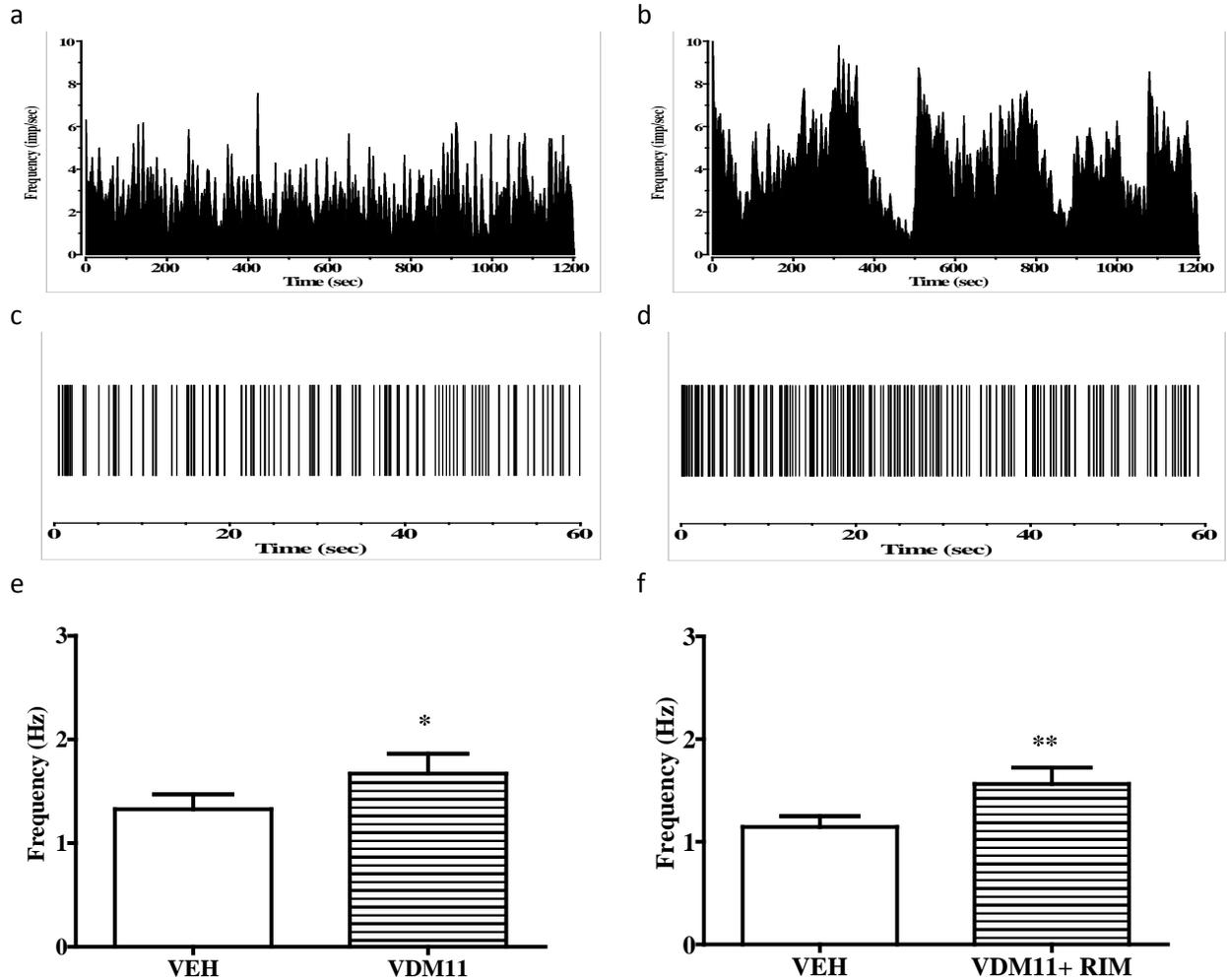
Figure 2.5.1. Effects of VDM11 effects on firing rate of hippocampal pyramidal cells

Representative firing rate of CA3 and CA1 principal cells following vehicle and VDM11 treatment (a, b) and following vehicle and VDM11 after rimonabant pretreatment(c, d). Representative raster plots, each lasting sixty seconds, obtained from the same cell represented (c, d).Means ± S.E.M. of cells firing frequency following vehicle and VDM11 (e) and following vehicle and VDM11 after rimonabant pretreatment (f). Analysis was carried out on 32 and 34 pyramidal neurons were tracked following vehicle and VDM11 (3.0 mg/kg) and following vehicle, VDM11 (3.0 mg/kg) after rimonabant (3.0 mg/kg) pretreatments
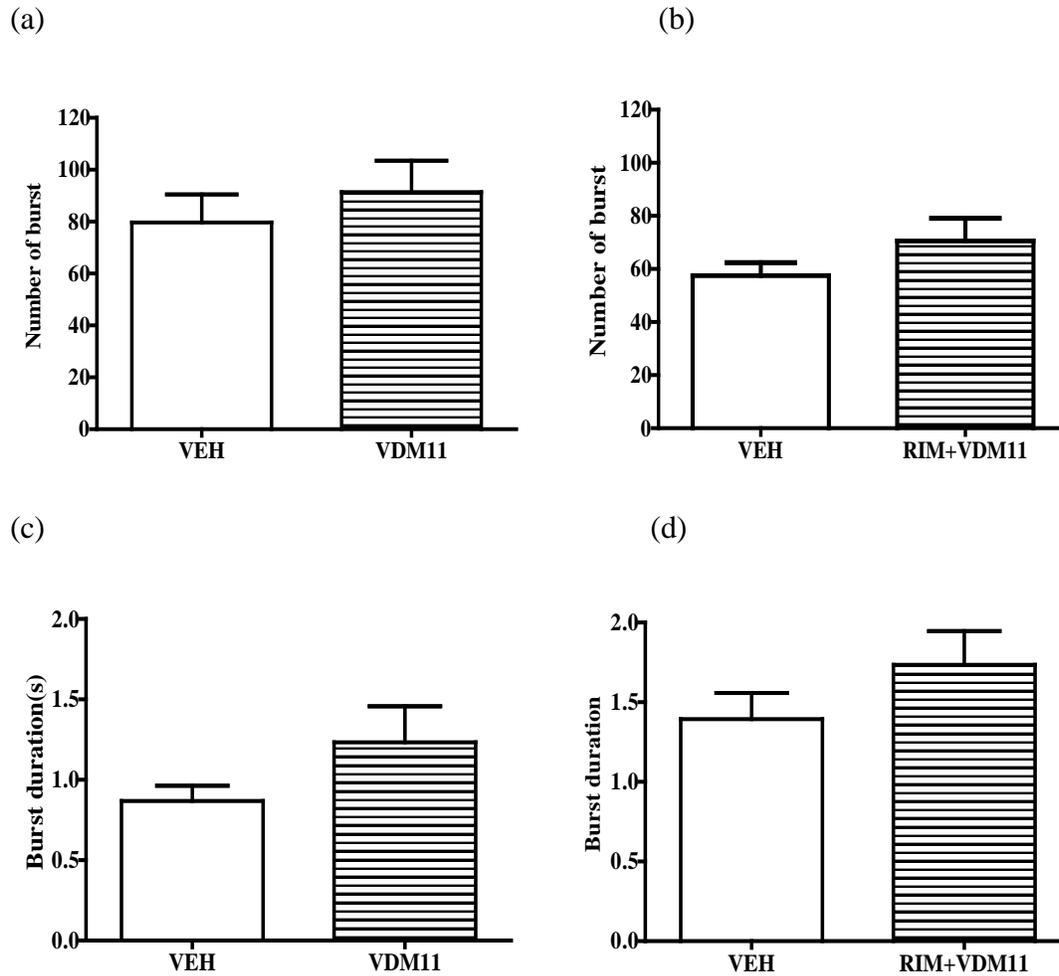
Figure 2.5.2 Effects of VDM11 on burst characteristics of hippocampal pyramidal cells

Means ± S.E.M. of hippocampal pyramidal cells number of burst and, burst duration respectively, following vehicle and VDM11 (a, c) and following vehicle and VDM11 after rimonabant pretreatment (b, d).Analysis was carried out on 32 CA3 and CA1 principal cells following vehicle and VDM11 (3.0 mg/kg) and 34 CA3 and CA1 principal cells following vehicle, VDM11 (3.0 mg/kg) after rimonabant (3.0 mg/kg) pretreatment.

(a)   (b)

(c)   (d)
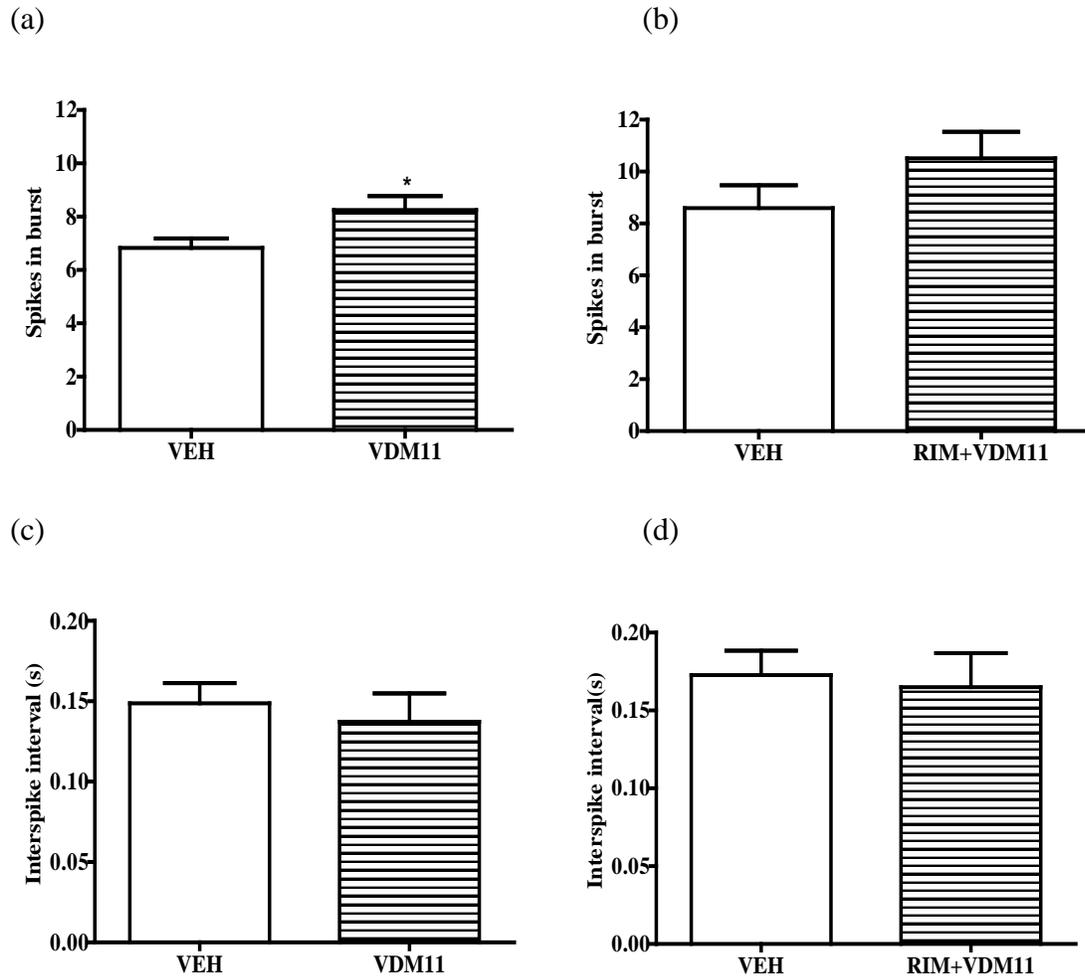
Figure 2.5.3. Effects of VDM11 on spike of hippocampal pyramidal cells

Means ± S.E.M. of hippocampal pyramidal cells of spikes per burst and inter-spike interval respectively, following vehicle and VDM11, and following vehicle (a, c) and VDM11 and rimonabant after pretreatment (b, d). Analysis was carried out on 32 CA3 and CA1 principal cells following vehicle and VDM11 (1mg/kg) and 34 CA3 and CA1 principal cells following vehicle, VDM11 (3.0 mg/kg) after rimonabant (3.0 mg/kg) pretreatment ($*p < 0.05$).

In addition, cross-correlogram analysis performed on four CA1-CA1, four CA3-CA3, and four CA3- CA1 cell pairs. These observations are further illustrated in Figure 2.5.4 that shows representative cross-correlograms for selected hippocampal pyramidal cells pairs between CA1 – CA1 (Figure 2.5.4a) CA3 – CA3 (Figure 2.5.4b) and CA3 – CA1 (Figure 2.5.4c).Results revealed that a 3 mg/kg VDM1 dose was not able to significantly reduce the average peak z-scores (CA1 - CA1: t=0.23, $p > 0.05$, Figure 2.5.3d, CA3 - CA3: t = 1.71, $p > 0.05$, Figure 3.5.3e; and CA3 - CA1: t = 0.20, $p > 0.05$, Figure 2.5.3f in comparison to vehicle treatment.
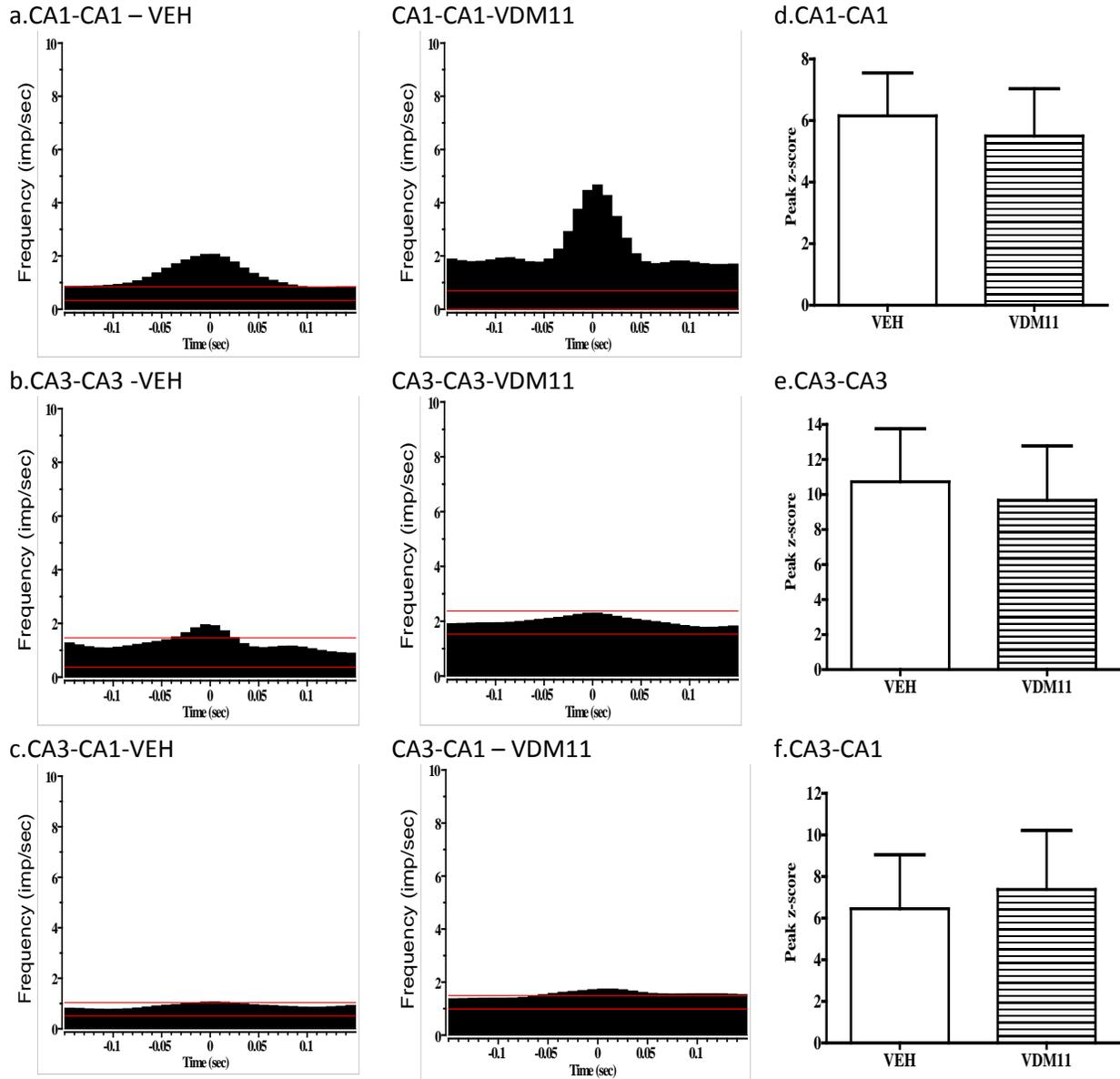
Figure 2.5.4. Effects of VDM11 on synchrony of hippocampal pyramidal cells

Representative cross-correlograms on selected cell pairs between CA3 – CA1 (a); CA3 – CA3 (b) and CA1 – CA1 (c) sub-fields, following vehicle and VDM11.Note: Bin size = 3ms; smoothed to 4 bins. The horizontal red lines in each cross-correlogram represent the 95% confidence limits. Mean ± S.E.M. of peak z-scores calculated from CA1 – CA1 (d); CA3 – CA3 (e) and CA3 –CA1 (f) following vehicle and VDM11 (3.0 mg/kg) treatments.

2.6 Result: Effects of URB597 on firing characteristics of hippocampal pyramidal cells

The effects of URB597 were assessed on firing characteristics of hippocampal pyramidal cells. URB597, a potent FAAH enzyme inhibitor, has being shown to increase AEA levels the brain. Analysis was carried out on 45 CA3 and CA1 cells following vehicle and URB597 (1mg/kg) and 50 CA3 and CA1 cells following vehicle, URB597 (1mg/kg) after rimonabant (3.0 mg/kg) pretreatment. Representative firing rate of CA3 and CA1 pyramidal cells following vehicle Fig. 2.6.1a,c and Fig. 2.6.1b,d. URB597 at a dose of 1.0 mg/kg dose did produced significant change in the firing frequency ($t = 2.17$, $p < 0.05$, fig. 2.6.1(e). Also, pretreatment with rimonabant tends to reverse the effect of URB597 back to control levels. Bursting and spike train analysis was carried out to determine the effect of URB597 on neuronal processing. As shown in figure 2.6.2a, URB597 at a dose of 1.0 mg/kg dose did not produce any change in the average number of bursts ($t = 0.67$, $p > 0.05$); no change in burst duration ($t = 0.33$, $p > 0.05$, fig. 2.6.2b), no change in spikes per burst ($t = 2.37$, $p > 0.05$, fig. 2.6.3a) and no produce no change in inter-spike interval ISI ($t = 0.52$, $p < 0.05$, fig. 2.6.3c). To assess whether URB597 effects are CB1 receptor mediated, we measure neuronal activities of hippocampal neurons in the presence of rimonabant. Single injection of URB597 (0.3 mg/kg, ip) did not produce any effect in the average number of bursts ($t = 0.51$, $p > 0.05$, fig 2.6.2d); no change in burst duration ($t = 0.67$, $p > 0.05$, fig. 2.6.2d), no change in spikes per burst ($t = 0.90$, $p > 0.05$, fig. 2.6.3b) and no produce no change in inter-spike interval ISI ($t = 1.42$, $p < 0.05$, fig. 2.6.3c).
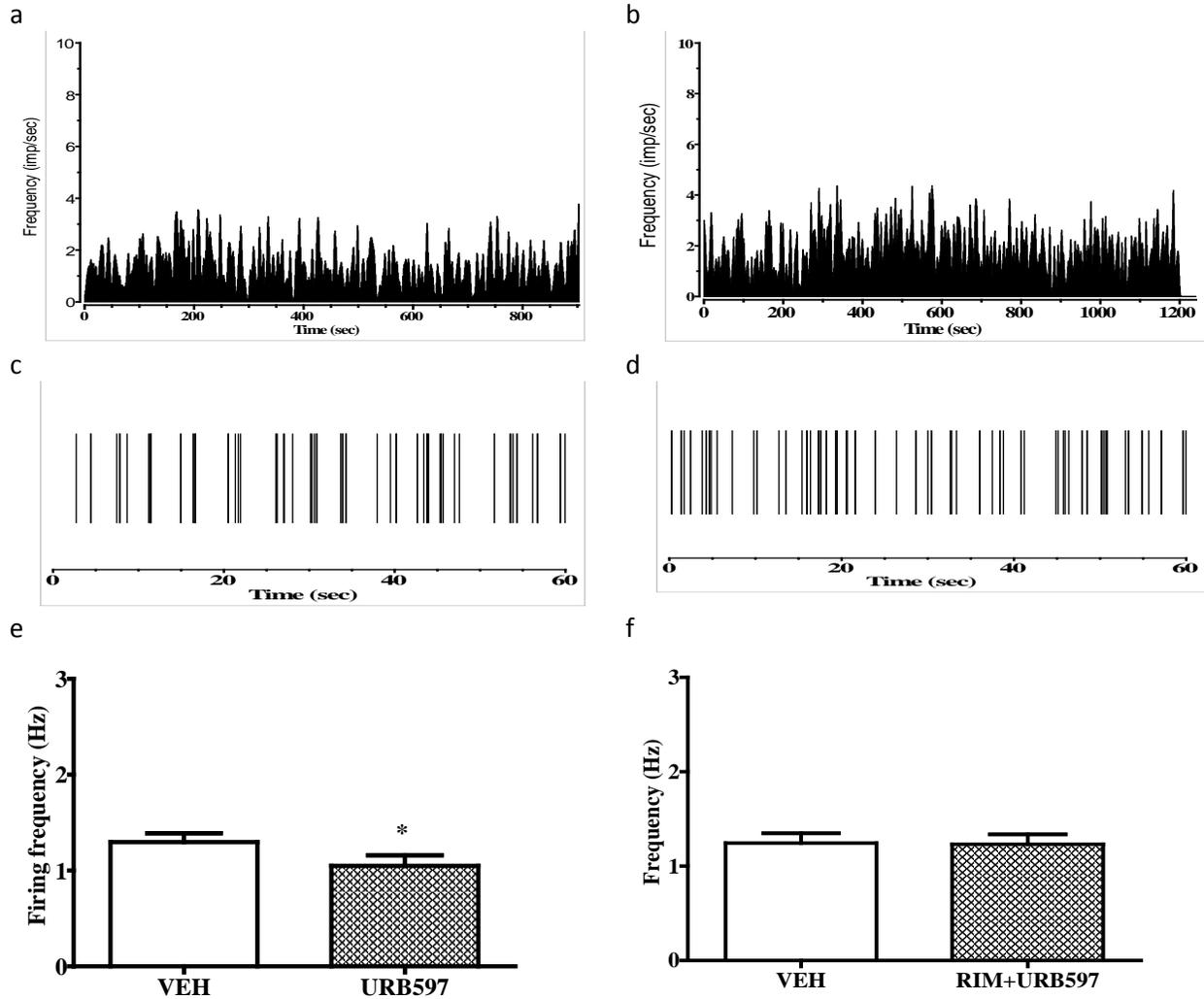
Figure 2.6.1. Effects of URB597 on firing rate of hippocampal pyramidal cells

Representative firing rates of CA3 and CA1 principal cells following vehicle and URB597 (1mg/kg) treatments respectively (a) and following vehicle and URB597 and rimonabant after pretreatment (b). Representative raster plots, each lasting sixty seconds, obtained from the same cell represented (c, d).Means ± S.E.M of firing rate of cells following vehicle and URB597 (e) and following vehicle and URB597 after rimonabant pretreatment (f). Analysis was carried out on 45 CA3 and CA1 cells following vehicle and URB597 (1mg/kg) and 50 CA3 and CA1 cells following vehicle, URB597 (1mg/kg) after rimonabant (3.0 mg/kg) pretreatment

(a)

(b)

(c)

(d)
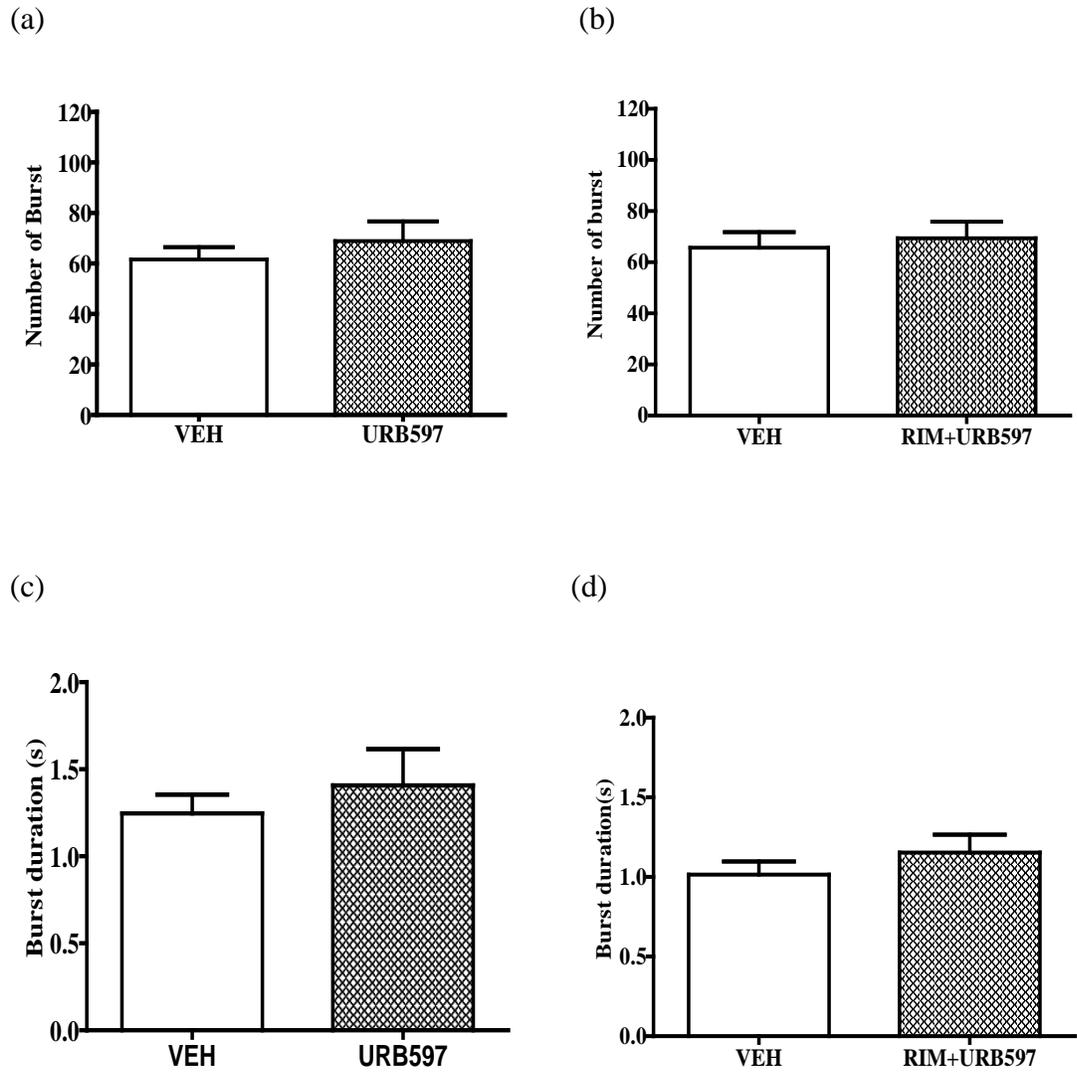
Figure 2.6.2. Effects of URB5971 on burst characteristics of hippocampal pyramidal cells

Mean± S.E.M. of number of bursts and burst duration of hippocampal pyramidal cells following

vehicle and URB597 (a, c) and following vehicle and URB597 and rimonabant pretreatments (b,

d). Analysis was carried out on 45 CA3 and CA1 cells following vehicle and URB597 (1mg/kg)

and 50 CA3 and CA1 cells following vehicle, URB597 (1mg/kg) after rimonabant (3.0 mg/kg)

pretreatment

(a) (b)

(c) (d)
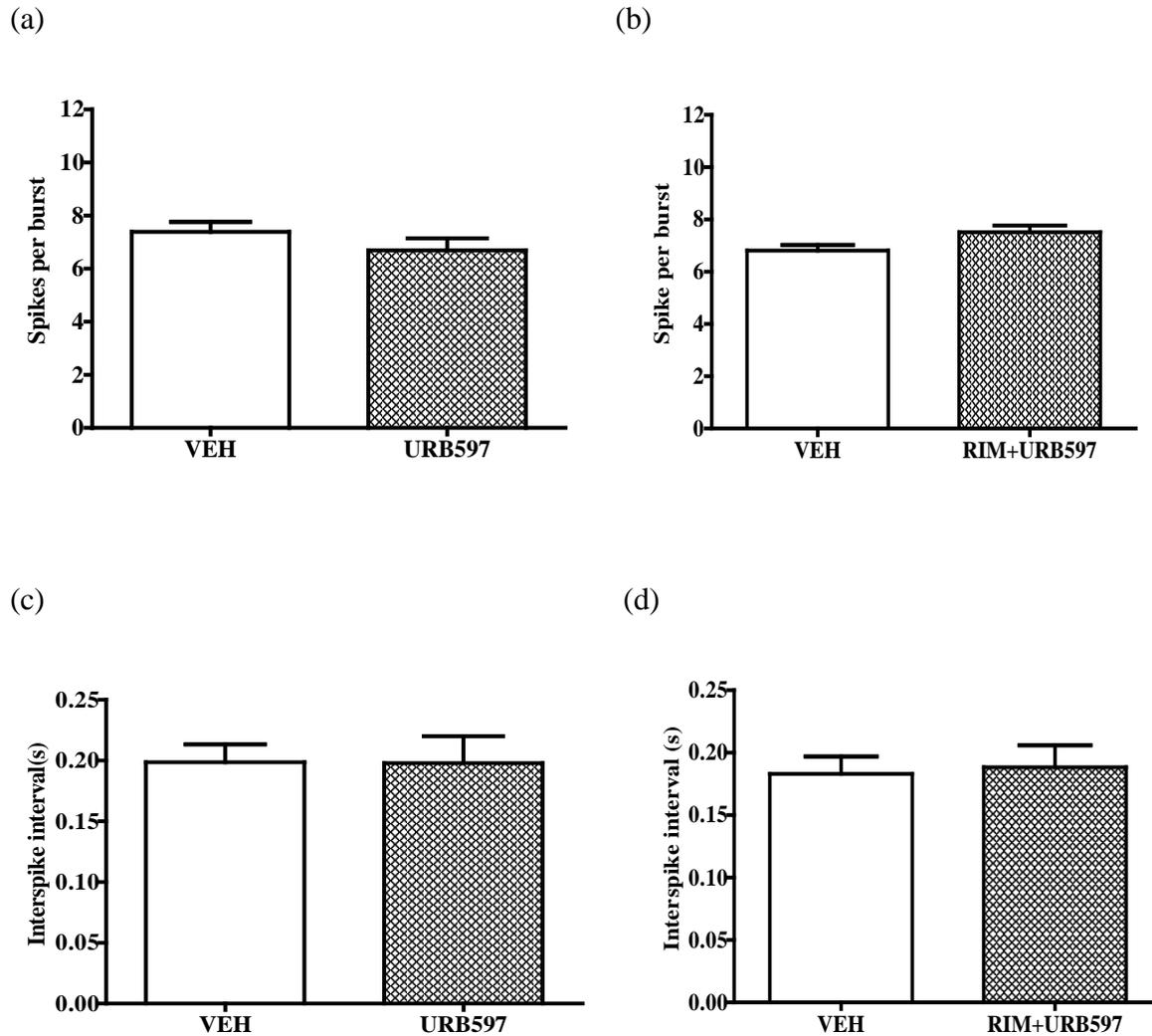
Figure 2.6.3. Effects of URB597 on spike of hippocampal pyramidal cells

Means± S.E.M. of spikes per burst and inter-spike interval of hippocampal pyramidal cells following vehicle and URB597 (a, c) and following vehicle and URB597 and rimonabant pretreatment (b, d). Analysis was carried out on 45 CA3 and CA1 cells following vehicle and URB597 (1mg/kg) and 50 CA3 and CA1 cells following vehicle, URB597 (1mg/kg) after rimonabant (3.0 mg/kg) pretreatment

There appears to be a reduction in effect in mean firing rates while pretreatment with rimonabant tends to reverse the effect but non-significant. Figure 2.6.4 shows representative cross-correlograms for selected hippocampal pyramidal cell pairs between CA3 – CA1 (fig. 2.6.4a); CA3 – CA3 (fig. 2.6.4b) and CA1 – CA1 (fig. 2.6.4c). Cross-correlogram analysis performed on six CA1-CA1, six CA3-CA3 and eight CA3- CA1 cell pairs, revealed that URB597 was not able to significantly reduce the average peak z-scores (CA1 - CA1: t=0.9308, p > 0.05, fig 2.6.4d; CA3 - CA3: t = 3.74, p > 0.05, fig. 2.6.4f) but significantly reduce the average peak z-scores CA3 - CA1: t = 4.40, p > 0.05, fig. 2.6.4e) in comparison to vehicle treatment.
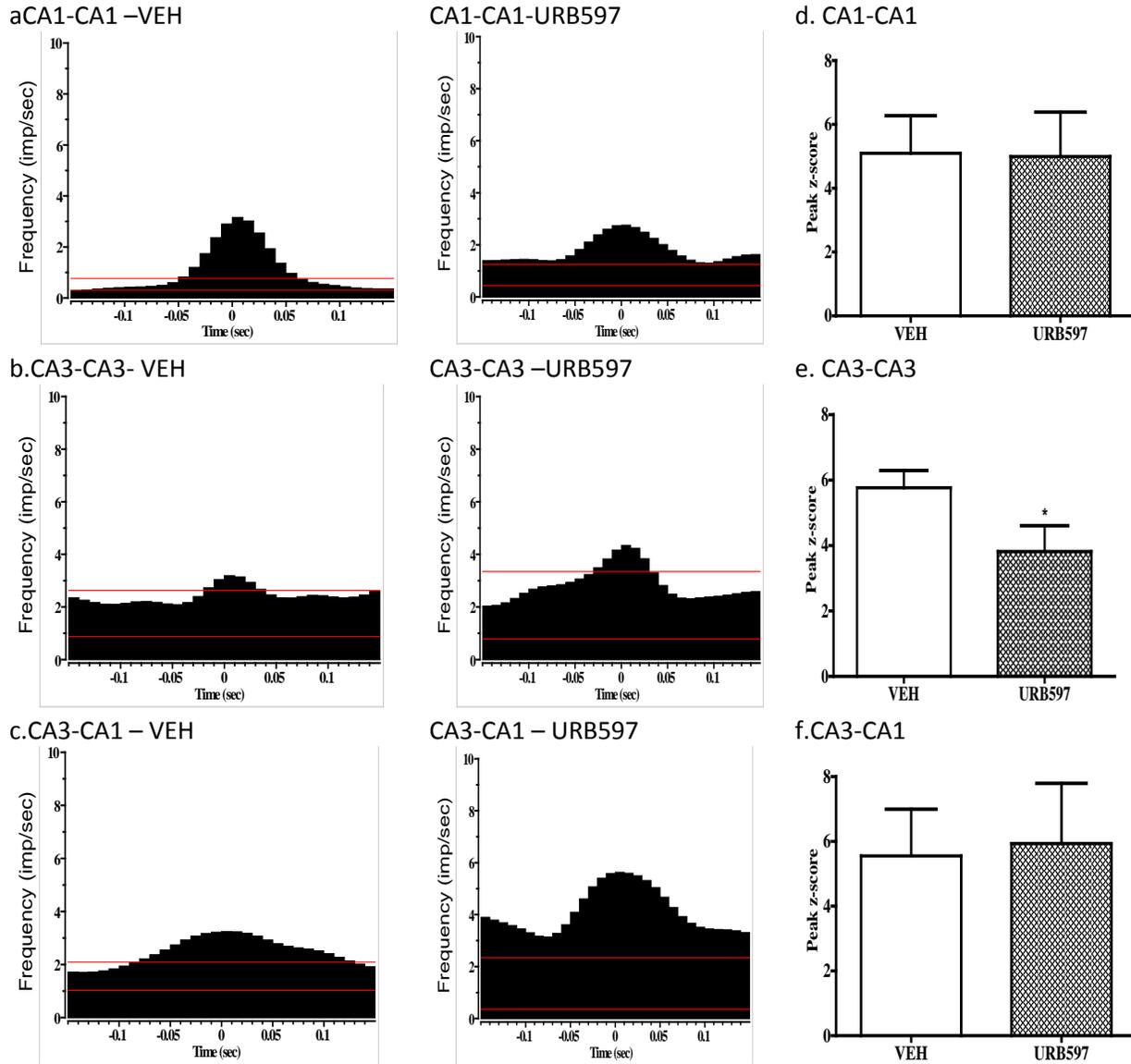
Figure 2.6.4 Effects of URB597 on synchrony of hippocampal pyramidal cells

Representative of URB597 on synchrony of pyramidal cells of CA3 – CA1 (a); CA3 – CA3 (b) and CA1 – CA1 (c) sub-fields, following vehicle and URB597. Note: Analysis performed with bin size of 3ms; smoothed to 4 bins. The horizontal red line each cross-correlogram represent the 95% confidence limits. Mean ± S.E.M. of peak z-scores calculated from CA1 - CA1 (d) CA3 - CA3 (e); and CA3 - CA1 (f) following vehicle and URB597 (1mg/kg) treatments.

Table 2. Effects of vehicle, *R*-methanandamide, VDM11 and URB597 on firing characteristics (firing rate, bursting, and cell synchrony) of hippocampal principal cells in anesthetized animals

Table 3a Effects of the effects of vehicle and *R*-methanandamide (N=40, df =39) and vehicle and *R*-methanandamide and rimonabant pretreatment (N=24, df =23), (t values and Mean ±S.E.M.)

Firing characteristics	t	vehicle	*R*-METH	t	vehicle	*R*-METH+ RIM
Firing rates	1.72	1.94±0.23	1.74±0.20	0.11	1.27±0.16	1.28±0.12
Number of Bursts	0.11	99.50±13.52	100.8±14.31	0.56	84.46±11.24	80.38±10.93
Burst duration	0.71	0.86±0.10	0.94±0.12	0.76	1.08±0.16	1.21±0.18
Spikes per burst	0.37	6.66±0.30	6.78±0.31	0.38	8.55±1.20	7.96±0.69
Interspike interval	0.87	0.13±0.01	0.15±0.20	0.62	0.15±0.02	0.17±0.01

Table 3b Effects of the effects of vehicle and VDM11 (N=32, df =31) and vehicle, and VDM11 and rimonabant pretreatment (N=34, df =33) (t values, and Mean ±S.E.M.)

Firing characteristics	t	vehicle	VDM11	t	vehicle	VDM11+ RIM
Firing rates	2.28	1.32±0.14	1.67±0.19	2.29	1.04±0.07	1.35±0.15
Number of Bursts	1.93	88.63±12.42	105.8±14.50	1.72	55.35±4.47	68.00±8.48
Burst duration	1.87	0.92±0.09	1.52±0.32	0.59	1.60±0.21	1.75±0.21
Spikes per burst	2.37	7.06±0.41	8.29±0.54	1.50	8.69±0.93	10.60±1.05
Interspike interval	0.52	0.14±0.01	0.16±0.02	1.52	0.20±0.01	0.24±0.02

Table 3c Effects of the effects of vehicle, URB597 (N=45, df=44), and vehicle and URB597 and rimonabant pretreatment t values (N=64, df=63), (df and Mean ±S.E.M.)

Firing characteristics	t	vehicle	URB597	t	vehicle	URB597+ RIM
Firing rates	2.17	1.28±0.09	1.03±0.11	0.12	1.39±0.13	1.37±0.11
Number of Bursts	0.67	67.69±6.06	62.87±7.56	0.51	71.52±7.33	68.00±6.10
Burst duration	0.33	1.36±0.12	1.43±0.24	0.67	1.26±0.12	1.34±0.11
Spikes per burst	0.88	7.20±0.34	6.68±0.45	0.90	8.33±0.61	7.68±0.33
Interspike interval	1.41	0.20±0.01	0.25±0.03	1.42	0.18±0.01	0.21±0.01

2.7. Discussion of results

For this study, we chose to utilize *R*-methanandamide, which has been shown to have a high affinity for CB_1 receptor and is highly stable (Abadji et al., 1994), as well as URB597, a stable FAAH inhibitor and VDM11, an anandamide membrane transporter inhibitor. VDM11, unlike other similar inhibitors, has little effect on anandamide hydrolysis or vanilloid TRPV1 receptors (Fowler et al., 2003). These compounds have been shown to cross the blood brain barrier and potentiate or enhance the endogenous levels anandamide in the brain (Bisogno et al., 2005). Furthermore, based on previous reports the administration of the antagonist, rimonabant, particularly at high concentration has been shown to selectively block the CB_1 receptor (Terranova et al., 1995b). We measured the acute effects of *R*-Methanandamide, URB597 and VDM11 on the firing frequency, bursting characteristics and synchrony of presumed CA3 and CA1 pyramidal neurons which fire between 0.5 – 6.0 Hz, located in CA3 and CA1 subfields. It is well known that in rat brain, spontaneous neuronal activities are responsible for control of network and how the neuronal activities in brain respond to stimuli (Henze and Buzsaki, 2001;Buzsaki, 1986). Based on this knowledge, we hypothesized that indirectly enhancing endocannabinoid levels in the brain would alter hippocampal neuronal activity in both anesthetized rats by modulating the neuronal firing characteristics. The results of this experiment are summarized in table 3.

Results showed that URB597 significantly decreased firing rates and did not alter the mean burst duration and interspike intervals, in anesthetized animals when compared with vehicle. However, VDM11 showed an increase in firing rates, increase in number of bursts, but not change in burst duration and interspike interval in anesthetic animals when compared with vehicle. In anesthetized animals, pretreatment with rimonabant was able to block most of these

effects. Only *R*-methanandamide disrupted the synchronous firing of principal cells within CA3 and not within CA1 or between CA3 and CA1 regions in behaving animals. Moreover, the neurophysiological changes that were altered by these compounds were different, suggesting that in anesthetized rats (1) *R*-methanandamide, URB597, and VDM11 can enhance anandamide levels in the brain leading to alteration in hippocampal firing characteristics, bursting and spike train in mechanisms that are distinct in anaesthetized. Alteration could be mediated through CB_1 receptor since rimonabant was able to block these effects of URB597 while the firing rates and bursting activities of VDM111 were potentiated in the presence of rimonabant. Also, the firing and bursting activities of *R*-methanandamide and URB597 were not altered by rimonabant. The neurophysiological changes that were altered by these compounds seems to change independently, as firing rates were altered whiles parameters like spike and burst duration were not significantly changed.

The results of this study are also consistent with other reports showing that Δ^9-THC and WIN55,212-2 alter hippocampal neuron activities by reducing firing rates and altered bursting which were reversible with rimonabant and AM251, another CB_1 receptor antagonist(Goonawardena et al., 2010a;Hampson and Deadwyler, 2000;Sullivan, 2000). These alterations are responsible for producing the observed deficits in spatial learning and memory that develop after acute cannabis (Δ^9-THC)(Deadwyler et al., 1990). The present results also confirm previous observations showing that *R*-methanandamide action is similar to other cannabinoids and acts as an analog of anandamide (Hanus et al., 1993;Jarbe et al., 1998). *In vitro* studies have also shown that *R*-methanandamide, at equivalent concentration, depressed excitatory transmission in the CA1 interneurons. Perhaps, at this dose, *R*-methanandamide effects on hippocampal firing characteristics cannot produce significant effects in anesthetic

animals. In addition, VDM11 produced a significant effect under anesthetized conditions as seen in increased firing rates, longer burst duration and increase interspike interval. The activity of the drug can occur through the activation of other non cannabinoid sensitive receptors. Also, *R*-methanandamide and URB597 effects on hippocampal firing characteristics are not significant in reducing basal number of spikes in anaesthetized animals than behaving animals. *R*-methanandamide can act on other receptors and might be dependent on the state of the pyramidal neurons and other unknown electrophysiological conditions. Since most of these effects are blocked by pretreatment with the CB_1 receptor antagonist, Rimonabant, it indicates VDM11 actions and URB597 actions occur thorough a CB_1 mediated mechanism.

CHAPTER 3: EFFECTS OF ANANDAMIDE ON SHORT TERM MEMORY AND ON NEURONAL FIRING CHARACTERISTIC DURING PERFORMANCE OF THE DNMS TASK

3.1. Rationale

Smoking of *cannabis* is one of the leading forms of drug abuse in the United States and other western societies, despite its reported high dependency and abuse potential in heavy users. Epidemiological studies have demonstrated that cannabis affects brain regions that are important for expression of short term memory (Battisti et al., 2010;Solowij and Battisti, 2008). The DNMS task, considered to be a test of short-term memory and the successful performance of the DNMS tasks depends on the integrity of medial temporal lobe structures (Zola-Morgan et al., 1986;Malkova et al., 1995). One way in which drugs produce their effects in the body is by altering processing of memory information in regions of the medial temporal lobe in particular the hippocampus (Freund et al., 2003;Deadwyler and Hampson, 2008). These effects include the modulation of overall neuronal firing rates during performance of tasks such as the radial arm maze and DNMS tasks. In the medial temporal lobe, cannabinoids activation of CB1 receptors has been shown to reduce the extent of memory encoding and retrieval of short term memory. In animal studies, synthetic compounds including WIN55, 212-2, CP55940, HU210 and anandamide have been shown to induce profound deficits in short-term memory (Deadwyler and Hampson, 2008;Robinson et al., 2008). In addition, these drugs produce deficits in performance in spatial learning tasks together with alterations in hippocampal neuronal activity. Experiments have shown that elevation of endocannabinoid levels also changes hippocampal neuronal activity leading to alterations in short-term memory in a CB_1 receptor-dependent manner (Lichtman et al., 2002).

Several studies done in rodents have provided substantial evidence of altered performances of a short term memory task by cannabinoids. Example of such studies include the radial arm maze, Morris water maze and DNMS task, were performances are altered by cannabinoid agents including Δ^9-THC, HU210 and WIN55,212-2(Deadwyler et al., 1990;Lichtman and Martin, 1996;Davies et al., 2002;Hernandez-Tristan et al., 2000;Ferrari et al., 1999). There is also considerable evidence showing that cannabinoid agents, Δ^9-THC, HU210 and WIN55, 212-2, alter neuronal properties in specific neurons of the hippocampal neurons (Terranova et al., 1995b). These alterations occur through activation of CB_1 receptors which are responsible for modulating neuronal activities and neurotransmission at the presynaptic and post synaptic levels (Wilson et al., 2001). However, the extent to which these neurophsyiological changes relate to performance of a behavioral task is still elusive and more information is needed on firing properties of these cells during performance. The most disruptive effects have been observed in neurons in the CA1 and CA3 subfields and dentate gyrus of the hippocampus, which express a high density of the CB_1 receptor. In these structures, alterations in neuronal firing activities are associated with impairment in learning and memory processes. These changes can also be altered at different stages of the task and cannabinoids disrupt neuronal firing selectively at specific phases of the task (Kirby et al., 2000;Heyser et al., 1993).

As observed in chapter two, under anesthetic conditions, enhanced anandamide level in the hippocampus altered neuronal firing characteristics. In rats, the following features have been observed; first, inhibition of neuronal FAAH enzyme activity leads to an elevation of endocannabinoid levels (Arreaza et al., 1997). Second, rat hippocampus neuronal activity is correlated with accuracy of working memory tasks (Lichtman and Martin, 1996;Deadwyler and Hampson, 1999). Third, WIN55,212-2, CP55940, HU210 and anandamide, have been shown to

induce profound deficits in DNMS performance(Hampson and Deadwyler, 1996). These finding suggests that modulation of the levels of endocannabinoids can alter the electrophysiological characteristics of hippocampal neurons and these changes are correlated with changes in behavior in a task.

Therefore, to study these changes, concomitant recordings were done in specific stages of the task to determine if there are alterations in neuronal ensemble activity occurring during specific stages. The measurement of motor activity during task was done to determine whether these drug treatments produce any deficit in locomotor. Locomotor is regarded as the time to move from the head entry device to the response lever and is indication for motivation of the animals. Here we investigated the effect of anandamide on neurophysiological characteristic of hippocampal cells and cell synchrony of hippocampal pyramidal cells in rats performing the DNMS task. We determined how these treatments affect cell firing rates, bursting and synchrony. We proposed that administration of R-methanandamide, VDM11 and URB597, could impair DNMS performance at a longer delay interval, and reduced hippocampal neuronal firing during the sample (encoding) phase but not during non-match phase (retrieval), and alter affect baseline firing and burst characteristics. Studying these electrophysiological changes may increase our knowledge of the effect of cannabinoids in the brain.

3.2. Objectives

(i) To examine how the exogenous cannabinoid agonist R-methanandamide, the endocannabinoid transporter inhibitor VDM11, and the FAAH inhibitor, URB597, affect short-term memory (STM) performance during the DNMS task at different delay intervals;;

(ii) To assess how the exogenous cannabinoid agonists R-methanandamide, the endocannabinoid transporter inhibitor VDM11, and the FAAH inhibitors, URB597, affect motor activity.

(iii)To examine how the exogenous cannabinoid agonist R-methanandamide, the endocannabinoid transporter inhibitor VDM11, and the FAAH inhibitor, URB597, influence concomitant hippocampal ensemble activity during sample (encoding) and non-match (recall) phases of the DNMS task;

(iv) To examine how the exogenous cannabinoid agonist R-methanandamide, the endocannabinoid transporter inhibitor VDM11, and the FAAH inhibitor, URB597, affect baseline firing and burst characteristics of hippocampal pyramidal cells isolated from CA3 and CA1 sub-fields in behaving rats;

(v) To examine how the exogenous cannabinoid agonist R-methanandamide, the endocannabinoid transporter inhibitor VDM11, and the FAAH inhibitor, URB597, affect spike train characteristics of hippocampal pyramidal cells isolated from CA3 and CA1 sub-fields in behaving rats;

(vi) To examine how the exogenous cannabinoid agonist R-methanandamide, the endocannabinoid transporter inhibitor VDM11, and the FAAH inhibitor, URB597, affect the synchronous firing between hippocampal pyramidal cells pairs located in CA3 - CA3; CA3 - CA1 and CA1 - CA1 hippocampal sub-fields in behaving rats.

3.3. Methods

3.3.1. Study design, drug preparation and administration

Subjects: (*N*=11) Male, Long-Evans rats (Harlan, Indianapolis, Indiana, USA), approximately 120-180 days old and weighing 280-350 grams were used as subjects. Animals were housed individually in plastic cages, in approved animal facilities on a 12h: 12h day/night cycle. *Ad libitum* food was always available, but prior to assessing behavior, animals were water regulated to eighty- three percent of their *ad libitum* body weight.

Drug preparation and Administration**:** *R*-Methanandamide, URB597, and VDM11 were from Cayman Chemical, Ann Arbor, Michigan, U.S.A. Stock solutions of cannabinoid agents were freshly prepared each day in a suspension of: 95% saline, 5% cremophor®(sigma, Steinheim, Germany). Solutions containing alcohol were stirred rapidly and placed under a stream of nitrogen gas to evaporate the alcohol. Control solutions consisted of saline and cremophor® with no cannabinoid agents.

Drug treatment and Schedule: A diagram illustrating the experimental protocol is presented in table 3. Animals with unilateral electrode implants were injected with the drug suspension intraperitoneally. Intraperitoneal administration was chosen because it was the more consistent method for dosing and timing of treatment in experiments of this kind. In all, the antagonists were administered fifteen minutes before cannabinoid agonists. Animals in the spatial task were assessed separately from anesthetized animals

Implantation of Microelectrode Arrays: The basic concept of electrode implantation was adopted from Hampson and Deadwyler lab (Hampson et al., 1993) at the department of Physiology and Pharmacology at Wake Forest School of Medicine. Each animal was anesthetized with ketamine hydrochloride (10 mg/kg) and xylazine hydrochloride (10 mg/kg). Once anesthetized, holes were

drilled in the exposed skull to hold support screws. Sixteen stainless steel, insulated electrodes were purchased as an array from a commercial source (Neurolinc, New York, New York, U.S.A). The arrays were built according to the following specifications: two rows of 40 micron diameter wires, spaced 200 microns on center and 800 microns between the rows. Arrangements were made with asymmetrical lengths such that the longer CA3 electrode tip was automatically placed ventral to the CA1 sub-field electrode tips (see Figure 1.5). Positioning of the center of the array was performed using a stereotaxic instrument were 3.4 mm posterior to bregma and 2.7 (lateral to midline) and a depth of 3.2 – 3.4 mm. (Deadwyler and Hampson, 1999;Paxinos et al., 1985). The longitudinal axis of the array was set at 30 degrees to the midline. Sealing of the exposed skull and securing the arrays was done with dental cement. The animal's breathing was monitored continuously and the body temperature maintained at 37°C with the use of a heat pad. Once implanted, animals were allowed a minimum of one week postsurgical recovery period. The scalp wound was treated periodically with Neosporin antibiotic and systemic injection of penicillin G (300 000 U, intramuscular) to prevent infection. Intramuscularly administered buprenorphine (0.01–0.05 mg/kg) was used for analgesia immediately following and six to nine hours after surgery. The animals were evaluated daily using a species-specific quantitative behavioral score. The multiunit recording arrays were monitored and neurons recorded to check for damage and stability.

3.3.2. Multiunit Recording Procedures: Anesthetized and behavioral task electrophysiological recording

Before the start of each recording session, rats were connected to a flexible recording cable. A commutator permits electrical continuity while allowing unrestrained movement within

the test apparatus. The head stage of each recording cable contained miniature unity-gain field effect transistors. After stabilization of the electrode array, selected principal cells with firing rates of 0.5–6 Hz were isolated and discriminated with a Multiunit Acquisition Processor (MAP) (Plexon Inc., Dallas, Texas, U.S.A). The parameters for isolation and discrimination of single unit activities were determined, recorded and saved from one session to another, using the MAP software package. The activities of these preselected CA3-CA1 principal cells were tracked and recorded following treatments. Waveform parameters were recalled on successive days and modified as necessary to discriminate unsorted new neurons or to identify previously recorded neurons.

3.3.3 Recording parameters: behavioral animal electrophysiology

Individual principal cell firing characteristics known as spike trains were analyzed using Neuroexplorer software (Nex Technologies, Massachusetts, U.S.A). The following parameters were calculated for each animal and for each selected cell throughout treatments: the average firing rate (FR, Hz), the average number and duration of bursts, the average number of spikes and inter-spike intervals (ISI). Furthermore, 'bursts' of the spikes were identified by: (1) calculating mean FR and ISI for all spikes, (2) identifying sequences of three or more consecutive spikes with ISIs, in which all ISIs were less than one-half the mean ISI, (3) 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. Bursts with S-values >10 were used to assess the mean burst duration, mean number of spikes in a burst, mean firing frequency and mean ISI within bursts. In addition, cross-correlograms between selected CA1 - CA1, CA3 - CA3 and CA1 - CA3 cell pairs were performed for all

animals. Each cross-correlogram represents the average firing frequency (bin size = 5 ms) from a single cell referenced to the firing of another cell through a window of ±0.15 seconds. The peak z-scores were calculated for each cross-correlogram as follows: (1) The peak value (that is the histogram maximum), (2) the mean (M) and standard deviation (S) from the background bin values are calculated. Peak z-score is equal to [(peak firing rate – mean of baseline firing rate) ÷ Standard deviation (S.D.) of baseline firing rate]. The background parameter was calculated from bins outside peak (i.e. bins that are less than peak value divided by 2 away from the bin with the histogram maximum). All peak z scores were averaged across the selected CA1 - CA1; CA3 - CA3; and CA1 - CA3 cell pairs.

3.3.4. Behavioral procedure- Apparatus and setup

The behavioral apparatus was similar to Hampson and Deadwyler (Hampson et al., 1993;Deadwyler et al., 1990). Briefly, the behavioral training sessions were conducted in an experimental Plexiglass chamber 43x43x50 cm located within a sound-attenuated chamber (Industrial Acoustics Co., Bronx, New York, U.S.A). On one side of the Plexiglas chamber are two retractable levers (Coulborn Instrument, Length Valley, PA, U.S.A) with a plastic drinking sprout mounted between the levers. On the other side of the chamber is a head entry device that contains a photo beam, mounted on a polypropylene rectangular block 3.5 cm above the chamber floor. A white cue light (Sylvania Pilot Light, 28v) was placed above the floor head entry device. A speaker for tone (67 DB, 1 KHz) presentation and a white noise speaker (90 db) are mounted above the chamber floor, on the opposite walls. Two houselights (25W each) are mounted at the top of the chamber. Water reward was controlled by computer activation of a solenoid valve that

emits a fixed amount (0.04 ml) into a sprout. All behavioral events were monitored by a video camera.

3.3.5. Behavioral procedure-Training and testing

All animals were placed on *ad libitum* food and water for 3-5 days. Water restrictions were applied on day 5 and day 6 before training on day 7 (day 1 on training). Each session or day 150 trials were given. On each successive trial, water was rewarded for 10 seconds and separated from the next trial by an inter-trial interval of 10 seconds. Training took place in stages. The first stage involved the association of the animal to a 'click' for water reward. This stage was then followed by a stage when both levers were presented. The next stage forms an association of the animal's reward with a nose-spoke into the head entry device. Usually, this stage was completed in less than an hour for 100 or more trials. It consisted of presentation of a sample lever, followed by a nose-poke and then by a lever press response during which the animals were rewarded for pressing a correct nonmatch lever. Pressing the wrong lever resulted in no water reward and a time out. Animals were fully trained when they perform this stage without assistance at 85% (± SEM) correct responding responses in a 100 trials per session of the task as shown in Figure 1.5. For each trial, the computer recorded the time in which the stimuli were presented and responses were made. From these data, percentage correct or errors, total performance was also measured in different delay intervals. All animals received humane care in accordance with the Animal Care and Use Committee (ACUC) at the Wake Forest University School of Medicine.

Table 3. Drug treatment and order of drug administration and electrophysiological recording during DNMS task

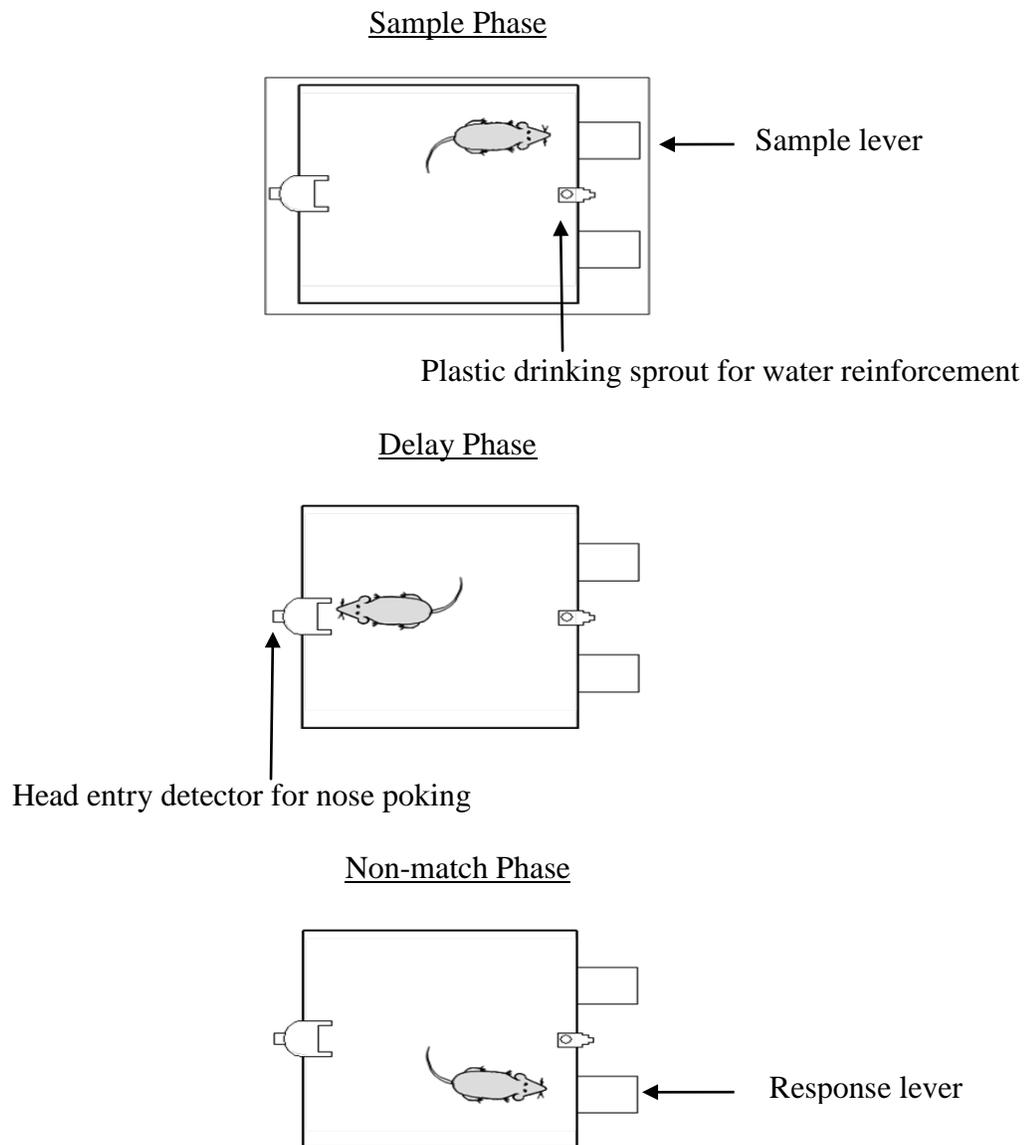| 15 min before recoding	60 min during performance of DNMS task
Vehicle (Cremophor)	recording
URB597 (1mg/kg)	recording
VDM11 (3mg/kg)	recording
R-methanandamide (3mg/kg)	recording

3.3.6. Data analysis

In this study the DNMS task data was analyzed using a one way measure ANOVA to assess differences between cannabinoid agent and vehicle treatment. A two-way repeated measures ANOVA was used to assess differences between cannabinoid agent and vehicle treatment for individual delay intervals.

Single neuron spike trains were analyzed using Neuroexplorer® software. The following parameters were computed for each selected principal cell across treatments: (1) mean frequency of firing (Hz), number of bursts , burst duration , spikes per burst and inter-spike interval (ISI) from sixty minute spike trains. In addition, cross-correlograms between selected CA1 - CA1; CA3 - CA3 and CA3 – CA1 cell pairs were carried out for all animals. Each cross-correlogram represents the mean firing frequency (bin size = 5ms) of a single cell referenced to the firing of another cell across a ± 0.15s time window. Peak z-scores were calculated for each cross-correlogram. Peak z-scores from hippocampal pyramidal cells that displayed significant increases in firing around sample and nonmatch responses phases of the DNMS task were determined from peri-event (± 1.5s) histograms, under control or treatment conditions.

The electrophysiological data was analyzed by Dunnett's test to compare the means between vehicle control and drug treatment across all parameters described above. The statistical significance level was set to $P<0.05$. All data were analyzed using the computer-based statistics package Graph pad Prism version 4.01. for Windows (Graph pad software, San Diego, California, U.S.A).

Figure 3.1. Diagram of the behavioral task (DNMS) task

Sample Phase

Sample lever

Plastic drinking sprout for water reinforcement

Delay Phase

Head entry detector for nose poking

Non-match Phase

Response lever

The three stages of the DNMS task, Sample, Delay and Non-match phases.

Pressing the sample phase results in light on at the nose poke devise. Pressing the correct

(opposite) lever during the non-match phase results in a water reward. In contrast, if the animal

presses incorrect lever during the non-match phase, this results in an 'error' response whereby

the animal receives no water reinforcement.

3.4 Results: Effects of *R*-methanandamide, VDM11 and URB597 on DNMS task

The DNMS task is a behavioral task considered to measure short-term memory. In this task, the successful performance has been shown to depend on the integrity of medial temporal lobe structures (Zola et al., 2000). Within the media temporal lobe, hippocampal neuronal firing characteristics are associated with performances during the sample (encoding) phase, delay and non-match phases (retrieval)(Deadwyler et al., 1996). However, it is still unclear how drug treatment alters an animal's behavior and how these alterations are related to changes in neuronal firing characteristics. Unlike analysis done in the anesthetized animal condition, analysis of the DNMS task was done for session lasting 60 minutes. The study examined the effects of performance of task, performance of DNMS task at different delay-interval epochs, locomotion time, and hippocampal activity during encoding (sample) and recall (non-match) phases of the DNMS task. All subjects were pre-trained to perform at 85% correct responses. This study assessed the mean percentage correct responses across 1l vehicle treated animals and 7 seven treated with *R*-methanandamide, VDM11 or URB597 with recordings were done on presumed pyramidal neurons which fire between $0.5 – 6.0$ Hz, located in CA3 and CA1 subfields. ANOVA revealed that R-methanandamide (3.0 mg/kg), VDM11 (3.0 mg/kg) and URB597 (1.0 mg/kg) produced no deficits in overall DNMS performance ($F_{(3,191)} =4.45$, $p>0.05$) Figure 3.4. A two-way ANOVA repeated measures using treatment group and delay as factors revealed that R-methanandamide but not VDM11 or URB597 produced significant impairments in DNMS performance at the 21-25 seconds delay interval epoch ($F_{(5,168}=21.22, P<0.05$ Figure.3..5). In comparison to vehicle, R-methanandamide, VDM11 and URB597 failed to show significant differences in DNMS performance at each of the other delay interval epochs that were less than twenty one seconds (all t's < 2.5 all p's > 0.05, Figure 3.5).
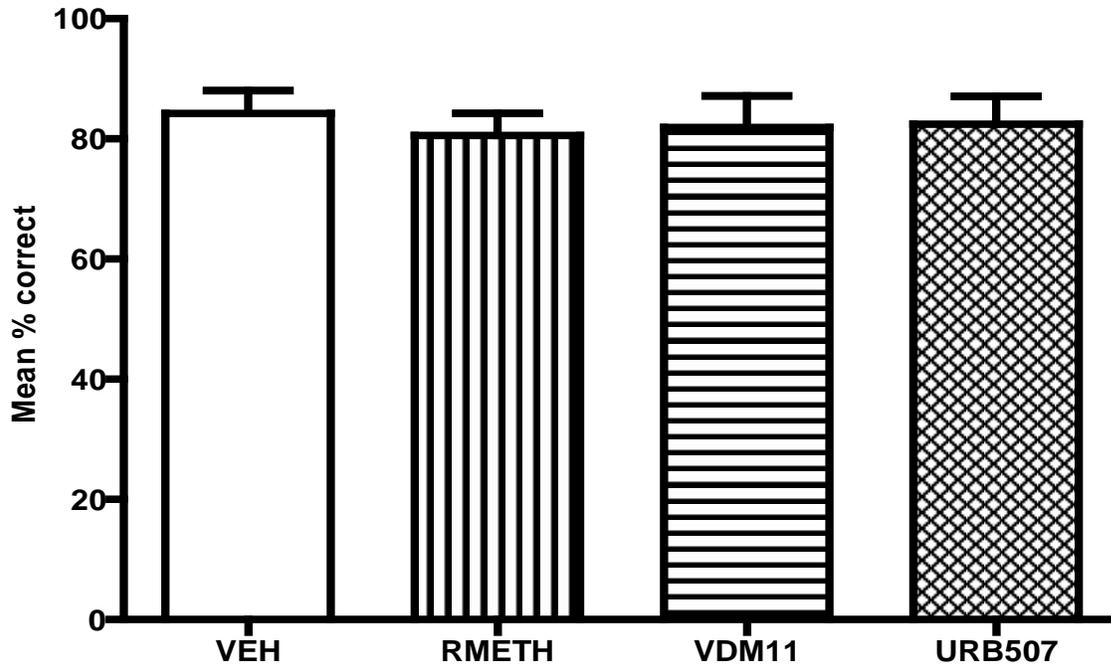
Figure 3.4. Percent correct responses of DNMS task following vehicle, *R*-methanandamide, VDM11 and URB597 treatments

Means ± SEM of percentage correct responses of DNMS task performance summed across all delay intervals (that is 1-30 seconds) following vehicle treatments (n = 10) and 3.0 mg/kg R-methanandamide; 3.0 mg/kg VDM11, and 0.3 mg/kg URB597(n = 7).

3.5. Results: Effects of *R*-methanandamide, VDM11 and URB597 at different delay intervals during DNMS task performance

Selected hippocampal principal cells that fired specifically during sample (n =14) and non-match (n = 14) responses following each treatment were assessed by one-way repeated measures ANOVAs. Comparing peak z-scores around the sample response during DNMS performance revealed that R-methanandamide produced a significant reduction on sample response firing (F (3, 55) =2.104 $p < 0.05$, Figure 3.6b) compared to vehicle. No overall significant effect of treatment (F (3, 55) = 2.03, $p > 0.05$, Figure 3.6b) was evident when peak z-scores were assessed around the non-match responses. Treatment of URB-597 (1.0 mg/kg) and VDM11 (3.0 mg/kg) did not produce any significant effects on overall hippocampal ensemble activity when peak z-scores were assessed around the sample (Figure 3.6b) and non-match (Figure 4.6b) responses.

The locomotor activity was assessed using a one–way repeated measures (ANOVA) and revealed no main effect of treatment at either correct trials (F(3,27) =0.17, $p > 0.05$, Figure 3.7a) or error trials (F(3,27)=0.37, $p > 0.05$, Figure. 3.7b) following *R*-methanandamide, VDM11 and URB597 treatments.
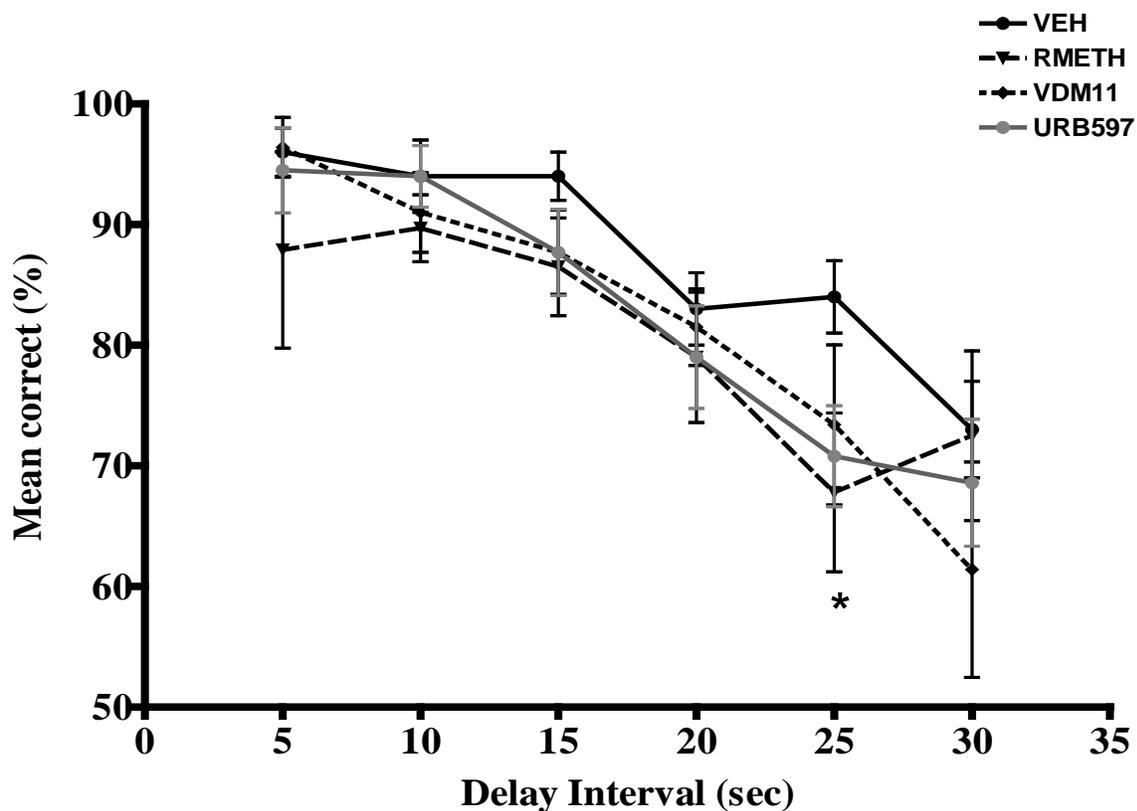
Figure 3.5. Percent correct responses of DNMS task at different delay intervals following vehicle, *R*-methanandamide, VDM11 and URB597 treatments

Means ± SEM of percentage correct responses of DNMS performance sorted by length of delay, in increments of 5 sec (1-5s; 6-10s; 11-15s; 16-20s; 21-25s and 26-30s) following the exposure of vehicle (n=10), 3.0 mg/kg R-methanandamide (n=7),; 3.0 mg/kg VDM11(n=7), and 1.0 mg/kg URB597 (n = 7)* p < 0.05 compared to vehicle.

3.6. Results: Effects of latency to respond to levers during DNMS task performance following *R*-methanandamide, VDM11 and URB597 treatments

(a) Correct
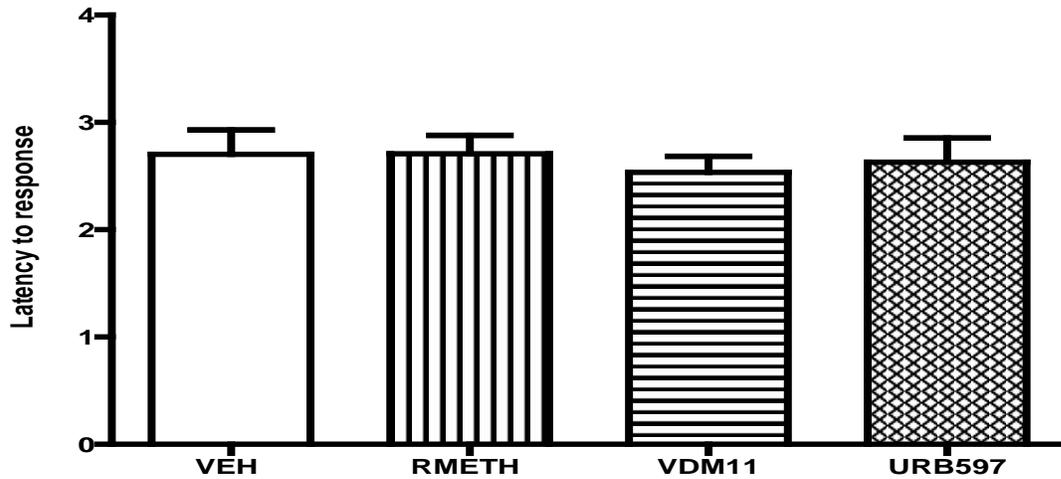
(b) Error
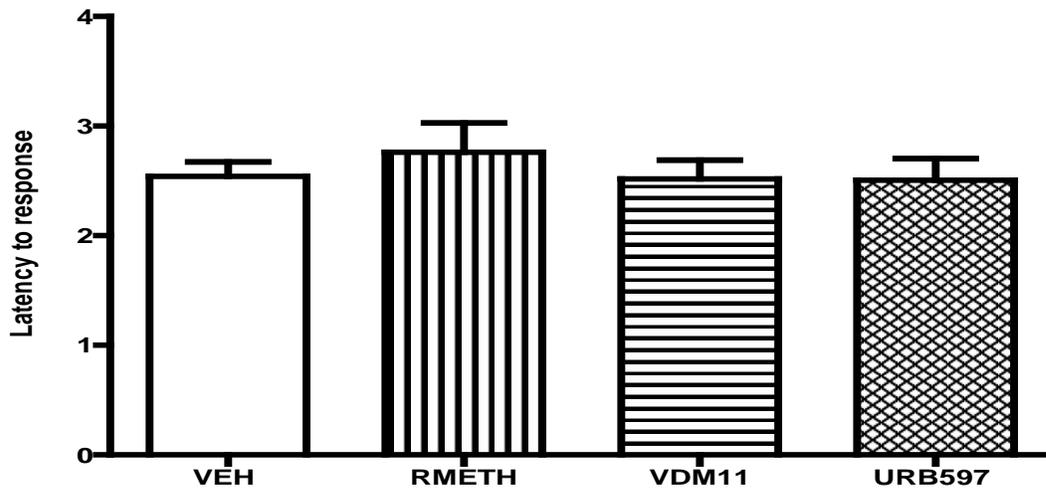
Figure 3.6.Effects of latency to respond to levers during DNMS performance following vehicle, *R*-methanandamide; VDM1 and URB597 treatments.

Mean ± SEM of latency to response levers during DNMS performance following vehicle (n=10), *R*-methanandamide, VDM11 (n = 7).and URB597 (n = 7) treatments.

3.7. Results: Effects of hippocampal ensemble activity during DNMS performance following vehicle, *R*-methanandamide, VDM11 and URB597 treatments

(a) Sample Phase (SR)
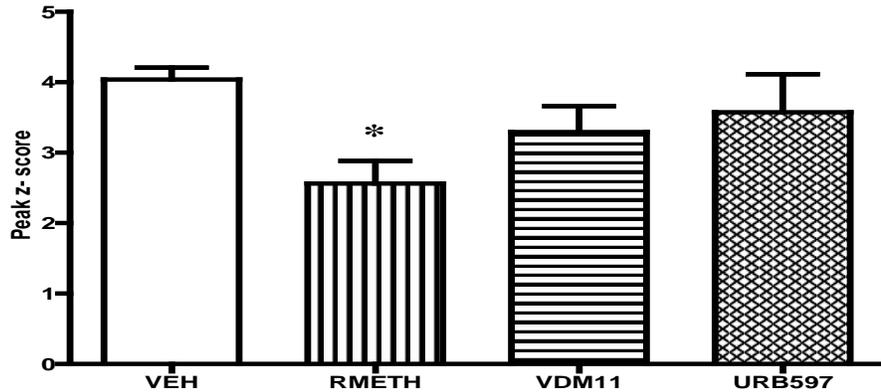
(b) Non-match Phase (NR)
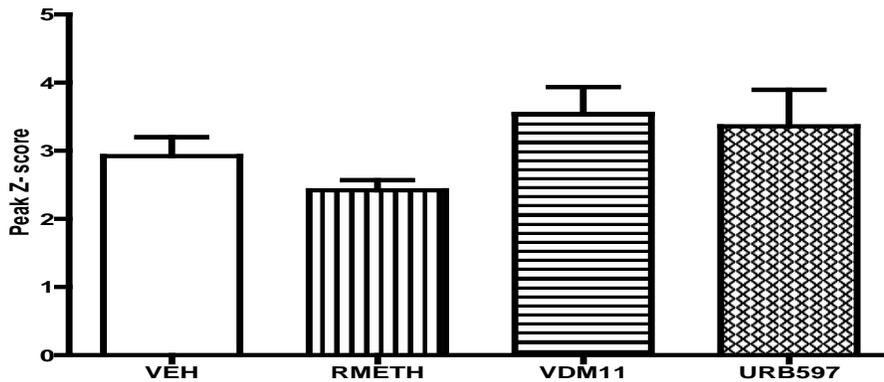
Figure 3.7. Effects on hippocampal ensemble activity during DNMS performance following vehicle, *R*-methanandamide, VDM11 and URB597 treatment Means ± SEM of peak z-scores of hippocampal principal cells firing around the sample (a) and non-match (b) phases following vehicle treatment or 3.0 mg/kg *R*-methanandamide; 3.0 mg/kg VDM11; 1.0 mg/kg URB597 treatments. The peak z-scores were derived from a total of 14 cells that fired around the sample and non-match responses. Asterisk (*) indicates $p < 0.05$ compared to vehicle.

3.8. Effects of *R*-methanandamide, VDM11 and URB597 on firing rate of hippocampal pyramidal cells during DNMS performance

Since *R*-methanandamide produced a delay and dose-dependent impairments in DNMS performance and a significant effect on the sample (encoding phase), while URB597 and VDM11 did not show any significant change, analysis was done to see if there is any related neuro- physiological changes in the hippocampal neurons during the entire sixty minute of the DNMS task. This is in relationship to other studies that demonstrated alterations in hippocampal ensemble activities in animals performing the DNMS task (Deadwyler et al., 2007;Hampson and Deadwyler, 1998). The mean overall firing rates was determine to determine the effects of *R*-methanandamide, VDM11 and URB597 on bursting of hippocampal, spiking and cell synchrony of hippocampal pyramidal cells during DNMS performance. *R*-methanandamide at a dose of 3.0 mg/kg, VDM11 at a dose of 3.0mg/kg and URB597 at a dose of 1.0 mg/kg does not produce significant changes in mean firing rates. To determine if these compounds alter bursting characteristic, analysis was done on the average number of bursts, spikes per burst, average burst duration and ISI in bursts.

(a)Vehicle
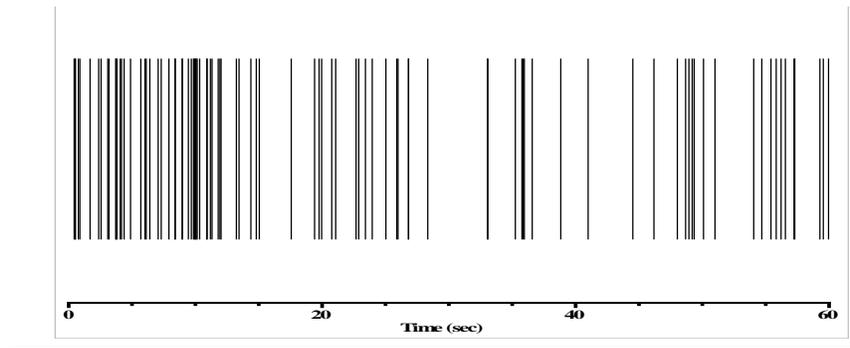
(b) R-methanandamide

(c) VDM11
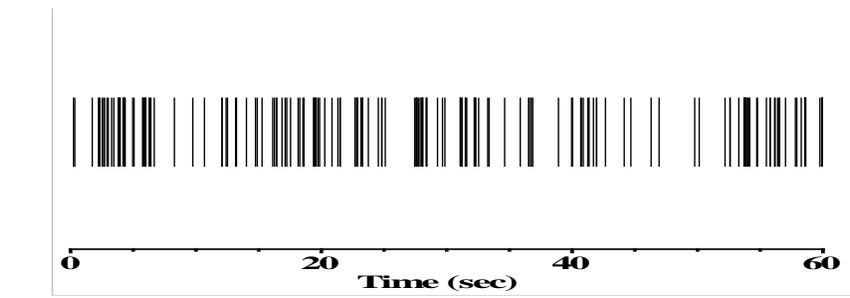
(d) URB597
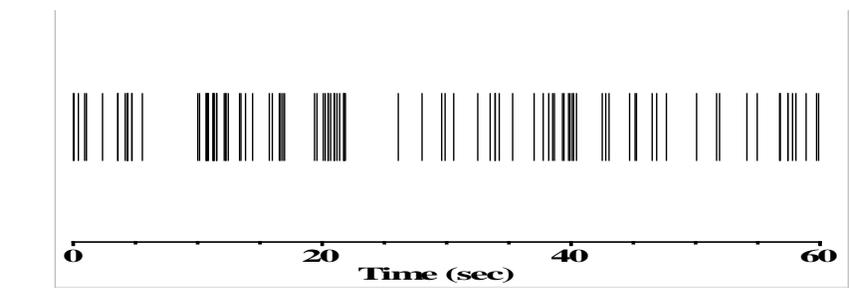
(e)

Figure 3.8. Effects of *R*-methanandamide, VDM11 and URB597 on firing rate of hippocampal pyramidal cells during DNMS performance

Representative raster plots obtained from the same cell represented across 60 seconds recording sessions following Vehicle (a), *R*-methanandamide (b), VDM11(c) and URB597 (d) treatments.

Means ± S.E.M. of firing rate from 40 CA3 and CA1 principal cells represented across 60 minutes recording sessions following vehicle, *R*-methanandamide, VDM11 and URB597, treatments (e).
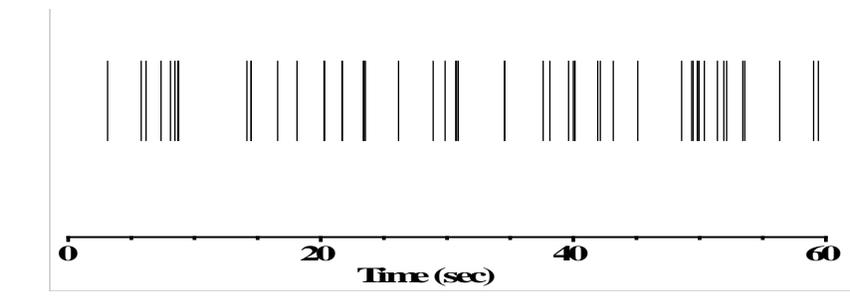
3.9. Results-Effects of *R*-methanandamide, VDM11 and URB597 on bursting and spike of hippocampal pyramidal cells during DNMS performance

Since *R*-methanandamide produced a delay and dose-dependent impairments in DNMS performance and a significant effect on the sample (encoding phase), while URB597 and VDM11 did not show any significant change, analysis was done to see if there is any related neuro- physiological changes in the hippocampal neurons during the entire sixty minute of the DNMS task. This is in relationship to other studies that demonstrated alterations in hippocampal ensemble activities in animals performing the DNMS task (Deadwyler et al., 2007;Hampson and Deadwyler, 1998). The main function of neuronal process of stimuli is their ability to produce action potentials which can be measured in the form of burst in which neurons. To determine the effect of this compound on neuronal processing, the overall burst characteristics, spiking and cell synchrony of hippocampal pyramidal cells during DNMS performance. *R*-methanandamide at a dose of 3.0 mg/kg does not produced any change in number of bursts and spikes per burst but significantly increased the average burst duration and ISI in bursts in comparison to vehicle. Also, VDM11 at a dose of 3.0mg/kg does not produce significant changes in the average number of bursts and spikes per burst but significantly increased the average burst duration and ISI in bursts compared to vehicle. Furthermore, URB597 does not increase the average number of bursts and spikes per burst but significantly increased the average burst duration and ISI in bursts in comparison to vehicle figure 3.6a, b, c, d). Figure 10 show representative cross-correlograms of CA1 and CA3 sub-fields.

(a)Number of Bursts

(b) Burst duration

(c) Spikes in Burst
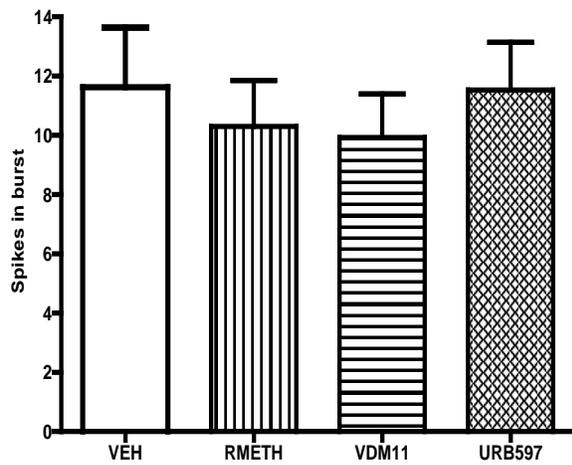
(d) Interspike interval(s)

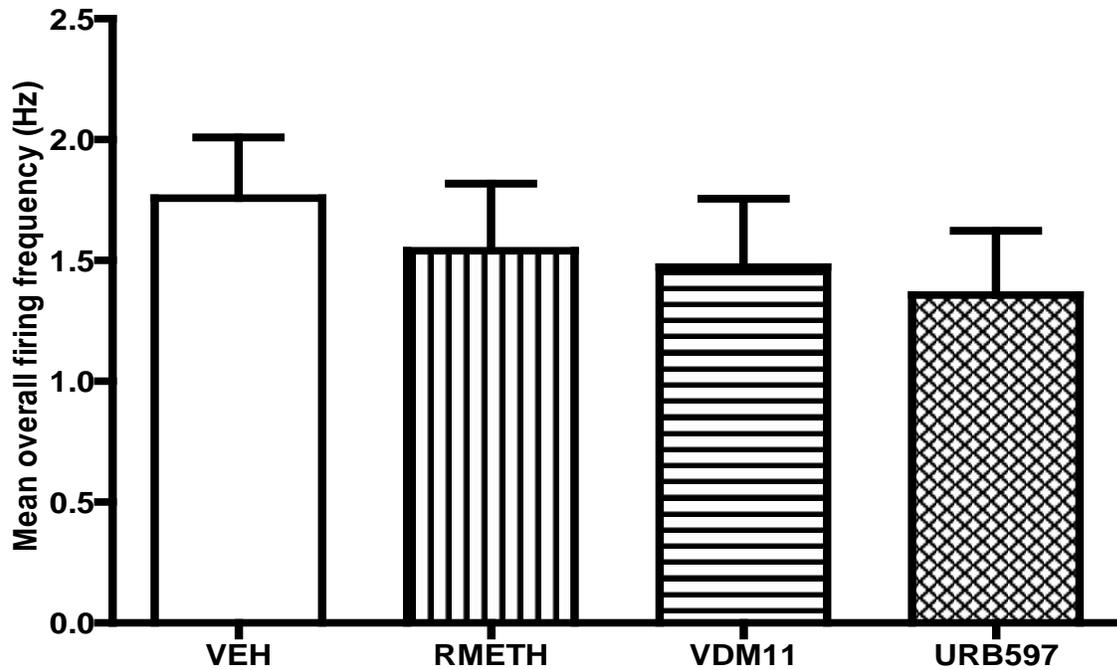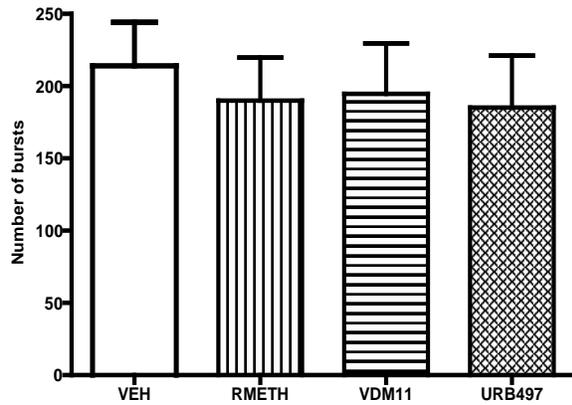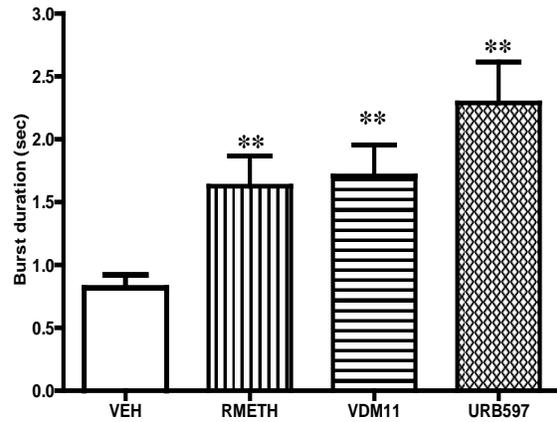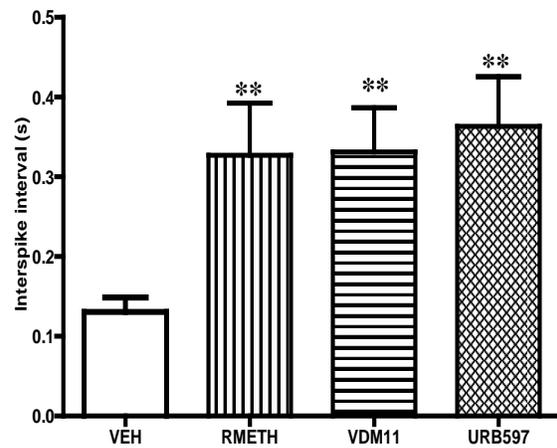Figure3.9. Effects of *R*-methanandamide, VDM11 and URB597 on bursting and spike of hippocampal pyramidal cells during DNMS performance. Means± S.E.M of number of burst (a) and bursts duration (b), spikes per burst(c) and inter-spike interval (d) following vehicle, *R*-methanandamide, VDM11 and URB597. Overall spike train analysis carried out on 40 CA3 and CA1 pyramidal cells (**$p < 0.01$, compared to vehicle).

3.10. Results: Effects of Vehicle, *R*-methanandamide, VDM11 and URB597 on cell synchrony of hippocampal pyramidal cells during DNMS performance

a. Vehicle   R-methanandamide   VDM11   URB597
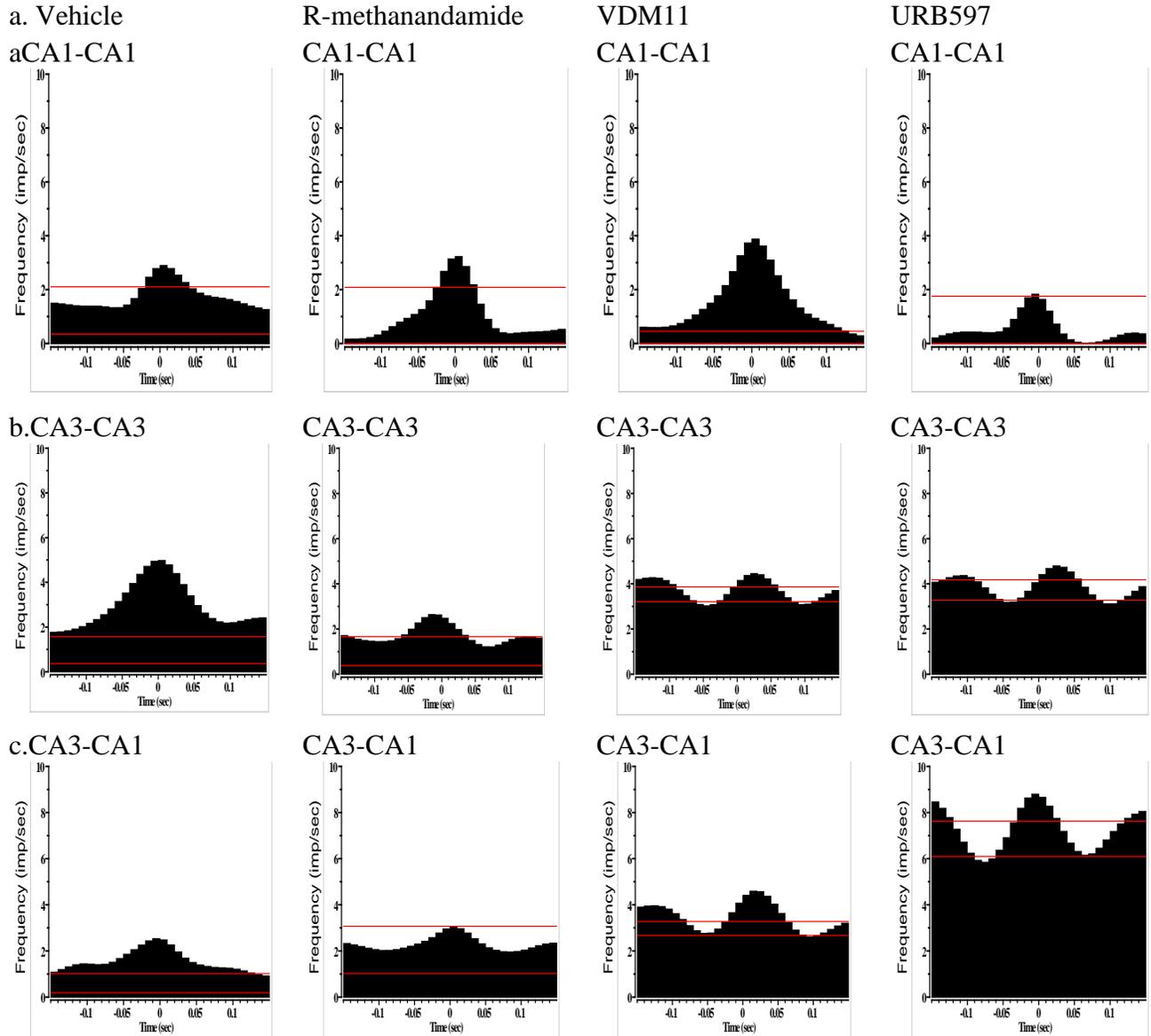
Figure 3.10. Effects of *R*-methanandamide, VDM11 and URB597 on cell synchrony of hippocampal pyramidal cells during DNMS performance .(a,b,c) show representative cross-correlograms of CA1 and CA3 sub-fields .The bin sizes were selected at 5 ms and each horizontal red lines in each cross-correlogram represent the 95% confidence limits.

e. CA1-CA1
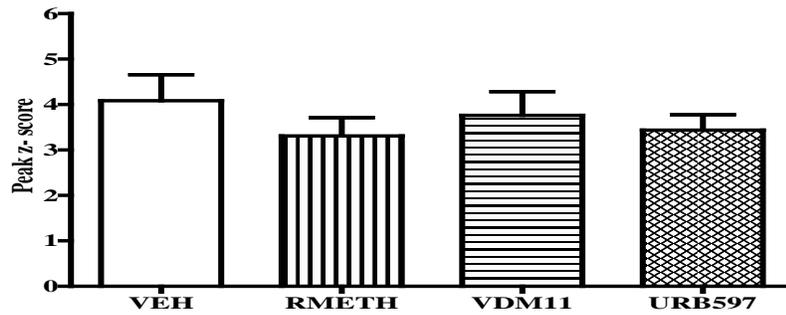
f. CA3-CA3
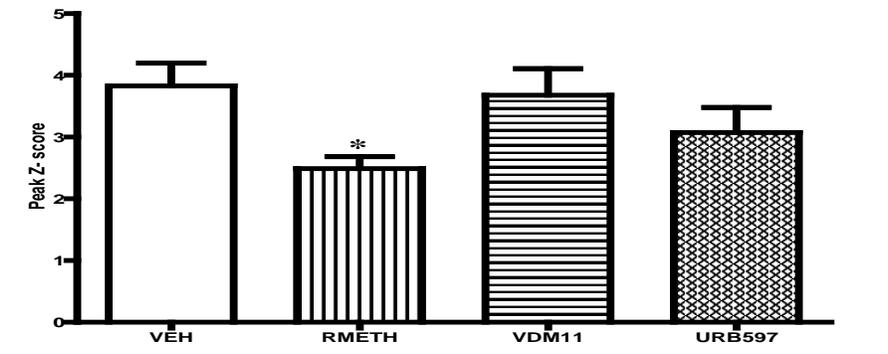
g. CA3-CA1
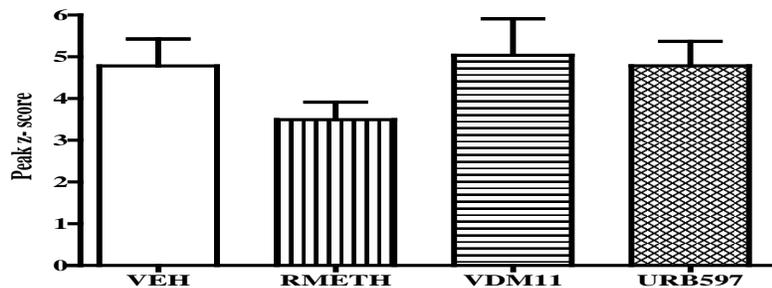
Mean ±S.E.M of peak z-scores calculated revealed that VDM11 and URB597 at this dose was not able to significantly reduce the average peak z-scores for selected cell pairs between CA1 – CA1, CA3 – CA3 and CA3 – CA1 (Figure 3.9e, f, g).However R-methanandamide at this dose was able to significantly reduce the average peak z-scores between CA3 - CA3 cell pairs: but not CA1 - CA1 cell pairs and CA3 - CA1cell pairs (Figure 3.9e, f, g).

Table 4 Effects of *R*-methanandamide, VDM11 and URB597 treatments on DNMS performance and on firing characteristics (firing rate, bursting, and cell synchrony) of hippocampal principal cells during performance of DNMS task

(F values, df and Mean ±S.E.M.)

	N	df	F values	VEH	R-METH	VDM1	URB597
Ensemble activity							
(a)Sample Phase	14	55	3.30	4.13±0.20	2.65±0.34	3.78±0.57	3.19±0.26
(b)Non-match Phase	14	55	0.98	2.84±0.31	2.67±0.28	3.36±0.32	3.18±0.36
Latency to response-							
Correct	7	27	0.17	2.70±0.22	2.70±0.17	2.53±0.15	2.62±0.22
Error	7	27	0.37	2.50±0.13	-2.76±0.26	2.51±0.17	2.50±0.19
Firing rates	40	159	2.81	1.76±0.25	1.42±0.27	1.39±0.28	1.56±0.35
Firing characteristics							
Number of Bursts	40	159	0.26	214.1±30.11	190±29.72	194.6±34.95	185.1±35.96
Burst duration	40	159	9.60	0.86±0.10	1.63±0.24	1.77±0.26	2.3±0.34
Spikes per burst	40	159	1.61	11.68±2.00	10.32±1.02	10.04±1.47	12.70±1.90
Interspike interval	40		6.66	0.13±0.01	0.28±0.06	0.31±0.05	0.34±0.06
Cell Synchrony							
CA1-CA1	12	47	1.66	4.08±0.57	3.30±0.40	3.76±0.52	3.43±0.33
CA3-CA3	10	39	2.88	3.82±0.36	2.48±0.19	3.67±0.43	3.07±0.40
CA3-CA1	12	39	0.04	3.80±0.34	3.49±0.41	3.11±0.47	3.89±0.36

3.11 Discussion of results

The changes in firing characteristics of this neuron have been shown to have critical role in the memory deficit produced by cannabinoids (Deadwyler et al., 1996;Kirby et al., 2000). Based on this knowledge, we hypothesized that indirectly enhancing endocannabinoid levels in the brain would alter hippocampal neuronal activity in performing animals and alter short-term memory processing by modulating the neuronal firing characteristics during certain phases of the DNMS task. Result from this study revealed that *R*-methanandamide, VDM11 and URB597 did not produce effects on overall DNMS performance (fig 3.4). Only *R*-methanandamide produced impairment in DNMS performance at the 21-25s delay intervals. Selected hippocampal principal cells that fired specifically during sample and non-match responses following each treatment were assessed and the peak z-scores calculated ±1.5 seconds during the phases for 60 minutes. This study showed that *R*-methanandamide was able to produce significant effects during the sample phase (fig. 3.6b) but no overall significant effects of treatment (fig. 3.6b) were evident around the non-match responses. Although, *R*-methanandamide elicited impairment in DNMS performance at 21-25s delay interval epoch, this cannot be regarded as a true of deficit as there was no overall drug effect. Overall hippocampal ensemble activity for URB597 and VDM11 treatment did not produce any significant effects around the sample (fig. 3.6a) and non-match (fig. 3.6b) phases. Animals had to maintain information in the sample phase and later retrieve that information in the non-match phase. There are reasons to speculate that rats injected with R-methanandamide failed to maintain information during the sample phase where the neuronal activities were altered and this may be related to the effects that are seen in the animals' behavior at the at twenty seconds delay.

In addition, *R*-methanandamide, VDM11 and URB597 treatments had no effect on general locomotion at either correct trials (fig. 3.7a) or error trials (fig. 3.7b). These results further suggest that these drugs at the given doses were not able to produce impairment in motor activity and that the effects could be mostly cognitive. It is possible that R-methanandamide was not able to alter performance of the task at longer delays and altered encoding but not recall of biological relevant events. Although the rats could run the task well, the doses use in this study might not be sufficient to produce a deficit in performance during the entire period of the task. Also, there was no alteration in response to either the correct responses or error responses. To determine the effect of this compound on neuronal processing, the overall burst characteristics and spike activity of neuronal pyramidal neurons was further examined.

Since many studies have demonstrated that activation of cannabinoid receptors impairs learning and cannabinoid receptor agonists impair learning and memory through the action on the hippocampus, the result from this study indicate that the neural firing changes that are occurring as an animal performed the task are altered by acute exposure to *R*-Methanandamide, VDM11 and URB597. There was consistent increase in mean burst duration and interspike intervals when compared with vehicle. However, the neuronal response parameters such as firing rate and number of burst were not significantly altered and may not be reflected in the animals' performance during the task.

CHAPTER 4: OVERALL DISCUSSION

4.1. Significance of the results

Considerable evidence suggests that cannabinoids impair hippocampal-dependent learning and memory processes, such as spatial learning and context-related memory tasks (Sullivan, 2000; Riedel and Davies, 2005). This study used a compound, *R*-methanandamide that has been shown to have a high affinity for CB_1 receptor and is highly stable(Abadji et al., 1994). In addition, the study used VDM11, which unlike other similar inhibitors, has little effect on anandamide hydrolysis (Fowler et al., 2003). The dose of the compounds used in this study has being similar to effect shown in other studies showing elevated anandamide levels in vivo ((Fowler et al., 2003). These compounds have been shown to cross the blood brain barrier and potentiate or enhance the endogenous levels anandamide in the brain (Bisogno et al., 2005;Marsicano et al., 2002). Furthermore, based on previous reports the administration of antagonist rimonabant, particularly at high concentration has been shown to selectively block the CB_1 receptor (Terranova et al., 1995b). One of the main processes by which neurons process information lie in their ability to produce action potentials which can be measured in the form of burst in which neurons, under certain conditions and long periods of quiescence, then a rapid firing of several spikes and a subsequent return to the quiescent state.

Overall, this study showed that in rats, *R*-Methanandamide, URB597, and VDM11 can indirectly enhance anandamide levels in the brain leading to alteration in hippocampal firing characteristics, bursting and spike train. These altered neuronal firing characteristics in mechanisms were distinct depending on the animal state. In anesthetized animals, VDM11 significantly increases while URB597 significantly decreases the average firing frequency of hippocampal pyramidal neurons. VDM11 also increases burst duration. Furthermore, URB597

but not R-Methanandamide or VDM11 produces significant increases in interburst intervals of hippocampal pyramidal neurons. In animals performing the DNMS task, *R*-Methanandamide, VDM11 and URB597 increase the burst duration of hippocampal pyramidal neurons. Overall, R-Methanandamide, VDM11 and URB597 all show differential modulation of hippocampal pyramidal neuron firing and bursting depending whether the animals were anesthetized or performing the DNMS task. Furthermore, URB597 but not R-Methanandamide or VDM11 produces significant increases in synchrony of hippocampal pyramidal neurons cell pairs. *R*-Methanandamide, VDM11 and URB597 all show differential modulation of hippocampal pyramidal neuron firing and bursting depending whether the animals were anesthetized or performing the DNMS task. These alterations of hippocampal neuronal activity may be responsible for producing some of the observed behavioral deficits in spatial learning and memory in rats. Neuronal characteristics can change independently of other parameters like spike and burst duration. This indicates that *R*-methanandamide, VDM11 and URB597, altered short-term memory differently and have different pharmacological profiles.

These results confirm previous observations showing that *R*-methanandamide possessed several behavioral and neuronal effects produced by Δ^9-THC, WIN55, 212-2, HU210 on hippocampal pyramidal firing patterns (Goonawardena et al., 2010a;Hampson and Deadwyler, 2000;Sullivan, 2000). These compound, Δ^9-THC, WIN55,212-2, HU210 have been shown to suppressed performance in DNMS task, radial maze, t-maze and water maze task (Hernandez-Tristan et al., 2000;Lichtman and Martin, 1996;Deadwyler and Hampson, 2008). In humans, activation of cannabinoid receptors in the brain is associated with loss coordination and movement caused by inhibition of motor skills and coordination. Furthermore, acute and higher doses of Δ^9-THC, CP55, 940 and anandamide suppressed motor activity and produced catalepsy

(Darmani and Pandya, 2000;Compton et al., 1993). This study showed that *R*-methanandamide caused a delay-dependent impairment at delay intervals greater than twenty one seconds. The doses use in study did not produce a deficit in motion during the task, suggesting that these drugs did not appear to affect other sensory abilities and the main effects could be cognitive. In vitro studies have also shown that *R*-methanandamide, at equivalent concentration, depressed excitatory transmission in the CA1 interneurons. Perhaps, at this dose, *R*-methanandamide effects on hippocampal firing characteristics can be produce mainly in behaving animals in increase burst duration and increase interspike interval through the activation other subset of hippocampal neurons or other non cannabinoid sensitive receptors. The drug effects on hippocampal firing characteristics are might be independent on the state of the pyramidal neurons and other unknown electrophysiological mechanisms.

Our results are consistent with the previous reports which showed that anandamide at higher does altered characteristics of pyramidal neurons and disrupted memory processing (Deadwyler et al., 1990;Deadwyler and Hampson, 1997). In the hippocampus, the activities of neurons can regularly alternate between burst firing and spiking modes and this mode are relevant to processes by which memories are form and stored in animals Moreover, several studies have shown that cannabinoid inhibition of firing occurs through the modulation of conductance at the GABA interneurons and glutamate neurons (Robbe et al., 2006;Itskov et al., 2008). In all, the integration and processing of information by the hippocampus is dependent on the activity and output of pyramidal neurons and the inhibitory action of interneurons, which synthesize and release GABA (Pastalkova et al., 2008;Itskov et al., 2008). In the hippocampus, selective activity of interneurons may change firing rates and modulate the synaptic strength of these inputs (Alger et al., 1996). Another mechanisms by which neuronal activity can be

regulated is through DSI, a form of short-term plasticity at GABAergic synapses (Kreitzer and Regehr, 2002;Alger, 2002). Results from our study suggested that, *R*-methanandamide, VDM11 and URB597 could have differential mechanism of action on the cannabinoid receptor. In addition, these drugs may also act on different receptors such as TRPV-1 or GPR55. URB597 has been shown to not only block the FAAH enzyme but also the MAGL lipase enzyme, an enzyme responsible for catabolism of 2AG. Furthermore, VDM11 has been shown to act as a partial agonist on the CB_1 receptor in addition to its transporter blocking effects (Szallasi and Di Marzo, 2000). VDM11, which unlike other similar inhibitors, has little effect on anandamide hydrolysis Anandamide is also a substrate for COX-2 enzyme and may also be TRP1 vallinoid agonists (Kozak and Marnett, 2002). The fact hippocampal pyramidal cells are mainly located in the CA1-CA3 regions of the hippocampus suggest that activation of the cannabinoid receptors in the neurons can occur through various mechanisms; however, the mechanism of activation with alteration in neuronal firing characteristics is not well defined.

Overall, it is also possible that anandamide production in the hippocampus, by indirectly inhibition of it hydrolysis production, may depend on the brain state. The use of anesthetics itself might influence the drug response as changes in average neuronal activities are more pronounced in awake animals than in anesthetized animals. This was shown despite the different length of the recording period. However, it remains unclear to what extent even basic properties of pyramidal neurons are influenced by anesthesia condition. It could be that, cannabinoid responses are mainly on a population of neurons while some neurons may not fully respond or are resistant to evoke spikes. In addition, these overall change in neuronal activates could due to the time course by which the drug acts on the neuron, the dose of drug or the type of anesthetic compound used in this study. However, it remains to be answered whether the changes in

neuronal firing patterns in anesthetized animals can be extrapolated to behavioral conditions. Arousal states have been shown to have profound effects in cognitive and behavioral processing involving in learning and other functions controlled by the hippocampus (Eichenbaum, 2001; Deadwyler and Hampson, 2008; Deadwyler et al., 2007; Manns and Eichenbaum, 2006).

The results in this study are difficult to interpret because compounds like *R*-methanandamide did not produce significant effects on neuronal activities in the anesthetized state but did so in the behaving state. It is also not clear why the effects of R-methanandamide are significant at twenty one second delay interval, and at sample phase but not non-match phase and not overall performance of the task. It is possible that that timing and the doses use are too small to show any significant effect. This alteration in hippocampal firings should be taken in caution because using FAAH inhibitor or inhibitors of the transporter in pharmacological studies in vivo alone does not entirely differentiate endocannabinoid actions as other receptor like vanilloid receptors have been recently found in brain regions, such as the hippocampus and cortex. Generally, the neuronal and behavioral effects produced by these cannabinoids in rats may vary as a function of dose, route of administration of the drug and the specific mechanisms of actions of drug used.

4.2 Future directions

The results from this study demonstrated that facilitation of anandamide levels in brain was closely related to changes in neurophysiology and memory processing. However, there are several unaddressed issues in study. The first challenge is training and learning of animals for the performance of the task and to test the effects of these drugs at higher doses and longer delays. This may provide further evidence for an involvement of the endocannabinoid system in memory processing in rats. In the present study, the time course of the anesthetic and the mechanism of action of the two effects were dissimilar. The second challenge of the study is to test chronic drug treatment and measure firing rates, as this may reflect the change in endocannabinoids levels induced by anandamide at the hippocampal synapse. The inclusion of a much higher dose would most likely have resulted in a significant effect of delay. Most studies have focused on the chronic effects of cannabinoid, rather on acute effects. However the use of other potent compounds and knowledge of the endocannabinoid systems may demonstrate how these compounds modulate hippocampal function and short term memory. The third challenge is to differentiate between inhibition of anandamide breakdown by FAAH and the role of 2-AG. Like anandamide, 2-AG is also synthesized and metabolized in hippocampal neuron. The main enzyme responsible for 2-AG metabolism is monoacylglycerol lipase which can be targeted with inhibitors such as URB602 and JZL184. The effects of these compounds on short term memory may provide evidence for an involvement of 2-AG in memory processing and further differentiate which of the main endocannabinoids are involved in producing these effects. The fourth and major challenge was to look at other the endocannabinoid pathways. COX-2, an enzyme that can also metabolize 2-AG or anandamide may also be studied. These pathways may regulate other unknown components of anandamide metabolism. Hence, targeting of putative

transporter and FAAH may not be the only means of influencing cannabinoid-mediated signaling and memory processing. The role of COX-2 in endocannabinoid signaling in neurons can be explored with the use of compounds that selectively inhibit COX-2.

Further studies will be needed to determine which of the several pharmacological actions are exerted by other receptor like the TRP1 vanilloid receptors. Many reports have shown that selective transport inhibitors can activates other receptor like the vanilloid receptor (Zygmunt et al., 2000a). Distinguish between the vanilloid-like and cannabinoid actions may also provide insight into the actions of anandamide at the cellular level. The fifth challenge is to assess the effects of *R*-methanandamide, VDM11 and URB597 on reversal learning. The reversal learning task has been shown to be associated with hippocampal activation by alteration of functional cell types in trained animals. In this type of task, a trained animal is allowed to respond differentially or in direct opposite to two stimuli such as matching to sample. This task may cause inhibition of the previously learned behavior and has been used as further neurophysiologic measurement of the hippocampal neurons and the extinction of memory processes. This may provide evidence for an involvement of the endocannabinoid system in behavioral flexibility or other adaptive behavior. This study has demonstrated some interesting findings but more experimentation is needed in terms of the number of animal subjects used, the type vehicle used in the anesthetized study are all important issues that need to be investigated in detail and the type anesthetic use in the anesthetic study.

4.3. Conclusion

In conclusion, we found that indirectly enhancing anandamide levels in the brain altered hippocampal neuronal firing rates. However, the relationships that exist between pyramidal neuron activity and endocannabinoid release are still not fully understood. It could be that different levels of anandamide occur when the animal is awake and performing a behavioral task, as opposed to under anesthetic conditions. We showed that the effects of anandamide on hippocampal neuronal signaling may be related to alteration of firing characteristics and that these effects of anandamide on pyramidal neurons is likely to have important consequences for information processing in the hippocampus. It is clear that in addition to the stable analogue of anandamide, FAAH inhibition and anandamide re-uptake transport blockade may be also be effective in elevating brain anandamide levels. In behaving rats, enhancing anandamide may be related to alteration in hippocampal firing characteristics at specific phases of the short term spatial memory task. Therefore, the modulation of the anandamide system might be responsible for producing the observed deficits in spatial learning and memory that develop after acute cannabis (Δ^9-THC) use. It is certain that endocannabinoid modulate pyramidal neurons in the hippocampus and alter their neuronal firing properties. Given the addictive and potentially therapeutic nature of marihuana, its extracts and synthetic analogues, the use of compounds that mimic the effect of direct acting cannabinoid agonists may be beneficial in studying the role of endocannabinoids in different physiological states.

Reference List

Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, Makriyannis A (1994) (R)-methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. J Med Chem 37:1889-1893.

Acquas E, Pisanu A, Marrocu P, Di CG (2000) Cannabinoid CB(1) receptor agonists increase rat cortical and hippocampal acetylcholine release in vivo. Eur J Pharmacol 401:179-185.

Acsady L, Gorcs TJ, Freund TF (1996) Different populations of vasoactive intestinal polypeptide-immunoreactive interneurons are specialized to control pyramidal cells or interneurons in the hippocampus. Neuroscience 73:317-334.

Acsady L, Kamondi A, Sik A, Freund T, Buzsaki G (1998) GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. J Neurosci 18:3386-3403.

Aggleton JP, Vann SD, Saunders RC (2005) Projections from the hippocampal region to the mammillary bodies in macaque monkeys. Eur J Neurosci 22:2519-2530.

Alger BE, Pitler TA (1995) Retrograde signaling at GABAA-receptor synapses in the mammalian CNS. Trends in Neurosciences 18:333-340.

Alger BE, Pitler TA, Wagner JJ, Martin LA, Morishita W, Kirov SA, Lenz RA (1996) Retrograde signalling in depolarization-induced suppression of inhibition in rat hippocampal CA1 cells. J Physiol 496 (Pt 1):197-209.

Alger E (2002) Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. Progress in Neurobiology 68:247-286.

Amaral DG, Witter MP (1989) The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 31:571-591.

Arreaza G, Devane WA, Omeir RL, Sajnani G, Kunz J, Cravatt BF, Deutsch DG (1997) The cloned rat hydrolytic enzyme responsible for the breakdown of anandamide also catalyzes its formation via the condensation of arachidonic acid and ethanolamine. Neurosci Lett 234:59-62.

Avraham Y, Menachem AB, Okun A, Zlotarav O, Abel N, Mechoulam R, Berry EM (2005) Effects of the endocannabinoid noladin ether on body weight, food consumption, locomotor activity, and cognitive index in mice. Brain Res Bull 65:117-123.

Balerio GN, Aso E, Berrendero F, Murtra P, Maldonado R (2004) Delta9-tetrahydrocannabinol decreases somatic and motivational manifestations of nicotine withdrawal in mice. Eur J Neurosci 20:2737-2748.

Bambico FR, Katz N, Debonnel G, Gobbi G (2007) Cannabinoids elicit antidepressant-like behavior and activate serotonergic neurons through the medial prefrontal cortex. J Neurosci 27:11700-11711.

Battisti RA, Roodenrys S, Johnstone SJ, Respondek C, Hermens DF, Solowij N (2010) Chronic use of cannabis and poor neural efficiency in verbal memory ability. Psychopharmacology (Berl) 209:319-330.

Bear MF, Malenka RC (1994) Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 4:389-399.

Beltramo M, Piomelli D (2000) Carrier-mediated transport and enzymatic hydrolysis of the endogenous cannabinoid 2-arachidonylglycerol. Neuroreport 11:1231-1235.

Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D (1997) Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 277:1094-1097.

Berger TW, Thompson RF (1978) Neuronal plasticity in the limbic system during classical conditioning of the rabbit nictitating membrane response. I. The hippocampus. Brain Res 145:323-346.

Biegon A, Kerman IA (2001) Autoradiographic study of pre- and postnatal distribution of cannabinoid receptors in human brain. Neuroimage 14:1463-1468.

Bisogno T, Ligresti A, Di Marzo V (2005) The endocannabinoid signalling system: biochemical aspects. Pharmacol Biochem Behav 81:224-238.

Bisogno T, Sepe N, Melck D, Maurelli S, De PL, Di Marzo V (1997) Biosynthesis, release and degradation of the novel endogenous cannabimimetic metabolite 2-arachidonoylglycerol in mouse neuroblastoma cells. Biochem J 322 (Pt 2):671-677.

Breivogel CS, Childers SR, Deadwyler SA, Hampson RE, Vogt LJ, Sim-Selley LJ (1999) Chronic delta9-tetrahydrocannabinol treatment produces a time-dependent loss of cannabinoid receptors and cannabinoid receptor-activated G proteins in rat brain. J Neurochem 73:2447-2459.

Bunsey M, Eichenbaum H (1996) Conservation of hippocampal memory function in rats and humans. Nature 379:255-257.

Buzsaki G (1986) Hippocampal sharp waves: their origin and significance. Brain Res 398:242-252.

Buzsaki G, Csicsvari J, Dragoi G, Harris K, Henze D, Hirase H (2002) Homeostatic maintenance of neuronal excitability by burst discharges in vivo. Cereb Cortex 12:893-899.

Cadas H, di TE, Piomelli D (1997) Occurrence and biosynthesis of endogenous cannabinoid precursor, N-arachidonoyl phosphatidylethanolamine, in rat brain. J Neurosci 17:1226-1242.

Cani PD, Montoya ML, Neyrinck AM, Delzenne NM, Lambert DM (2004) Potential modulation of plasma ghrelin and glucagon-like peptide-1 by anorexigenic cannabinoid compounds, SR141716A (rimonabant) and oleoylethanolamide. Br J Nutr 92:757-761.

Carta G, Nava F, Gessa GL (1998) Inhibition of hippocampal acetylcholine release after acute and repeated Delta9-tetrahydrocannabinol in rats. Brain Res 809:1-4.

Chakrabarti A, Onaivi ES, Chaudhuri G (1995) Cloning and sequencing of a cDNA encoding the mouse brain-type cannabinoid receptor protein. DNA Seq 5:385-388.

Cheer JF, Wassum KM, Heien ML, Phillips PE, Wightman RM (2004) Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. J Neurosci 24:4393-4400.

Chevaleyre V, Castillo PE (2003) Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron 38:461-472.

Chevaleyre V, Takahashi KA, Castillo PE (2006) Endocannabinoid-mediated synaptic plasticity in the CNS. Annu Rev Neurosci 29:37-76.

Childers SR, Pacheco MA, Bennett BA, Edwards TA, Hampson RE, Mu J, Deadwyler SA (1993) Cannabinoid receptors: G-protein-mediated signal transduction mechanisms. Biochem Soc Symp 59:27-50.

Cohen C, Perrault G, Voltz C, Steinberg R, Soubrie P (2002) SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav Pharmacol 13:451-463.

Collins DR, Pertwee RG, Davies SN (1995) Prevention by the cannabinoid antagonist, SR141716A, of cannabinoid-mediated blockade of long-term potentiation in the rat hippocampal slice. Br J Pharmacol 115:869-870.

Compton DR, Rice KC, De Costa BR, Razdan RK, Melvin LS, Johnson MR, Martin BR (1993) Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. J Pharmacol Exp Ther 265:218-226.

Cota D, Marsicano G, Lutz B, Vicennati V, Stalla GK, Pasquali R, Pagotto U (2003) Endogenous cannabinoid system as a modulator of food intake. Int J Obes Relat Metab Disord 27:289-301.

Cota D, Tschop MH, Horvath TL, Levine AS (2006) Cannabinoids, opioids and eating behavior: the molecular face of hedonism? Brain Res Rev 51:85-107.

Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996) Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. Nature 384:83-87.

Cravatt BF, Lichtman AH (2002) The enzymatic inactivation of the fatty acid amide class of signaling lipids. Chem Phys Lipids 121:135-148.

Daniel H, Levenes C, Crepel F (1998) Cellular mechanisms of cerebellar LTD. Trends Neurosci 21:401-407.

Darmani NA, Pandya DK (2000) Involvement of other neurotransmitters in behaviors induced by the cannabinoid CB1 receptor antagonist SR 141716A in naive mice. J Neural Transm 107:931-945.

Davies SN, Pertwee RG, Riedel G (2002) Functions of cannabinoid receptors in the hippocampus. Neuropharmacology 42:993-1007.

Davis M, Whalen PJ (2001) The amygdala: vigilance and emotion. Mol Psychiatry 6:13-34.

De MN, De PL, Orlando P, Daniele F, Fezza F, Di Marzo V (2003) Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis 2:5.

De PL, Cascio MG, Di Marzo V (2004) The endocannabinoid system: a general view and latest additions. Br J Pharmacol 141:765-774.

Deadwyler SA, Bunn T, Hampson RE (1996) Hippocampal ensemble activity during spatial delayed-nonmatch-to-sample performance in rats. J Neurosci 16:354-372.

Deadwyler SA, Goonawardena AV, Hampson RE (2007) Short-term memory is modulated by the spontaneous release of endocannabinoids: evidence from hippocampal population codes. Behav Pharmacol 18:571-580.

Deadwyler SA, Hampson RE (1997) The significance of neural ensemble codes during behavior and cognition. Annu Rev Neurosci 20:217-244.

Deadwyler SA, Hampson RE (1999) Anatomic model of hippocampal encoding of spatial information. Hippocampus 9:397-412.

Deadwyler SA, Hampson RE (2008) Endocannabinoids modulate encoding of sequential memory in the rat hippocampus. Psychopharmacology (Berl) 198:577-586.

Deadwyler SA, Heyser CJ, Michaelis RC, Hampson RE (1990) The effects of delta-9-THC on mechanisms of learning and memory. NIDA Res Monogr 97:79-93.

Deutsch DG, Chin SA (1993) Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. Biochem Pharmacol 46:791-796.

Deutsch DG, Glaser ST, Howell JM, Kunz JS, Puffenbarger RA, Hillard CJ, Abumrad N (2001) The cellular uptake of anandamide is coupled to its breakdown by fatty-acid amide hydrolase. J biol Chem 276:6967-6973.

Deutsch DG, Lin S, Hill WA, Morse KL, Salehani D, Arreaza G, Omeir RL, Makriyannis A (1997) Fatty acid sulfonyl fluorides inhibit anandamide metabolism and bind to the cannabinoid receptor. Biochem Biophys Res Commun 231:217-221.

Devane WA, Dysarz FA, III, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34:605-613.

Di Marzo V (1999) Biosynthesis and inactivation of endocannabinoids: relevance to their proposed role as neuromodulators. Life Sci 65:645-655.

Di Marzo V, Deutsch DG (1998) Biochemistry of the endogenous ligands of cannabinoid receptors. Neurobiol Dis 5:386-404.

Diamond A, Zola-Morgan S, Squire LR (1989) Successful performance by monkeys with lesions of the hippocampal formation on AB and object retrieval, two tasks that mark developmental changes in human infants. Behav Neurosci 103:526-537.

Doherty J, Dingledine R (2003) Functional interactions between cannabinoid and metabotropic glutamate receptors in the central nervous system. Curr Opin Pharmacol 3:46-53.

Eichenbaum H (2000) A cortical-hippocampal system for declarative memory. Nat Rev Neurosci 1:41-50.

Eichenbaum H (2004) Hippocampus: cognitive processes and neural representations that underlie declarative memory. Neuron 44:109-120.

Eichenbaum H, Dudchenko P, Wood E, Shapiro M, Tanila H (1999) The hippocampus, memory, and place cells: is it spatial memory or a memory space? Neuron 23:209-226.

Eichenbaum H, Dusek J, Young B, Bunsey M (1996a) Neural mechanisms of declarative memory. Cold Spring Harb Symp Quant Biol 61:197-206.

Eichenbaum H, Schoenbaum G, Young B, Bunsey M (1996b) Functional organization of the hippocampal memory system. Proc Natl Acad Sci U S A 93:13500-13507.

Eichenbaum H, Yonelinas AP, Ranganath C (2007) The medial temporal lobe and recognition memory. Annu Rev Neurosci 30:123-152.

Elphick MR, Egertova M (2001) The neurobiology and evolution of cannabinoid signalling. Philos Trans R Soc Lond B Biol Sci 356:381-408.

Eubanks LM, Rogers CJ, Beuscher AE, Koob GF, Olson AJ, Dickerson TJ, Janda KD (2006) A molecular link between the active component of marijuana and Alzheimer's disease pathology. Mol Pharm 3:773-777.

Evans DM, Lake JT, Johnson MR, Howlett AC (1994) Endogenous cannabinoid receptor binding activity released from rat brain slices by depolarization. J Pharmacol Exp Ther 268:1271-1277.

Farrant M, Kaila K (2007) The cellular, molecular and ionic basis of GABA(A) receptor signalling. Prog Brain Res 160:59-87.

Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, Lai Y, Ma AL, Mitchell RL (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol 48:443-450.

Felder CC, Nielsen A, Briley EM, Palkovits M, Priller J, Axelrod J, Nguyen DN, Richardson JM, Riggin RM, Koppel GA, Paul SM, Becker GW (1996) Isolation and measurement of the endogenous cannabinoid receptor agonist, anandamide, in brain and peripheral tissues of human and rat. FEBS Lett 393:231-235.

Ferrari F, Ottani A, Vivoli R, Giuliani D (1999) Learning impairment produced in rats by the cannabinoid agonist HU 210 in a water-maze task. Pharmacol Biochem Behav 64:555-561.

Ferraro L, Tomasini MC, Gessa GL, Bebe BW, Tanganelli S, Antonelli T (2001) The cannabinoid receptor agonist WIN 55,212-2 regulates glutamate transmission in rat cerebral cortex: an in vivo and in vitro study. Cereb Cortex 11:728-733.

Ferrer B, Bermudez-Silva FJ, Bilbao A, Alvarez-Jaimes L, Sanchez-Vera I, Giuffrida A, Serrano A, Baixeras E, Khaturia S, Navarro M, Parsons LH, Piomelli D, Rodriguez de FF (2007) Regulation of brain anandamide by acute administration of ethanol. Biochem J 404:97-104.

Fowler CJ, Tiger G, Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, Ramos JA (2003) Inhibition of fatty acid amidohydrolase, the enzyme responsible for the metabolism of the endocannabinoid anandamide, by analogues of arachidonoyl-serotonin. J Enzyme Inhib Med Chem 18:225-231.

Freedman NJ, Lefkowitz RJ (1996) Desensitization of G protein-coupled receptors. Recent Prog Horm Res 51:319-351.

Freund TF, Buzsaki G (1996) Interneurons of the hippocampus. Hippocampus 6:347-470.

Freund TF, Katona I, Piomelli D (2003) Role of endogenous cannabinoids in synaptic signaling. Physiol Rev 83:1017-1066.

Fride E, Mechoulam R (1993) Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. Eur J Pharmacol 231:313-314.

Frotscher M, Kugler P, Misgeld U, Zilles K (1988) Neurotransmission in the hippocampus. Adv Anat Embryol Cell Biol 111:1-103.

Gardner EL (2005) Endocannabinoid signaling system and brain reward: emphasis on dopamine. Pharmacol Biochem Behav 81:263-284.

Gatley SJ, Lan R, Pyatt B, Gifford AN, Volkow ND, Makriyannis A (1997) Binding of the non-classical cannabinoid CP 55,940, and the diarylpyrazole AM251 to rodent brain cannabinoid receptors. Life Sci 61:L-7.

Gerard C, Mollereau C, Vassart G, Parmentier M (1990) Nucleotide sequence of a human cannabinoid receptor cDNA. Nucleic Acids Res 18:7142.

Gifford AN, Ashby CR, Jr. (1996) Electrically evoked acetylcholine release from hippocampal slices is inhibited by the cannabinoid receptor agonist, WIN 55212-2, and is potentiated by the cannabinoid antagonist, SR 141716A. J Pharmacol Exp Ther 277:1431-1436.

Giuffrida A, Beltramo M, Piomelli D (2001) Mechanisms of endocannabinoid inactivation: biochemistry and pharmacology. J Pharmacol Exp Ther 298:7-14.

Giuffrida A, Parsons LH, Kerr TM, Rodriguez de FF, Navarro M, Piomelli D (1999) Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci 2:358-363.

Giuffrida A, Rodriguez de FF, Nava F, Loubet-Lescoulie P, Piomelli D (2000) Elevated circulating levels of anandamide after administration of the transport inhibitor, AM404. Eur J Pharmacol 408:161-168.

Glass M, Dragunow M, Faull RL (1997) Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience 77:299-318.

Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D (2005) Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc Natl Acad Sci U S A 102:18620-18625.

Goonawardena AV, Riedel G, Hampson RE (2010a) Cannabinoids alter spontaneous firing, bursting, and cell synchrony of hippocampal principal cells. Hippocampus.

Goonawardena AV, Robinson L, Hampson RE, Riedel G (2010b) Cannabinoid and cholinergic systems interact during performance of a short-term memory task in the rat. Learn Mem 17:502-511.

Gulyas AI, Megias M, Emri Z, Freund TF (1999) Total number and ratio of excitatory and inhibitory synapses converging onto single interneurons of different types in the CA1 area of the rat hippocampus. J Neurosci 19:10082-10097.

Haj-Dahmane S, Shen RY (2009) Endocannabinoids suppress excitatory synaptic transmission to dorsal raphe serotonin neurons through the activation of presynaptic CB1 receptors. J Pharmacol Exp Ther 331:186-196.

Hajos N, Katona I, Naiem SS, Mackie K, Ledent C, Mody I, Freund TF (2000) Cannabinoids inhibit hippocampal GABAergic transmission and network oscillations. Eur J Neurosci 12:3239-3249.

Hall W, Degenhardt L, Teesson M (2004) Cannabis use and psychotic disorders: an update. Drug Alcohol Rev 23:433-443.

Hampson RE, Deadwyler SA (1996) Ensemble codes involving hippocampal neurons are at risk during delayed performance tests. Proc Natl Acad Sci U S A 93:13487-13493.

Hampson RE, Deadwyler SA (1998) Role of cannabinoid receptors in memory storage. Neurobiol Dis 5:474-482.

Hampson RE, Deadwyler SA (1999) Cannabinoids, hippocampal function and memory. Life Sci 65:715-723.

Hampson RE, Deadwyler SA (2000) Cannabinoids reveal the necessity of hippocampal neural encoding for short-term memory in rats. J Neurosci 20:8932-8942.

Hampson RE, Deadwyler SA (2003) Temporal firing characteristics and the strategic role of subicular neurons in short-term memory. Hippocampus 13:529-541.

Hampson RE, Heyser CJ, Deadwyler SA (1993) Hippocampal cell firing correlates of delayed-match-to-sample performance in the rat. Behav Neurosci 107:715-739.

Hampson RE, Jarrard LE, Deadwyler SA (1999) Effects of ibotenate hippocampal and extrahippocampal destruction on delayed-match and -nonmatch-to-sample behavior in rats. J Neurosci 19:1492-1507.

Hampson RE, Simeral JD, Deadwyler SA (2001) What ensemble recordings reveal about functional hippocampal cell encoding. Prog Brain Res 130:345-357.

Hampson RE, Simeral JD, Kelly EJ, Deadwyler SA (2003) Tolerance to the memory disruptive effects of cannabinoids involves adaptation by hippocampal neurons. Hippocampus 13:543-556.

Hanus L, Gopher A, Almog S, Mechoulam R (1993) Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. J Med Chem 36:3032-3034.

Hao S, Avraham Y, Mechoulam R, Berry EM (2000) Low dose anandamide affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice. Eur J Pharmacol 392:147-156.

Hassabis D, Chu C, Rees G, Weiskopf N, Molyneux PD, Maguire EA (2009) Decoding neuronal ensembles in the human hippocampus. Curr Biol 19:546-554.

Henze DA, Buzsaki G (2001) Action potential threshold of hippocampal pyramidal cells in vivo is increased by recent spiking activity. Neuroscience 105:121-130.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, De Costa BR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. J Neurosci 11:563-583.

Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, De Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A 87:1932-1936.

Hernandez-Tristan R, Arevalo C, Canals S, Leret ML (2000) The effects of acute treatment with delta9-THC on exploratory behaviour and memory in the rat. J Physiol Biochem 56:17-24.

Herremans AH, Hijzen TH (1997) The delayed-conditional-discrimination task improves measurement of working memory in rats. Neurosci Biobehav Rev 21:371-379.

Heyser CJ, Hampson RE, Deadwyler SA (1993) Effects of delta-9-tetrahydrocannabinol on delayed match to sample performance in rats: alterations in short-term memory associated with changes in task specific firing of hippocampal cells. J Pharmacol Exp Ther 264:294-307.

Hillard CJ, Manna S, Greenberg MJ, DiCamelli R, Ross RA, Stevenson LA, Murphy V, Pertwee RG, Campbell WB (1999) Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). J Pharmacol Exp Ther 289:1427-1433.

Hillard CJ, Wilkison DM, Edgemond WS, Campbell WB (1995) Characterization of the kinetics and distribution of N-arachidonylethanolamine (anandamide) hydrolysis by rat brain. Biochim Biophys Acta 1257:249-256.

Hoffman DA, Magee JC, Colbert CM, Johnston D (1997) K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. Nature 387:869-875.

Hogestatt ED, Jonsson BA, Ermund A, Andersson DA, Bjork H, Alexander JP, Cravatt BF, Basbaum AI, Zygmunt PM (2005) Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. J biol Chem 280:31405-31412.

Howlett AC (1985) Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. Mol Pharmacol 27:429-436.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG (2002) International Union of Pharmacology. XXVII. Classification of Cannabinoid Receptors. Pharmacological Reviews 54:161-202.

Howlett AC, Bidaut-Russell M, Devane WA, Melvin LS, Johnson MR, Herkenham M (1990) The cannabinoid receptor: biochemical, anatomical and behavioral characterization. Trends Neurosci 13:420-423.

Howlett AC, Qualy JM, Khachatrian LL (1986) Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol Pharmacol 29:307-313.

Howlett AC, Scott DK, Wilken GH (1989) Regulation of adenylate cyclase by cannabinoid drugs. Insights based on thermodynamic studies. Biochem Pharmacol 38:3297-3304.

Howlett AC, Song C, Berglund BA, Wilken GH, Pigg JJ (1998) Characterization of CB1 cannabinoid receptors using receptor peptide fragments and site-directed antibodies. Mol Pharmacol 53:504-510.

Hsieh C, Brown S, Derleth C, Mackie K (1999) Internalization and recycling of the CB1 cannabinoid receptor. J Neurochem 73:493-501.

Huffman JW, Yu S, Showalter V, Abood ME, Wiley JL, Compton DR, Martin BR, Bramblett RD, Reggio PH (1996) Synthesis and pharmacology of a very potent cannabinoid lacking a phenolic hydroxyl with high affinity for the CB2 receptor. J Med Chem 39:3875-3877.

Hurst DP, Lynch DL, Barnett-Norris J, Hyatt SM, Seltzman HH, Zhong M, Song ZH, Nie J, Lewis D, Reggio PH (2002) N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-p yrazole-3-carboxamide (SR141716A) interaction with LYS 3.28(192) is crucial for its inverse agonism at the cannabinoid CB1 receptor. Mol Pharmacol 62:1274-1287.

Irving AJ, Coutts AA, Harvey J, Rae MG, Mackie K, Bewick GS, Pertwee RG (2000) Functional expression of cell surface cannabinoid CB(1) receptors on presynaptic inhibitory terminals in cultured rat hippocampal neurons. Neuroscience 98:253-262.

Itskov V, Pastalkova E, Mizuseki K, Buzsaki G, Harris KD (2008) Theta-mediated dynamics of spatial information in hippocampus. J Neurosci 28:5959-5964.

Jagerovic N, Fernandez-Fernandez C, Goya P (2008) CB1 cannabinoid antagonists: structure-activity relationships and potential therapeutic applications. Curr Top Med Chem 8:205-230.

Jarbe TU, Sheppard R, Lamb RJ, Makriyannis A, Lin S, Goutopoulos A (1998) Effects of delta-9-tetrahydrocannabinol and (R)-methanandamide on open-field behavior in rats. Behav Pharmacol 9:169-174.

Jbilo O, Ravinet-Trillou C, Arnone M, Buisson I, Bribes E, Peleraux A, Penarier G, Soubrie P, Le FG, Galiegue S, Casellas P (2005) The CB1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. FASEB J 19:1567-1569.

Kathmann M, Weber B, Zimmer A, Schlicker E (2001) Enhanced acetylcholine release in the hippocampus of cannabinoid CB(1) receptor-deficient mice. Br J Pharmacol 132:1169-1173.

Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La RG, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D (2003) Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med 9:76-81.

Katona I, Sperlagh B, Magloczky Z, Santha E, Kofalvi A, Czirjak S, Mackie K, Vizi ES, Freund TF (2000) GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. Neuroscience 100:797-804.

Kelai S, Hanoun N, Aufrere G, Beauge F, Hamon M, Lanfumey L (2006) Cannabinoid-serotonin interactions in alcohol-preferring vs. alcohol-avoiding mice. J Neurochem 99:308-320.

Khazipov R, Ragozzino D, Bregestovski P (1995) Kinetics and Mg2+ block of N-methyl-D-aspartate receptor channels during postnatal development of hippocampal CA3 pyramidal neurons. Neuroscience 69:1057-1065.

Kim J, Isokawa M, Ledent C, Alger BE (2002) Activation of muscarinic acetylcholine receptors enhances the release of endogenous cannabinoids in the hippocampus. J Neurosci 22:10182-10191.

Kirby MT, Hampson RE, Deadwyler SA (2000) Cannabinoid receptor activation in CA1 pyramidal cells in adult rat hippocampus. Brain Res 863:120-131.

Kirkham TC, Williams CM (2004) Endocannabinoid receptor antagonists: potential for obesity treatment. Treat Endocrinol 3:345-360.

Koga D, Santa T, Hagiwara K, Imai K, Takizawa H, Nagano T, Hirobe M, Ogawa M, Sato T, Inoue K, . (1995) High-performance liquid chromatography and fluorometric detection of arachidonylethanolamide (anandamide) and its analogues, derivatized with 4-(N-chloroformylmethyl-N-methyl)amino-7-N,N-dimethylaminosulp honyl-2,1 ,3- benzoxadiazole (DBD-COCl). Biomed Chromatogr 9:56-57.

Kozak KR, Marnett LJ (2002) Oxidative metabolism of endocannabinoids. Prostaglandins Leukot Essent Fatty Acids 66:211-220.

Kreitzer AC, Regehr WG (2002) Retrograde signaling by endocannabinoids. Curr Opin Neurobiol 12:324-330.

Lan R, Gatley J, Lu Q, Fan P, Fernando SR, Volkow ND, Pertwee R, Makriyannis A (1999) Design and synthesis of the CB1 selective cannabinoid antagonist AM281: a potential human SPECT ligand. AAPS PharmSci 1:E4.

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F, Bohme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, Parmentier M (1999) Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. Science 283:401-404.

Lee AK, Manns ID, Sakmann B, Brecht M (2006) Whole-cell recordings in freely moving rats. Neuron 51:399-407.

Lenz RA, Alger BE (1999) Calcium dependence of depolarization-induced suppression of inhibition in rat hippocampal CA1 pyramidal neurons. J Physiol 521 Pt 1:147-157.

Lichtman AH (2000) SR 141716A enhances spatial memory as assessed in a radial-arm maze task in rats. Eur J Pharmacol 404:175-179.

Lichtman AH, Dimen KR, Martin BR (1995) Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. Psychopharmacology (Berl) 119:282-290.

Lichtman AH, Hawkins EG, Griffin G, Cravatt BF (2002) Pharmacological activity of fatty acid amides is regulated, but not mediated, by fatty acid amide hydrolase in vivo. J Pharmacol Exp Ther 302:73-79.

Lichtman AH, Martin BR (1996) Delta 9-tetrahydrocannabinol impairs spatial memory through a cannabinoid receptor mechanism. Psychopharmacology (Berl) 126:125-131.

Lichtman AH, Shelton CC, Advani T, Cravatt BF (2004) Mice lacking fatty acid amide hydrolase exhibit a cannabinoid receptor-mediated phenotypic hypoalgesia. Pain 109:319-327.

Liu J, Li H, Burstein SH, Zurier RB, Chen JD (2003) Activation and binding of peroxisome proliferator-activated receptor gamma by synthetic cannabinoid ajulemic acid. Mol Pharmacol 63:983-992.

Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim HY, Kunos G (2006) A biosynthetic pathway for anandamide. Proc Natl Acad Sci U S A 103:13345-13350.

Lopez-Rodriguez ML, Viso A, Ortega-Gutierrez S, Fowler CJ, Tiger G, de LE, Fernandez-Ruiz J, Ramos JA (2003) Design, synthesis and biological evaluation of new endocannabinoid transporter inhibitors. Eur J Med Chem 38:403-412.

Lupica CR, Riegel AC, Hoffman AF (2004) Marijuana and cannabinoid regulation of brain reward circuits. Br J Pharmacol 143:227-234.

Maccarrone M, Attina M, Cartoni A, Bari M, Finazzi-Agro A (2001) Gas chromatography-mass spectrometry analysis of endogenous cannabinoids in healthy and tumoral human brain and human cells in culture. J Neurochem 76:594-601.

Maccarrone M, Cartoni A, Parolaro D, Margonelli A, Massi P, Bari M, Battista N, Finazzi-Agro A (2002) Cannabimimetic activity, binding, and degradation of stearoylethanolamide within the mouse central nervous system. Mol Cell Neurosci 21:126-140.

Mackie K (2005) Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol299-325.

Mackie K, Hille B (1992) Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. Proc Natl Acad Sci U S A 89:3825-3829.

Magee J, Hoffman D, Colbert C, Johnston D (1998) Electrical and calcium signaling in dendrites of hippocampal pyramidal neurons. Annu Rev Physiol 60:327-346.

Maguire EA, Hassabis D (2011) Role of the hippocampus in imagination and future thinking. Proc Natl Acad Sci U S A 108:E39.

Makara JK, Mor M, Fegley D, Szabo SI, Kathuria S, Astarita G, Duranti A, Tontini A, Tarzia G, Rivara S, Freund TF, Piomelli D (2005) Selective inhibition of 2-AG hydrolysis enhances endocannabinoid signaling in hippocampus. Nat Neurosci 8:1139-1141.

Maldonado R, Valverde O, Berrendero F (2006) Involvement of the endocannabinoid system in drug addiction. Trends Neurosci 29:225-232.

Malkova L, Mishkin M, Bachevalier J (1995) Long-term effects of selective neonatal temporal lobe lesions on learning and memory in monkeys. Behav Neurosci 109:212-226.

Manns JR, Hopkins RO, Reed JM, Kitchener EG, Squire LR (2003) Recognition memory and the human hippocampus. Neuron 37:171-180.

Marsicano G, Lutz B (2006) Neuromodulatory functions of the endocannabinoid system. J Endocrinol Invest 29:27-46.

Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglgansberger W, Di Marzo V, Lutz B (2002) The endogenous cannabinoid system controls extinction of aversive memories. Nature 418:530-534.

Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, Johnson MR, Melvin LS, Mechoulam R, Ward SJ (1991) Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. Pharmacol Biochem Behav 40:471-478.

Martin SJ, Grimwood PD, Morris RG (2000) Synaptic plasticity and memory: an evaluation of the hypothesis. Annu Rev Neurosci 23:649-711.

Matsuda LA (1997) Molecular aspects of cannabinoid receptors. Crit Rev Neurobiol 11:143-166.

Matsuda LA, Bonner TI, Lolait SJ (1993) Localization of cannabinoid receptor mRNA in rat brain. J Comp Neurol 327:535-550.

Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, . (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50:83-90.

Mechoulam R, Parker LA, Gallily R (2002) Cannabidiol: an overview of some pharmacological aspects. J Clin Pharmacol 42:11S-19S.

Megias M, Emri Z, Freund TF, Gulyas AI (2001) Total number and distribution of inhibitory and excitatory synapses on hippocampal CA1 pyramidal cells. Neuroscience 102:527-540.

Misner DL, Sullivan JM (1999) Mechanism of cannabinoid effects on long-term potentiation and depression in hippocampal CA1 neurons. J Neurosci 19:6795-6805.

Monk CS, Zhuang J, Curtis WJ, Ofenloch IT, Tottenham N, Nelson CA, Hu X (2002) Human hippocampal activation in the delayed matching- and nonmatching-to-sample memory tasks: an event-related functional MRI approach. Behav Neurosci 116:716-721.

Moore NL, Greenleaf AL, Acheson SK, Wilson WA, Swartzwelder HS, Kuhn CM (2010) Role of cannabinoid receptor type 1 desensitization in greater tetrahydrocannabinol impairment of memory in adolescent rats. J Pharmacol Exp Ther 335:294-301.

Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. Nature 297:681-683.

Nemeth B, Ledent C, Freund TF, Hajos N (2008) CB1 receptor-dependent and -independent inhibition of excitatory postsynaptic currents in the hippocampus by WIN 55,212-2. Neuropharmacology 54:51-57.

Nicoll RA, Kauer JA, Malenka RC (1988) The current excitement in long-term potentiation. Neuron 1:97-103.

Nicoll RA, Malenka RC (1995) Contrasting properties of two forms of long-term potentiation in the hippocampus. Nature 377:115-118.

O'Keefe J, Dostrovsky J (1971) The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Res 34:171-175.

O'Keefe J, Recce ML (1993) Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus 3:317-330.

Ohno-Shosaku T, Maejima T, Kano M (2001) Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron 29:729-738.

Olbrich HG, Braak H (1985) Ratio of pyramidal cells versus non-pyramidal cells in sector CA1 of the human Ammon's horn. Anat Embryol (Berl) 173:105-110.

Onaivi ES, Chakrabarti A, Chaudhuri G (1996) Cannabinoid receptor genes. Prog Neurobiol 48:275-305.

Oropeza VC, Mackie K, Van Bockstaele EJ (2007) Cannabinoid receptors are localized to noradrenergic axon terminals in the rat frontal cortex. Brain Res 1127:36-44.

Ottersen OP, Storm-Mathisen J (1986) Excitatory amino acid pathways in the brain. Adv Exp Med Biol 203:263-284.

Otto T, Eichenbaum H (1992) Neuronal activity in the hippocampus during delayed non-match to sample performance in rats: evidence for hippocampal processing in recognition memory. Hippocampus 2:323-334.

Pacher P, Batkai S, Kunos G (2006) The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 58:389-462.

Palmer SL, Thakur GA, Makriyannis A (2002) Cannabinergic ligands. Chem Phys Lipids 121:3-19.

Pastalkova E, Itskov V, Amarasingham A, Buzsaki G (2008) Internally generated cell assembly sequences in the rat hippocampus. Science 321:1322-1327.

Paxinos G, Watson C, Pennisi M, Topple A (1985) Bregma, lambda and the interaural midpoint in stereotaxic surgery with rats of different sex, strain and weight. J Neurosci Methods 13:139-143.

Pertwee RG (2006) The pharmacology of cannabinoid receptors and their ligands: an overview. Int J Obes (Lond) 30 Suppl 1:S13-S18.

Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA (2010) International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB and CB. Pharmacol Rev 62:588-631.

Pertwee RG, Ross RA (2002) Cannabinoid receptors and their ligands. Prostaglandins Leukot Essent Fatty Acids 66:101-121.

Piomelli D (2003) The molecular logic of endocannabinoid signalling. Nat Rev Neurosci 4:873-884.

Piomelli D, Beltramo M, Glasnapp S, Lin SY, Goutopoulos A, Xie XQ, Makriyannis A (1999) Structural determinants for recognition and translocation by the anandamide transporter. Proc Natl Acad Sci U S A 96:5802-5807.

Piomelli D, Tarzia G, Duranti A, Tontini A, Mor M, Compton TR, Dasse O, Monaghan EP, Parrott JA, Putman D (2006) Pharmacological profile of the selective FAAH inhibitor KDS-4103 (URB597). CNS Drug Rev 12:21-38.

Pontecorvo MJ, Sahgal A, Steckler T (1996) Further developments in the measurement of working memory in rodents. Brain Res Cogn Brain Res 3:205-213.

Raisman G, Ebner FF (1983) Mossy fibre projections into and out of hippocampal transplants. Neuroscience 9:783-801.

Ranganathan M, D'Souza DC (2006) The acute effects of cannabinoids on memory in humans: a review. Psychopharmacology (Berl) 188:425-444.

Razdan RK (1986) Structure-activity relationships in cannabinoids. Pharmacol Rev 38:75-149.

Reyes BA, Rosario JC, Piana PM, Van Bockstaele EJ (2009) Cannabinoid modulation of cortical adrenergic receptors and transporters. J Neurosci Res 87:3671-3678.

Rhee MH, Vogel Z, Barg J, Bayewitch M, Levy R, Hanus L, Breuer A, Mechoulam R (1997) Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. J Med Chem 40:3228-3233.

Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, . (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350:240-244.

Rinaldi-Carmona M, Barth F, Millan J, Derocq JM, Casellas P, Congy C, Oustric D, Sarran M, Bouaboula M, Calandra B, Portier M, Shire D, Breliere JC, Le Fur GL (1998) SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J Pharmacol Exp Ther 284:644-650.

Robbe D, Montgomery SM, Thome A, Rueda-Orozco PE, McNaughton BL, Buzsaki G (2006) Cannabinoids reveal importance of spike timing coordination in hippocampal function. Nat Neurosci 9:1526-1533.

Robinson L, Goonawardena AV, Pertwee R, Hampson RE, Platt B, Riedel G (2010) WIN55,212-2 induced deficits in spatial learning are mediated by cholinergic hypofunction. Behav Brain Res 208:584-592.

Robinson L, Hinder L, Pertwee RG, Riedel G (2003) Effects of delta9-THC and WIN-55,212-2 on place preference in the water maze in rats. Psychopharmacology (Berl) 166:40-50.

Robinson L, McKillop-Smith S, Ross NL, Pertwee RG, Hampson RE, Platt B, Riedel G (2008) Hippocampal endocannabinoids inhibit spatial learning and limit spatial memory in rats. Psychopharmacology (Berl) 198:551-563.

Rodriguez de FF, Del A, I, Bermudez-Silva FJ, Bilbao A, Cippitelli A, Navarro M (2005) The endocannabinoid system: physiology and pharmacology. Alcohol Alcohol 40:2-14.

Ryberg E, Vu HK, Larsson N, Groblewski T, Hjorth S, Elebring T, Sjogren S, Greasley PJ (2005) Identification and characterisation of a novel splice variant of the human CB1 receptor. FEBS Lett 579:259-264.

Salamone JD, McLaughlin PJ, Sink K, Makriyannis A, Parker LA (2007) Cannabinoid CB1 receptor inverse agonists and neutral antagonists: effects on food intake, food-reinforced behavior and food aversions. Physiol Behav 91:383-388.

Schacter DL (1992) Implicit knowledge: new perspectives on unconscious processes. Proc Natl Acad Sci U S A 89:11113-11117.

Shen M, Piser TM, Seybold VS, Thayer SA (1996) Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci 16:4322-4334.

Showalter VM, Compton DR, Martin BR, Abood ME (1996) Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. J Pharmacol Exp Ther 278:989-999.

Silva de Melo LC, Cruz AP, Rios Valentim SJJ, Marinho AR, Mendonca JB, Nakamura-Palacios EM (2005) Delta(9)-THC administered into the medial prefrontal cortex disrupts the spatial working memory. Psychopharmacology (Berl) 183:54-64.

Simic G, Kostovic I, Winblad B, Bogdanovic N (1997) Volume and number of neurons of the human hippocampal formation in normal aging and Alzheimer's disease. J Comp Neurol 379:482-494.

Skovronsky DM, Lee VM, Trojanowski JQ (2006) Neurodegenerative diseases: new concepts of pathogenesis and their therapeutic implications. Annu Rev Pathol 1:151-170.

Solowij N, Battisti R (2008) The chronic effects of cannabis on memory in humans: a review. Curr Drug Abuse Rev 1:81-98.

Somogyi P, Freund TF, Hodgson AJ, Somogyi J, Beroukas D, Chubb IW (1985) Identified axo-axonic cells are immunoreactive for GABA in the hippocampus and visual cortex of the cat. Brain Res 332:143-149.

Song C, Howlett AC (1995) Rat brain cannabinoid receptors are N-linked glycosylated proteins. Life Sci 56:1983-1989.

Squire LR (1993) The hippocampus and spatial memory. Trends Neurosci 16:56-57.

Squire LR, Davis HP (1981) The pharmacology of memory: a neurobiological perspective. Annu Rev Pharmacol Toxicol 21:323-356.

Squire LR, Zola-Morgan S (1991) The medial temporal lobe memory system. Science 253:1380-1386.

Staddon JE, Cerutti DT (2003) Operant conditioning. Annu Rev Psychol 54:115-144.

Stella N, Schweitzer P, Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. Nature 388:773-778.

Stubbs L, Chittenden L, Chakrabarti A, Onaivi E (1996) The gene encoding the central cannabinoid receptor is located in proximal mouse Chromosome 4. Mamm Genome 7:165-166.

Sugiura T, Kobayashi Y, Oka S, Waku K (2002) Biosynthesis and degradation of anandamide and 2-arachidonoylglycerol and their possible physiological significance. Prostaglandins Leukot Essent Fatty Acids 66:173-192.

Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 215:89-97.

Sugiura T, Kondo S, Sukagawa A, Tonegawa T, Nakane S, Yamashita A, Ishima Y, Waku K (1996) Transacylase-mediated and phosphodiesterase-mediated synthesis of N-arachidonoylethanolamine, an endogenous cannabinoid-receptor ligand, in rat brain microsomes. Comparison with synthesis from free arachidonic acid and ethanolamine. Eur J Biochem 240:53-62.

Sullivan JM (2000) Cellular and molecular mechanisms underlying learning and memory impairments produced by cannabinoids. Learn Mem 7:132-139.

Sundram S, Copolov D, Dean B (2005) Clozapine decreases [3H] CP 55940 binding to the cannabinoid 1 receptor in the rat nucleus accumbens. Naunyn Schmiedebergs Arch Pharmacol 371:428-433.

Suzuki A, Josselyn SA, Frankland PW, Masushige S, Silva AJ, Kida S (2004) Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J Neurosci 24:4787-4795.

Swanson LW, Wyss JM, Cowan WM (1978) An autoradiographic study of the organization of intrahippocampal association pathways in the rat. J Comp Neurol 181:681-715.

Szallasi A, Di Marzo V (2000) New perspectives on enigmatic vanilloid receptors. Trends Neurosci 23:491-497.

Tarzia G, Duranti A, Gatti G, Piersanti G, Tontini A, Rivara S, Lodola A, Plazzi PV, Mor M, Kathuria S, Piomelli D (2006) Synthesis and structure-activity relationships of FAAH inhibitors: cyclohexylcarbamic acid biphenyl esters with chemical modulation at the proximal phenyl ring. ChemMedChem 1:130-139.

Tarzia G, Duranti A, Tontini A, Piersanti G, Mor M, Rivara S, Plazzi PV, Park C, Kathuria S, Piomelli D (2003) Design, synthesis, and structure-activity relationships of alkylcarbamic acid aryl esters, a new class of fatty acid amide hydrolase inhibitors. J Med Chem 46:2352-2360.

Teng E, Squire LR (1999) Memory for places learned long ago is intact after hippocampal damage. Nature 400:675-677.

Terranova JP, Michaud JC, Le FG, Soubrie P (1995a) Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors. Naunyn Schmiedebergs Arch Pharmacol 352:576-579.

Terranova JP, Michaud JC, Le FG, Soubrie P (1995b) Inhibition of long-term potentiation in rat hippocampal slices by anandamide and WIN55212-2: reversal by SR141716 A, a selective antagonist of CB1 cannabinoid receptors. Naunyn Schmiedebergs Arch Pharmacol 352:576-579.

Terranova JP, Storme JJ, Lafon N, Perio A, Rinaldi-Carmona M, Le FG, Soubrie P (1996) Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716. Psychopharmacology (Berl) 126:165-172.

Tsou K, Mackie K, Sanudo-Pena MC, Walker JM (1999) Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. Neuroscience 93:969-975.

Tucci SA, Rogers EK, Korbonits M, Kirkham TC (2004) The cannabinoid CB1 receptor antagonist SR141716 blocks the orexigenic effects of intrahypothalamic ghrelin. Br J Pharmacol 143:520-523.

Tulving E (1987) Multiple memory systems and consciousness. Hum Neurobiol 6:67-80.

Tulving E (1992) Memory systems and the brain. Clin Neuropharmacol 15 Suppl 1 Pt A:327A-328A.

Twitchell W, Brown S, Mackie K (1997) Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. J Neurophysiol 78:43-50.

Valjent E, Mitchell JM, Besson MJ, Caboche J, Maldonado R (2002) Behavioural and biochemical evidence for interactions between Delta 9-tetrahydrocannabinol and nicotine. Br J Pharmacol 135:564-578.

Valverde O, Ledent C, Beslot F, Parmentier M, Roques BP (2000) Reduction of stress-induced analgesia but not of exogenous opioid effects in mice lacking CB1 receptors. Eur J Neurosci 12:533-539.

Vandevoorde S, Lambert DM (2007) The multiple pathways of endocannabinoid metabolism: a zoom out. Chem Biodivers 4:1858-1881.

White NM, McDonald RJ (2002) Multiple parallel memory systems in the brain of the rat. Neurobiol Learn Mem 77:125-184.

Wiley JL, Compton DR, Dai D, Lainton JA, Phillips M, Huffman JW, Martin BR (1998) Structure-activity relationships of indole- and pyrrole-derived cannabinoids. J Pharmacol Exp Ther 285:995-1004.

Wiley JL, Martin BR (2002) Cannabinoid pharmacology: implications for additional cannabinoid receptor subtypes. Chem Phys Lipids 121:57-63.

Wilson RI, Kunos G, Nicoll RA (2001) Presynaptic specificity of endocannabinoid signaling in the hippocampus. Neuron 31:453-462.

Wilson RI, Nicoll RA (2001) Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 410:588-592.

Wilson RI, Nicoll RA (2002) Endocannabinoid signaling in the brain. Science 296:678-682.

Wolff MC, Leander JD (2003) SR141716A, a cannabinoid CB1 receptor antagonist, improves memory in a delayed radial maze task. Eur J Pharmacol 477:213-217.

Yoshida T, Hashimoto K, Zimmer A, Maejima T, Araishi K, Kano M (2002) The cannabinoid CB1 receptor mediates retrograde signals for depolarization-induced suppression of inhibition in cerebellar Purkinje cells. J Neurosci 22:1690-1697.

Zola SM, Squire LR, Teng E, Stefanacci L, Buffalo EA, Clark RE (2000) Impaired recognition memory in monkeys after damage limited to the hippocampal region. J Neurosci 20:451-463.

Zola-Morgan S, Squire LR, Amaral DG (1986) Human amnesia and the medial temporal region: enduring memory impairment following a bilateral lesion limited to field CA1 of the hippocampus. J Neurosci 6:2950-2967.

Zygmunt PM, Julius I, Di M, I, Hogestatt ED (2000a) Anandamide - the other side of the coin. Trends Pharmacol Sci 21:43-44.

Zygmunt PM, Sorgard M, Petersson J, Johansson R, Hogestatt ED (2000b) Differential actions of anandamide, potassium ions and endothelium-derived hyperpolarizing factor in guinea-pig basilar artery. Naunyn Schmiedebergs Arch Pharmacol 361:535-542.

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EDUCATION

M.S. Physiology and Pharmacology, Wake Forest University, Winston-Salem, NC 2007 - 2011

M.S Biology, North Carolina Central University Durham, N.C   2004 – 2007

B.Sc (Hons) Biology, North Carolina Central University Durham, N.C   2002 – 2004

TRAINING

Research Assistant   Fall 2002 –Spring 2004
Biomedical Biotechnology Research Institute, Durham, N.C

Peer Program   Summer 2004
Duke University Medical Center and University of North Carolina Chapel Hill, Durham, N.C

Medical Education Development Program   Summer 2003
Wake Forest University School of Medicine, Winston-Salem, NC
Summer research opportunity program for minority student

AWARDS

Dean's list   2002-2004
GlaxoSmithKline Scholar   2003-2004
Golden Key Honor Society Inductee   2002-2003

PUBLICATIONS-COAUTHOR

Moorman PG, **Sesay J**, Nwosu V, Kane JG, de Cotret AR, Worley K, Millikan R. Cyclooxygenase-2 polymorphism (Val511Ala), nonsteroidal anti-inflammatory drug use and breast cancer in African American women. Cancer Epidemiology Biomarkers Prevention. 2005

Sesay J, Somasundaram C, Diz DI, Bukoski RD, Howlett AC Awumey EM.
N18TG2 neuroblastoma cells express a Calcium ion sensing receptor coupled to intracellular Calcium ion signaling. Submitted.-ASPET- Molecular Pharmacology

Chan RH, Song D, Goonawardena AV, Bough S, **Sesay J**, Hampson RE, Deadwyler SA, Berger TW. Changes of hippocampal CA3-CA1 population nonlinear dynamics across different training sessions in rats performing a memory-dependent task. Conference Proc IEEE Eng Med Biol Soc. 2010

Goonawardena AV*, **Sesay J***, Sexton CA, Riedel G, Hampson RE.

Pharmacological elevation of anandamide impairs short-term memory by altering the neurophysiology in the hippocampus- Neuropharmacology. 2011(*Contributed equally)

THESIS

Effect of enhancing anandamide levels on hippocampal neurophysiology and short-term memory processing in rats:
Advisor: Robert Hampson. PhD. Wake Forest University

Measurement of intracellular calcium ions mobilization in Neuroblastoma cells:
Advisors: Emmanuel M Awumey, PhD, Allyn Howlett, PhD. and. North Carolina Central University

PROFESIONAL AND SOCIETY MEMBERSHIPS:

International Cannabinoid Research Society	2005- present
Society for Neuroscience	2008 -present

ABSTRACTS, PRESENTATIONS AT PROFESSIONAL MEETINGS

Annual Biomedical Research Conference for Minority Students, Dallas, TX	2004
Annual Meeting of American Cancer Society, Anaheim, CA	2005
Annual Meeting of the International Cannabinoid Research Society, Chicago, IL	2009
Annual Meeting of the International Cannabinoid Research Society, Lund, Sweden	2010
Annual Meeting of the Society for Neuroscience. Washington DC	2008
Annual Meeting of the Society for Neuroscience. Chicago, IL	2009
Annual Meeting of the Society for Neuroscience. San Diego, CA	2010
Annual Meeting of the Society for Neuroscience. Washington DC	2011

ACTIVITIES

Member- Black Graduate Student Association, Wake Forest University	2007-present
Member- Brain Awareness Council, Wake Forest University School of Medicine	2007-present
Member - Big Brothers Big Sisters, Wake Health	2010-2011