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# THE EFFECT OF AGONISTS AND ANTAGONISTS ON THE PRESYNAPTIC METABOTROPIC GLUTAMATE RECEPTORS

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## ABSTRACT

*The metabotropic glutamate receptors (mGluRs) modify the excitatory glutamate transmission in the brain. As the part of the temporal lobe the mGluRs are positioned both pre and post synaptically on the synaptic terminals. Our investigation is based on finding the role of group III mGluRs in synaptic facilitation and depression in controlling synchronous network activity in the medial entorhinal cortex (mEC). It was found that selective group III mGluR agonist ACPT-1 (20μM) suppressed the oscillations in both the superficial and deep layers of the mEC, with lowering of the gamma frequency in superficial layer and increase in the deep layers of the mEC. The selective group III mGluR antagonist UBP-1112 (20μM) enhanced the oscillations in both the superficial and deep layers of the mEC, with further lowering in the superficial layer and further increase in the deep layers of the mEC. The data thus indicate that pharmacological manipulations of the presynaptic receptors will affect the network activity and synchrony in the medial entorhinal cortex (mEC).*

## KEY WORDS

*Metabotropic Glutamate Receptors, medial entorhinal cortex.*

## 1. INTRODUCTION

The past three decades has highlighted the evolution of glutamate as an important excitatory neurotransmitter in the vertebral nervous system. Besides being involved in various intermediary metabolic functions it also influences the synaptic transmission and the neuronal plasticity [1, 2]. The glutamate plays a critical role in interacting with the diverse amount of receptors present in the brain to generate its activity. Also glutamate has been involved in various cognitive and pathological conditions.

The involvement of glutamate in various conditions like amnesia, stroke, and schizophrenia has evolved the need to develop strategies which would modulate the activity of the glutamate via glutamate receptor [3, 4]. Since the pervasive nature of the glutamate receptor function, drugs that intervene with activity of glutamate receptor function are expected to affect the glutamate functioning thereby causing disturbance to brain functioning.

The physiological function of the body is mediated by glutamate activating various ligand gated ion channels, NMDA (N-methyl-D-aspartate), AMPA (α-amino-.3-hydroxy-5-methyl-isoxazole- 4-propionate) and the Kainate receptor (KA) [5, 6]. They interact with various subclasses of receptors instigate the opening the Na+ and Ca+2 channels by the receptor itself or by the presence of the sensitive voltage ion channels [7, 8].

The past two decades has emphasized the evidence of the metabotropic glutamate receptors (mGluRs) in a way to modulate the network activity via new class of receptors, the G-Protein coupled receptors (GPCRs) [3]. The mGluRs has been divided into eight subtypes and three groups viz Group I comprising of (mGluR1 and mGluR5); Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7, mGluR8).

The Group I mGluR are associated with the G-Proteins, coupled with Phospholipase C (PLC) would cleave the Phosphatidyl inositol bis-phosphate (PIP2) into Inositol triphosphate (IP3) and Diacylglycerol (DAG). The IP3 would interact with IP3 receptors causing increase in the intercellular calcium levels and DAG on other hand would activate PKC (Protein Kinase C) [3, 9]

The Group II and III mGluRs inhibit Adenylate cyclase (AC) activity thus producing decrease in the secondary messenger cyclic adenosine monophosphate (cAMP)[3, 9]. The activation of Group II and III mGluR would thus produce decrease in the intercellular calcium [8]. Thus the activation of Group I mGluR causes increase in the calcium [8].

The mGluRs modulate the neuronal network activity and has been involved in various other functions as neuronal plasticity and the transmitter release [2]. They have been also involved in determining the dynamic balance between the inhibition and excitation of the neurons in the central nervous system (CNS) [8].

There has been evidence of the generation of gamma oscillatory activity by the activation of kainate receptors [10]. On the insertion of electrodes onto the brain slices, we were able to see the oscillatory activity which is due to the tonically active mGluR in the entorhinal cortex. This point out the differentiated control of the synchrony in neurons providing afferent input to hippocampus.

### 1.1. The Perforant Path

The perforant path provides input into the hippocampus. The axons arises evidently from layers II/III and less prominently from layers IV/V. Axons arising from layers II/IV project into the granule cells of the dentate gyrus and the pyramidal cells of CA3 region[11].

Axons arising from layers III/V project into CA1 pyramidal cells and the subiculum[11]. The perforant path can thus be segregated into two distinct medial perforant pathway (MPP) and lateral perforant pathway (LPP)[11]. The concept of LTP was first generated in this pathway. The input provided in the form of unidirectional network by hippocampus forms connections with dentate gyrus and CA3 via perforate path[11]. The CA3 pyramidal neurons receive input from the Dentate gyrus via Mossy fiber network. They further send axons to CA1 pyramidal cells via Schaffer collateral pathway (SCP)[11]. The CA1 pyramidal receive input directly from perforant path and send axons to the subiculum[11].

The entorhinal cortex receives high process sensory input from every sensory modality as well as input relating to ongoing cognitive processes [11]. The layers II/IV provides input of carry axons projecting into Dental gyrus and CA3 pyramidal neurons and layers III/V will provide input to that of subiculum and CA1 pyramidal cells [11].

There have been studies to demonstrate the modulator activity of the pre-synaptic mGluRs. The suppression of the P/Q Ca+2 channels and the activation of K+ channels is the important pharmacological mechanism for the reduction of glutamate release [2. 3].

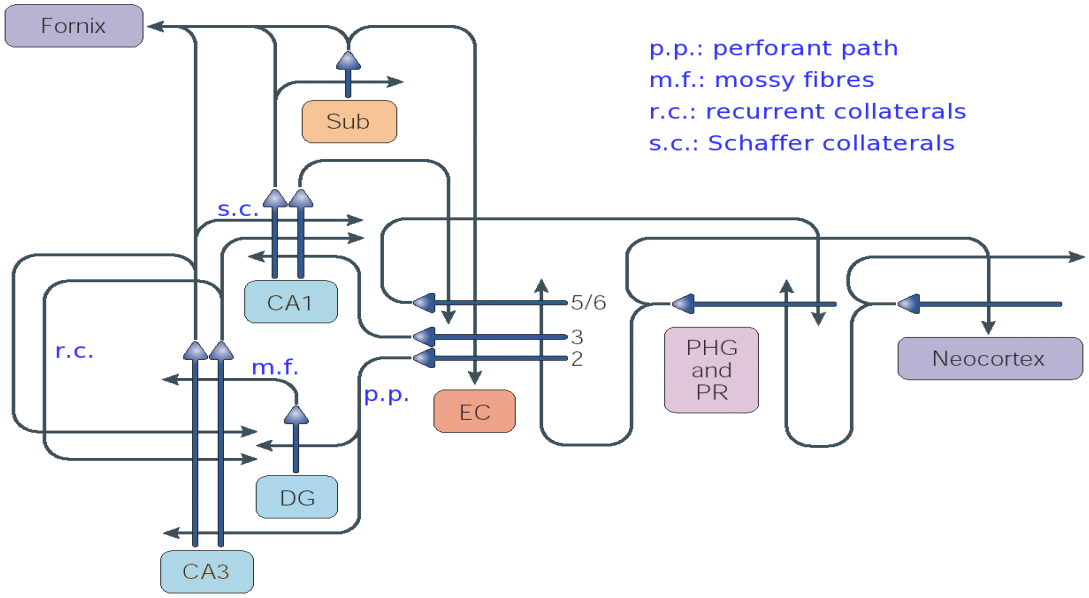



**Figure 1.** The Perforant Path (Taken from Neuroscience – Bear, Connors & Paradiso)

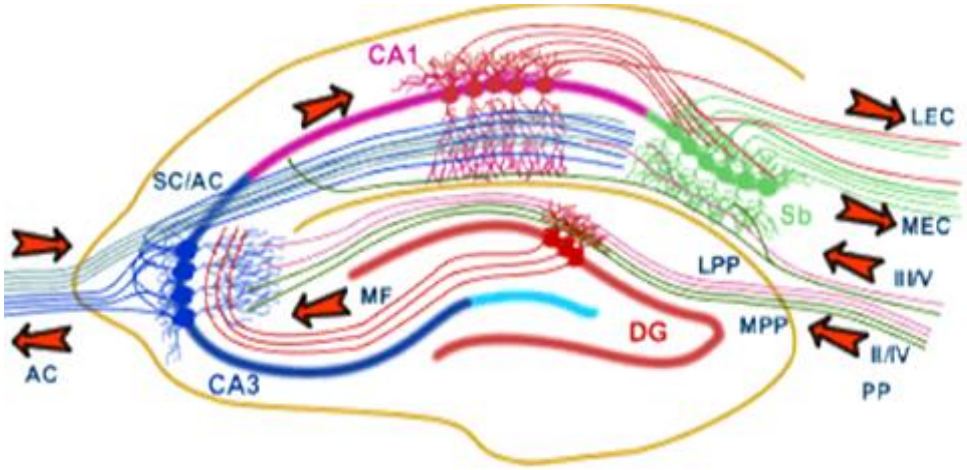



**Figure 2.** The Hippocampal network

(Taken from http://www.bristol.ac.uk/synaptic/pathways/

There have been studies carried by Billups and colleagues that the activation of mGluRs will change the state of presynaptic terminal and will not produce depression of the synaptic current [12]

There has been evidence of the decrease in the glutamate release in layer II and increase in layer V of mGluR activation [13].

There has also been indication of an increase in synaptic activity with redistribution of synaptic efficacy [14]. These studies has emphasized on the importance of synaptic depression as an essential set point in generation of network synchrony between the neuronal cells [15]. This synaptic depression influences the mGluR activity. Thus the critical role highlighting the importance of presynaptic mGluR in setting the rate of synaptic depression within the pyramidal neurons is an important set point to determine network synchrony [15]. The group III mGluR has been found to have time and cell dependent effects influencing the functional mechanism of synaptic mechanism.

## 2. AIMS

1. The project is expected to find the effect of mGluR agonists and mGluR antagonists on the Group III metabotropic Glutamate Receptors of the medial entorhinal cortex (mEC).
2. The project is expected to find the role of presynaptic receptors on the network activity of whole neurons.

## 3. OBJECTIVES

The main objective underlying the project is to find the role of metabotropic glutamate receptors (mGluRs) in synaptic facilitation and depression in controlling synchrony of the oscillatory activities produced by the neurons by the pharmacological manipulation of the presynaptic receptors in the theta, beta and the gamma frequency ranges by the agonists and antagonists.

## 4. MATERIALS AND METHODS

Slices of combined EC-hippocampal from adult Wistar rats (50-70 g) were used to perform the extracellular recording experiments. All the experiments were performed in accordance with the UK animals (Scientific Procedures act 1986) and European Communities Council Directive 1986 (86/609/EEC) and the Bath, Bristol and Aston university ethical review documents. The rats were anaesthetized with isoflurane and $N_2/O_2$ and then decapitated to remove the brain, which was immediately placed in an oxygenated artificial cerebrospinal fluid (ACSF) apparatus. The brain slices of 450 µm were prepared using Vibroslice (Campden instruments, Loughborough, UK) and stored in ACSF continuously bubbled with 95% $O_2$/5% $CO_2$ at room temperature. After an hour of recovery period the slices were placed on small square lens tissue and the neurons were visualized using differential interference contrast optics and an infrared video camera.

### 4.1. EXTRACELLULAR RECORDING:

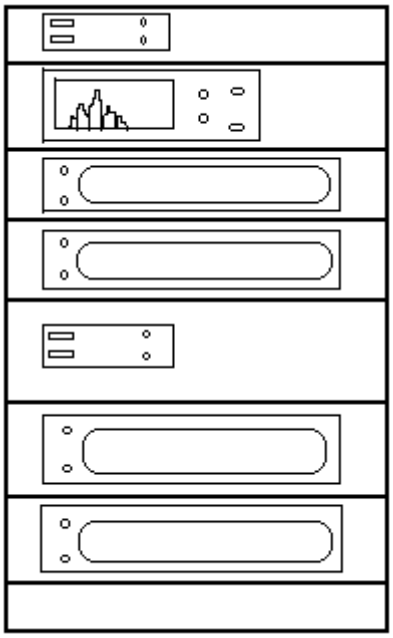
After an hour of recovery the slices were transferred to a recording chamber mounted on the stage of Olympus. The chamber was well perfused with oxygenated ACSF warmed at 32°C with a flow rate of 2ml/min. The ACSF is comprised of NaCl (126),KCl (3.25), $NaH_2PO_4$ (1.25), $NaHCO_3$ (24), MgSO4 (2), $CaCl_2$ (2.5), and D-glucose (10) and the solution is maintained at a pH of 7.4. All the salts used in the preparation of the ACSF and Sucrose cutting solution were of the Analar grade and purchased from Fischer Scientific apart from sodium chloride which was obtained from Sigma Aldrich. The other compounds such as Indomethacin, Uric acid and Ascorbic acid were obtained from Sigma. The standard drugs ACPT-1 and UBP-1112 were purchased from Tocris.

In the recording chamber, the slices were left to equilibrate for 45-60 minutes to induce oscillatory activity before any recordings were started. To induce the oscillations, kainic acid at 200-400nM was added to the ACSF bath. The drugs Kainic Acid, ACPT-1 and UBP-1112 were purchased from Tocris Cookson (UK). The drugs, group III agonist ACPT-1 and group III antagonist UBP-1112 were applied to the slices at 20µM and 20µM respectively. Glass microelectrodes made from filament 1.2mm O.D borosilicate glass (sutter) had open tip resistance of 2-4mΩ and were filled with ACSF. Electrodes had then been positioned on head stages at opposite sides of the bath and

lowered in to layers II and V of the EC. The whole cell extracellular recordings were made from the medial entorhinal cortex region and oscillations were recorded using an NPI EXT-01 extracellular amplifier and filtered using an NPI LHBF48X signal conditioner and Clampex 10.2 software (Molecular devices, USA). Data was analysed off-line using Clampfit, sigma plot and DaDisp. The changes in the power of oscillations were studied at 60s epoch of time. A clearer idea of changes in specific frequency bands was obtained at 30-90Hz for gamma oscillations and 15-29Hz for beta oscillations. T-tests were used to analyze the significant changes in the power of oscillations in the different drug periods.

**4.2. THE RECORDING INSTRUMENT:**

It consists of oscilloscope. Also two humbugs which could absorb the high frequency noise of 50Hz, thereby eliminating it to interfere with the oscillatory activity. We have two amplifiers to amplify the slow and fast oscillations thus making a good recording data to maximize the miniscal and miniature potentials. Every action potential generated will produce an oscillatory event and the activity is recorded.



**Fihgure 3**: The recording chamber

**4.3. DETAILED METHODOLOGICAL PROCEEDURE:**

The activation of Group III mGluRs produces depressive effect by the inhibition of Adenylate cyclase system. The ACPT-1 acts as a selective agonist at Group III mGluR thus facilitating this effect and UBP-1112 as a selective antagonist reverses this effect. Initially the Medial Entorhinal Cortex (mEC) slices were cut and placed in an interface chamber with bubbled ACSF (Artificial cerebrospinal fluid) prepared at 300-310 mOsm. Sucrose cutting solution was used initially to get the brain slices and later aCSF prepared and is bubbled with $O_2$.The rat is sacrificed by euthanasia (EEC/Animal Scientific Act). The slices are placed in the interface chamber for stabilization for 30-45 minutes with aCSF bubbled in. The forceps are thus used to place the slices on the recording chamber with the bubbled aCSF that flows onto the slice getting collected in a small beaker for recirculation. The important component is the heater which provides the heating of the ACSF that flows onto the slice creating moist and humid environment as *In vivo*. The slice is then kept for stabilization for an hour. Later Kainic acid (KA) is added to activate the Kainate receptor facilitating oscillations. The set up is then kept for half an hour for stabilization. After a stable mode of oscillations is seen drugs have been added. The Clampfit software was used for analyzing the data and Clampex software used for recording of the oscillatory activity. The drugs used are ACPT-1 and UBP-1112 as agonist and antagonist which were prepared from standard Tocris reagents of 20 µM each. The electrodes were prepared from Sutter instrument and pulled with 2-4mΩ resistance between the electrodes for the extracellular recording. The electrodes were then filled with aCSF. It was fixed in the holders with silver wire inserted. The fine and coarse adjustments of the electron microscope was then used and penetrated enough to touch the extracellular surface of the slice. As soon as it touches the cell we could see the oscillations on the monitor. We then record the oscillations and give it 20-30 mins for stabilization. Then the tags of control, ACPT-1 or UBP-1112 were then inserted. The set recording is then analysed to check for its stability. The layer II and layer V are analysed separately. The initial time period with the final time period of 1 min is taken and filtered at 50 Hz to eliminate all the noise

created with oscillatory activity and the recording system. A power spectrum was then drawn for the initial clump of data and the final clump in layer II and the final clump in layer II and seen whether they are overlapping with no significant change seen in the spectrum with bell shaped curve. A peak power v/s log frequency spectrum is then obtained and for better analyzing we take the log linear form of the spectrum.

The EC –hippocampal slices were prepared from P45 – P50 rats and the electrodes were inserted into the superficial (layer II) and the deep (layer V) of the mEC which was maintained at 32 – 34˚C.

The recordings were then made from both the layers II and V of Group III metabotropic Glutamate Receptor. Kainic acid is added (250 nM) to induce the gamma oscillatory activity (greater than 30Hz).

The flow is maintained at uniform rate a moist environment is created for the slice to produce good oscillations. The slice is then kept for stabilization for one hour in presence of Kainic Acid.

The recording is then started and after 20 minutes of recording, the power spectrum is obtained for the control period. The initial time period is compared with the final period of recording for no significant change in the spectrum with the overlapping spectrum curves.

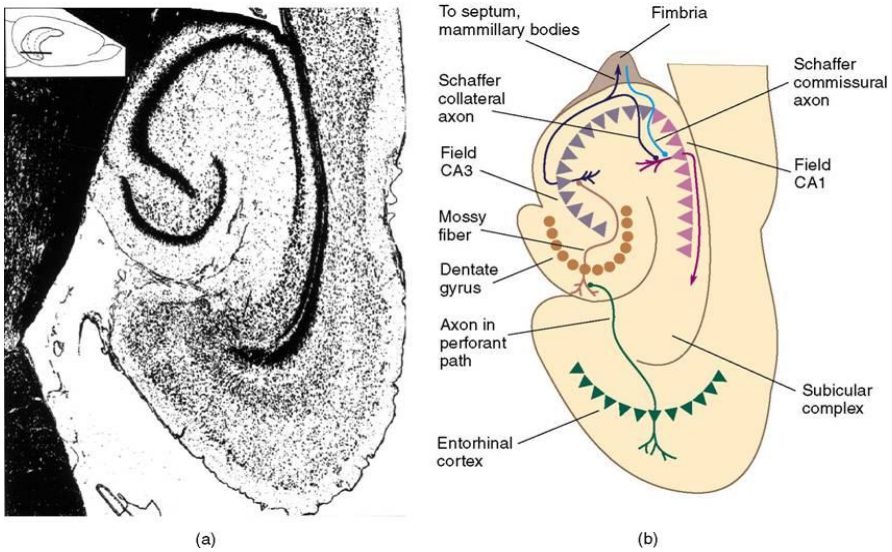



**Figure 4. The Entorhinal-Hippocampal Slice**
**(Taken from Neuroscience – Bear, Connors & Paradiso)**

When we see a stabilized curve we proceed further with the drug ACPT-1 which is selective Group III mGluR agonist. A 20 µM concentration of 100 µL is pipette out and poured down into running aCSF bubbled with $O_2$. It is then allowed to run for some time with the tag on. The agonist ACPT-1 will act by agonizing the Group III mGluR effect viz it depresses or reduces the power of oscillations in a pronounced manner. The effect can be demonstrated by building a power spectrum of the initial ACPT-1 period and the final ACPT-1 period. The curve then shows a significant decrease in the power of oscillations when compared to the initial ACPT-1 period or the final control (Kainic Acid) period. A Bar chart is drawn in an excel worksheet to demonstrate the decrease in power (power v/s log frequency) and peak frequency.

The drugs added after the addition of ACPT-1. This drug acts as an antagonist at the group III mGluR. This drug tends to reverse the depressive effect. The bath of ACSF is washed with UBP1112. The output pipe is allowed to flow out into empty cylinder. Output of UBP-1112 (20µM) & 250 nM of Kainic acid is added in cylinder of 100ml ACSF and supported with the previous cylinder. The recording is started and set for a period of 20-30 mins to stabilize. The recording is

then stopped and clump of recording of first one minute and last minute is taken and filtered by notch filter at 50Hz between cursors one and two or three and four. Either way band pass filter can also be performed on that clump. A power spectrum is generated for UBP-1112 putting cursors 1 and 2 as initial and 3 and 4 as final. The peak power spectrum of Control, ACPT-1 and UBP-1112 also compared with the control as 100%, ACPT-1 reduces the power and UBP-1112 brings it back either to control (K.A) or also more than that of control.

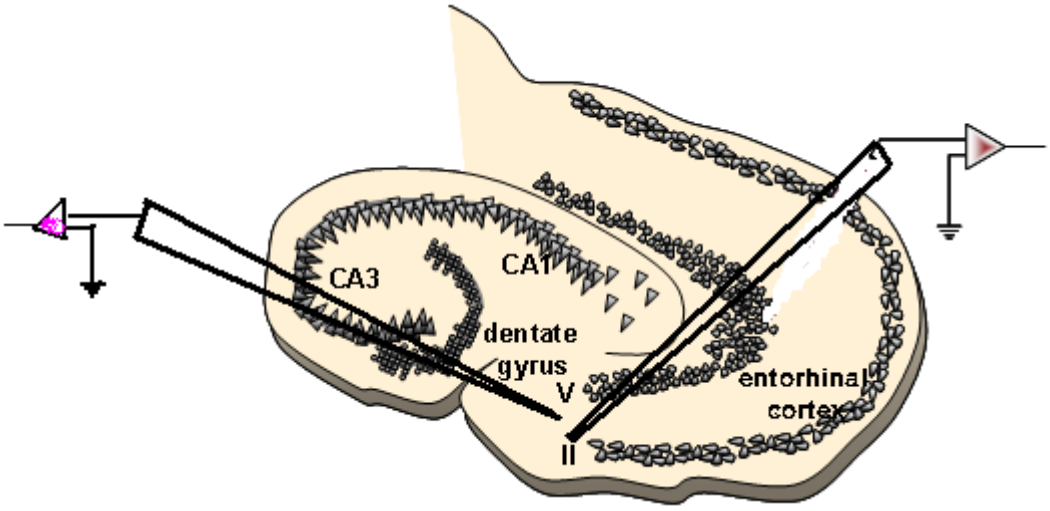



**Figure 5. The Insertion of Electrodes in Superficial and Deep layer of Medial Entorhinal Cortex (Taken from Neuroscience – Bear, Connors & Paradiso)**

## 5. RESULTS

### 5.1. EFFECTS OF ACPT-1 & UBP-1112 ON GROUP-III MGLUR IN SUPERFICIAL LAYERS OF MEDIAL ENTORHINAL CORTEX (MEC):

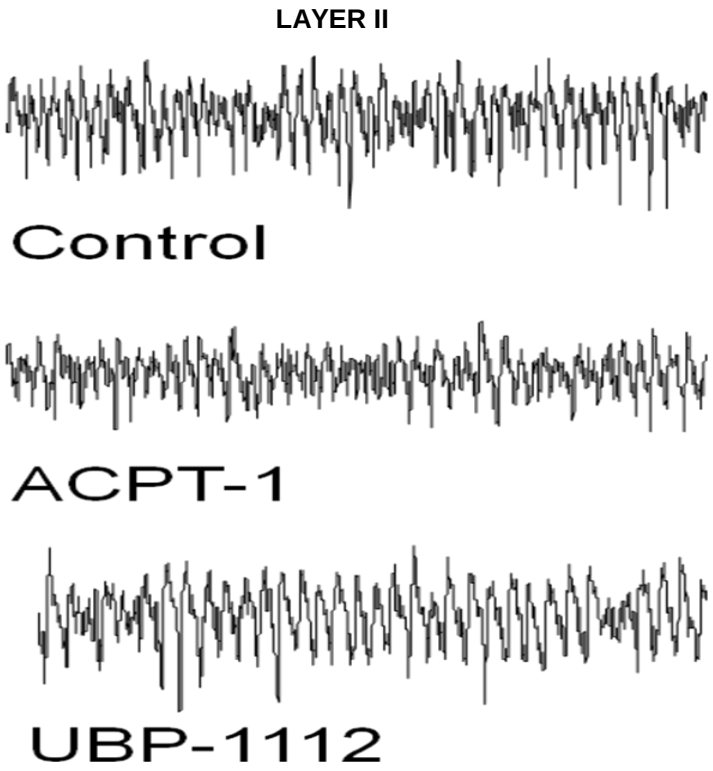
The activation by the Kainic Acid (250 nM), the layer II shows both Theta (4-12 Hz) and Gamma (30 – 80 Hz) oscillatory activity.

The mean peak power of the control (kainic acid) oscillation in the gamma range was $30.78 \times 10^{-12}$ $\mu V^2$ (n=5). The mean peak control gamma frequency was found to be 42.60± 1.87 Hz. Later after the recording of the kainic acid is taken, we apply group III selective mGluR agonist (ACPT-1). The experiment is then allowed to stabilize with ACPT in a concentration of 20 µM into 100ml of ACSF cylinder for 20-30 mins and then recording is started. The recording is then continued for 20 mins after stable oscillations are seen. The initial and final drug periods are notch filtered at 50Hz each. An epoch of one minute is taken from both the initial and final drug periods and the power spectrum is produced. The log linear form of the curve is then produced for better distinction. It was found that a mean peak power has decreased from $30.78 \times 10^{-12}$ $\mu V^2$ to $17.78\times 10^{-12}$ $\mu V^2$ (n=5). The mean peak ACPT-1 gamma frequency was found to be 34.54 ± 5.3 Hz. The pooled data thus suggest that ACPT-1 did reduce the peak power from $30.78 \times 10^{-12}$ $\mu V^2$ to $17.78\times 10^{-12}$ $\mu V^2$ with all the recordings shared decrease peak power compared to that of control. The mean peak gamma frequency is also reduced from 42.60 ± 1.87 Hz to 34.54 ± 5.93 Hz following perfusion of selective group III mGluR agonist.

Followed by the addition of agonist selective group III mGluR antagonist (UBP-1112) was added at the concentration of 20µM into 100ml of ACSF cylinder. The antagonist UBP-1112 added in the cylinder was kept for stabilization for 20-30 minutes. Later recording was started and oscillations were recorded for a period of 20 minutes. The recorded data was then notch filtered at 50 Hz. The initial drug period is compared to that of final drug period and a power spectrum was obtained with the epoch of one minute drug period. The log linear form of the curve is then produced for better distinction.

It was found that the antagonist (UBP-1112) increases the much gamma oscillatory power from $17.78 \times 10^{-12}$

$\mu V^2$ to 26.15 × $10^{-12}$ $\mu V^2$ in the layer II (superficial layer). The mean peak gamma frequency was found to reduce from 34.54 ± 5.93 Hz to 32.47 ± 5.83 Hz.



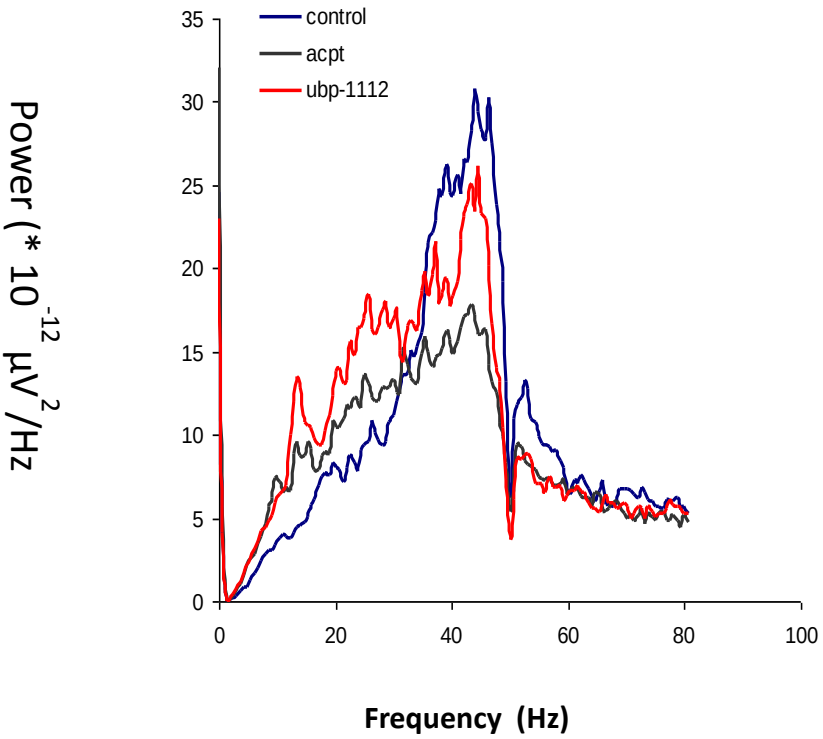
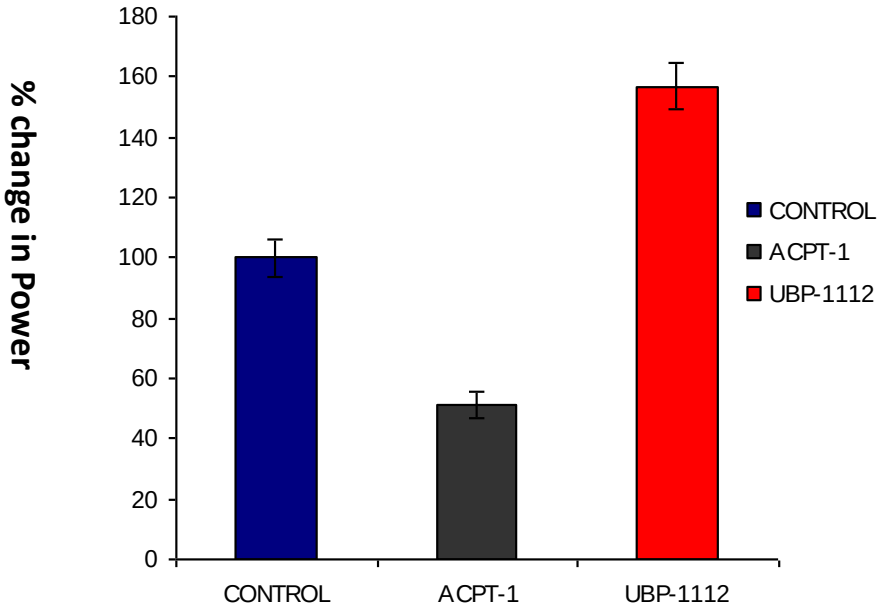




**Figure 6: The raw traces showing the effect of control, ACPT-1 and UBP-1112 on Group III mGluR in the superficial layer (layer II) of the mEC. The ACPT-1 (20µM) and UBP-1112 (20 µM) were applied. (A) The plot of the pooled power spectrum during drug application in the layer II showing the effects of ACPT-1 and UBP-1112. (B) The bar chart indicating the % change in the peak power in the presence of ACPT-1 and UBP-1112. (C) The bar chart showing the changes in the frequency in the presence of ACPT-1 and UBP-1112.**


% change in Power
180
160
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ACPT-1
UBP-1112
CONTROL
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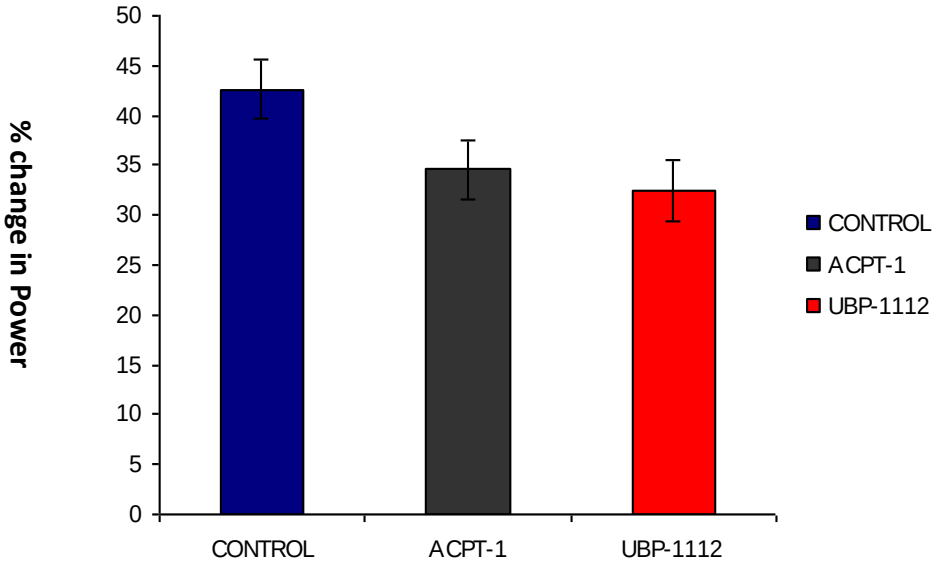
% change in Power
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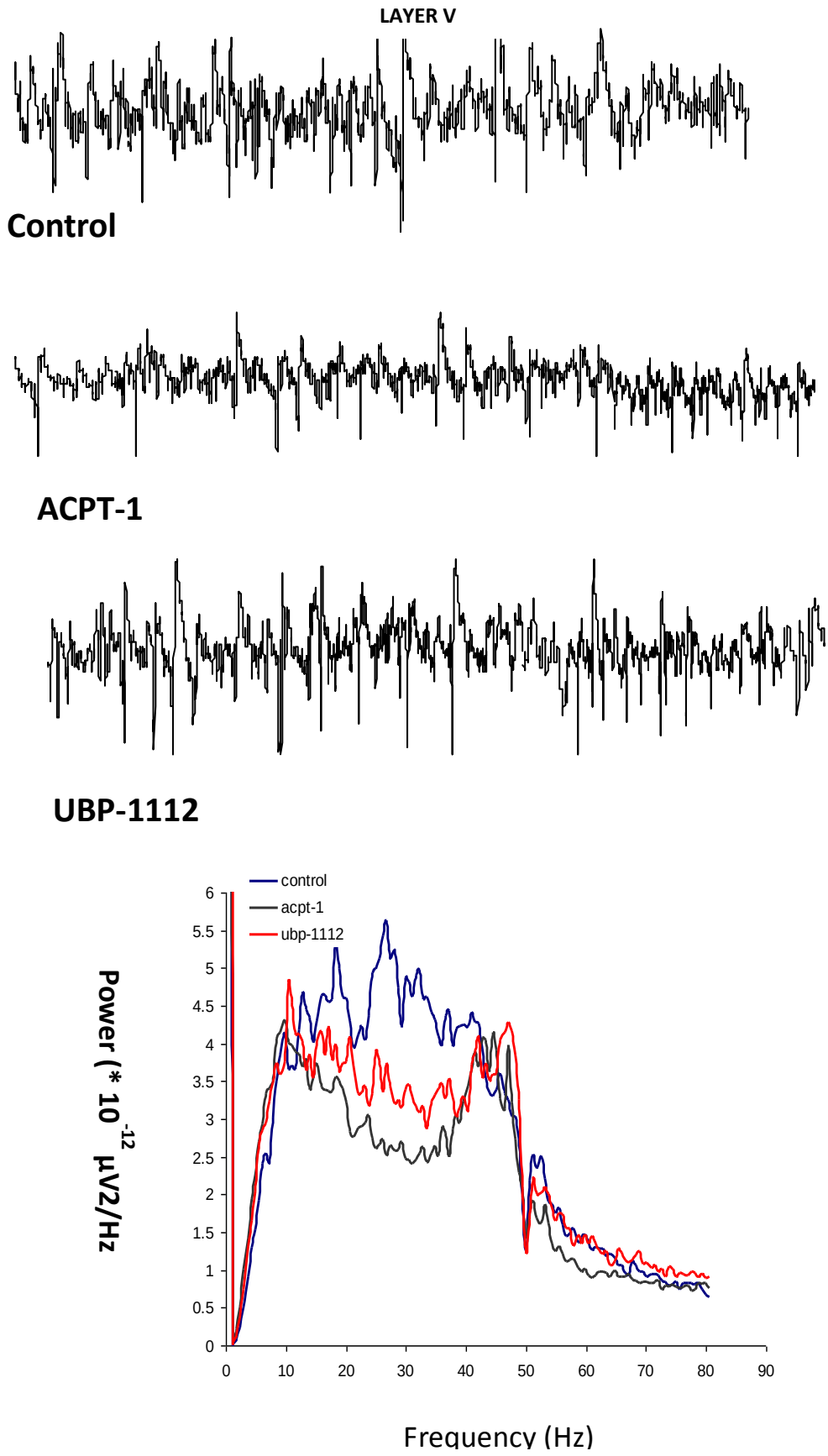
LAYER V
Control
ACPT-1
UBP-1112
control
acpt-1
ubp-1112
Power (* 10 -12 µV2/Hz
Frequency (Hz)
0
0.5
1
1.5
2
2.5
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6
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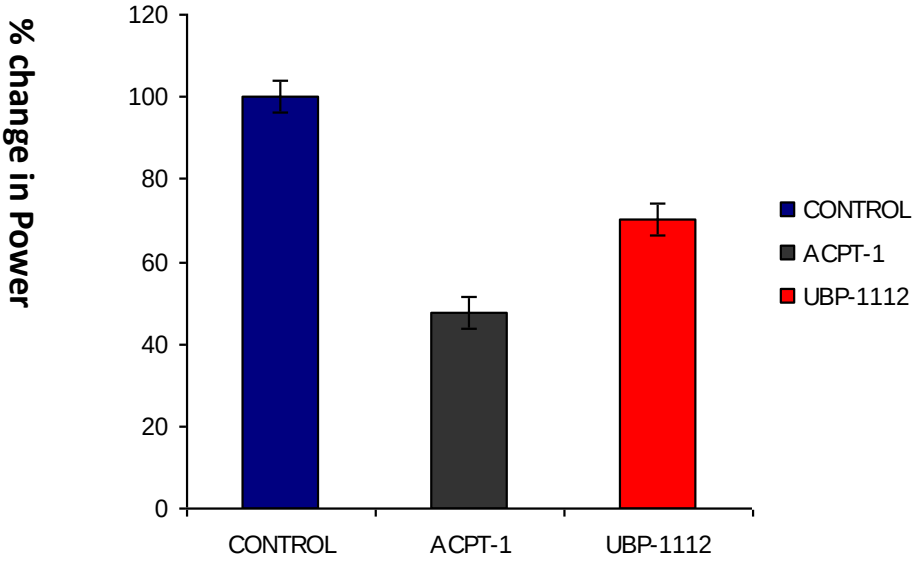


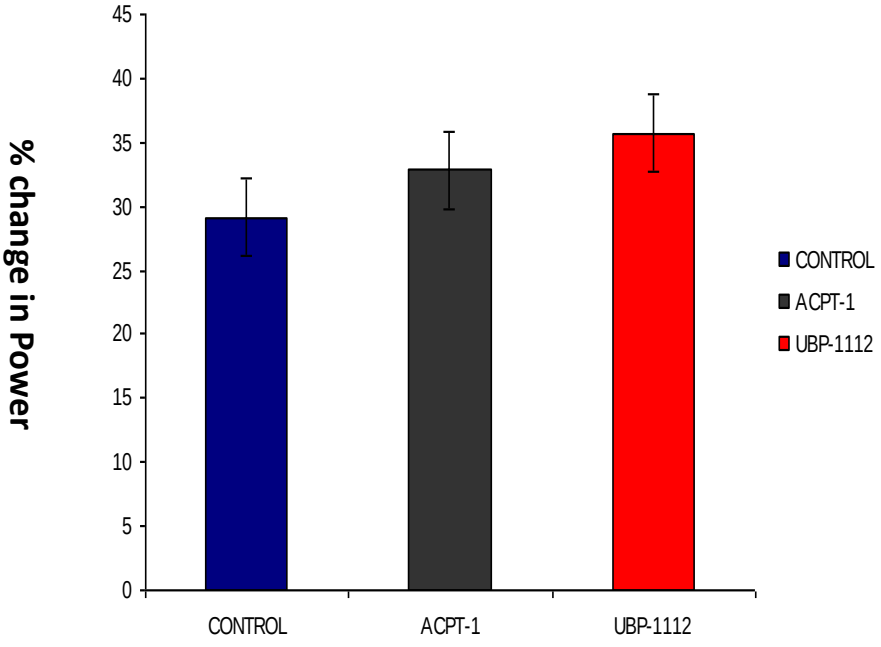


**Figure 7: The raw traces showing the effect of control, ACPT-1 and UBP-1112 on the Group-III mGluR in the deep layer (layer V) of the mEC. The ACPT-1 (20µM) and UBP-1112 (20 µM) were applied. (A) The plot of the pooled power spectrum during drug application in the layer II showing the effects of ACPT-1 and UBP-1112. (B) The bar chart indicating the % change in the peak power in the presence of ACPT-1 and UBP-1112. (C) The bar chart showing the changes in the frequency in the presence of ACPT-1 and UBP-1112.**

**5.2. EFFECTS OF ACPT-1 & UBP-1112 ON GROUP-III MGLUR IN DEEP LAYERS OF MEDIAL ENTORHINAL CORTEX (MEC):**

Following the above experiment forward, we did the recording from the deep layer (layer V) of the mEC and found that there was decrease in the oscillatory activity compared to that of the superficial layer both in frequency and amplitude. The mean control peak power for control (kainic acid 250 nM) was found to be $5.62 \times 10^{-12}$ $\mu V^2$ and the mean peak gamma frequency was found to be 29.14 ± 4.35 Hz.

Later after the recording of the control the group III mGluR agonist (ACPT-1) was added. The experiment is then allowed to stabilize in the presence of 20 µM of ACPT-1 for 20-30 minutes and then recording is started. The recording is continued for a period of 20 minutes and then stopped. The recorded data is the analysed. The initial & the final drug periods are taken & a power spectrum is obtained. The log linear form of curve is then produced for better distinction. It was found that the mean peak gamma power has decreased from $5.62 \times 10^{-12}$ $\mu V^2$ to $4.74 \times 10^{-12}$ $\mu V^2$ (n=4). The mean peak ACPT-1 gamma frequency was found to be 32.80 ± 8.09 Hz.

The peak power spectrum thus indicates that ACPT-1 has decreased the peak power from $5.62 \times 10^{-12}$ $\mu V^2$ to $4.74 \times 10^{-12}$ $\mu V^2$.

The mean peak frequency was found to have increased from 29.14 ± 4.3Hz to 32.80 ± 8.09 Hz. Followed by the addition of ACPT-1, group III mGluR antagonist UBP-1112 was added in a concentration of 20 µM in fresh 100ml of ACSF cylinder. The slice is kept for stabilization in presence of UBP-1112 for a period of 20-30 minutes. Later on recording is started for a period of 20 minutes. The recorded data was then notch filtered at 50Hz each. The initial drug period is compared to that of the final drug period & a power spectrum was obtained. Usually the epoch of one minute was taken & the power spectrum was obtained. The log linear form of curve is used for better distinction. From the data, we take the mean of the values of all the experiments conducted & draw a mean peak power spectrum for control, ACPT-1 & UBP-1112. It was found that the UBP-1112 has increased the mean peak gamma power from $4.31 \times 10^{-12}$ $\mu V^2$ to $4.79 \times 10^{-12}$ $\mu V^2$. The mean peak gamma frequency was found to have increased from 32.80 ± 8.09 Hz on addition of UBP-1112.

There has been an indication of the presence of theta (4-12Hz) peaks in the deep layer (layer V) on the addition of control, ACPT-1 & UBP-1112 but the prominent peaks were obtained in gamma range (30-80 Hz). Thus in the layer V (deep layer), ACPT-1 decreased the mean peak power & UBP-1112 brings it back to some extent.

The data presented in the normal brain slice of the rat indicate that selective group III mGluR agonist (ACPT-1) decreases the peak gamma power in both the superficial and deep layers & the selective group III mGluR antagonist (UBP-1112) increases the mean peak gamma peak in both the superficial & deep layers. There have been peaks observed in the theta range showing the sensitivity of the deep layers to low frequency ranges.

In the superficial layers the mean peak gamma frequency has been shown to decrease on addition of ACPT-1 & further lowering of frequency was observed on the addition of UBP-1112.

In the deep layers, there has been increase in the peak frequency on ACPT-1 addition, followed by further increase in addition of UBP-1112.

## 6. DISCUSSION

The project is expected to discover the role of presynaptic mGluR in synaptic depression and synaptic facilitation with emphasis on measuring the effect on oscillations in the gamma frequency range. The modulation of the network activity is carried out by the action of selective Group III mGluR agonist ACPT-1 and selective Group III mGluR antagonist UBP-1112 directly on the network of whole neurons. The IPSPs generated on the addition of ACPT-1 were generally of greater power and amplitude in the superficial layer compared to the deep layer of the mEC. The selective group III mGluR agonist ACPT-1 tends to suppress the oscillatory activity both in power and amplitude in the superficial layer. The

deep layer has an indication of decreased oscillatory power. However ACPT-1 has caused a decrease in the oscillatory power in the deep layer.

The selective group III mGluR antagonist UBP-1112 has enhanced the oscillatory activity in both the superficial and deep layers of the mEC. There has been indication that the superficial layer produces higher power of oscillations when compared to the deep layers of the mEC. The UBP-1112 has enhanced the oscillatory power with a greater power frequency in the superficial layer and the deep layer also witnessed an enhancement of the oscillatory activity with oscillations in the theta (4-12 Hz) frequency range.

The selective group III mGluR agonist ACPT-1 and antagonist UBP-1112 also modulate the frequency at which the oscillations are generated. The ACPT-1 has decreased the frequency and UBP-1112 has decreased it more in the superficial layer with both the frequency changes corresponding to the gamma (30-80 Hz) frequency range. In contrast with above ACPT-1 increase the frequency and UBP-1112 boosted it more with both changes in the frequency corresponding to the gamma frequency range in the deep layer of the mEC.

The selective group III mGluR agonist ACPT-1 also modulates the frequency mode at which the oscillations are generated. The ACPT-1 decreases the frequency and UBP-1112 decreases it more with both frequency changes corresponding to the superficial layer of the mEC in the gamma frequency range. The deep layer of the mEC witnessed increase in the frequency on addition of ACPT-1 and further augmentation with UBP-1112 with both the frequency changes corresponding to the gamma frequency range.

There has been evidence from the previous literature that the deep layer of the mEC is driven by the action potential dependent evoked release prominently due to the NMDAR mediated EPSPs (excitatory post synaptic potentials) with weak synaptic inhibition [16]. The superficial layer of the mEC on the other hand is driven by the action potential independent release prominently by the inhibitory post synaptic potentials (IPSPs) due to the stimulation of the GABA [17, 18, 19]. Thus superficial layer II of the mEC has dominant inhibition factor over excitation and the deep layer V of the mEC has predominant excitation factor over inhibition. Since the mGluR activation produces decreased glutamate release by decrease in the voltage gated calcium current[20, 21, 22] the superficial layer driven by action potential independent GABA mediated IPSPs are less affected by the decrease in the calcium release but the deep layer being Action potential dependant is affected much likely due to the changes in the calcium. Thus the deep layers of the mEC are more likely to be affected by the mGluR ligands as it is action potential dependent.

The effect of ACPT-1 and UBP-1112 on the superficial and the deep layer has been demonstrated in the current study. The effects of the group III mGluR agonist on the deep layer has more pronounced inhibition compared to the superficial layer. This effect might be due to the dominance of inhibition (IPSPs) over excitation in the superficial layer is camouflaged by the mGluR effects.

The decreased oscillatory activity in the deep layer of mEC during the experimentation proceedure might be expected as a result of activation of the mGluR during the ongoing oscillatory activity and further activation by agonists will not increase the power of oscillations. This has been confirmed by the profound inhibition in power of gamma oscillations in the superficial layer, thus pointing out the importance of mGluR in maintaining the oscillatory activity.

There has been evidence in the previous literature about the dual role of mGluRs having autoreceptor function at the glutamatergic synapses and hetero receptor function at the GABA ergic synapses [23, 24, 25]. The GABA release can not only be modulated by the GABA receptors but also by the mGluRs acting presynaptically to inhibit GABA [26, 27]. This modulatory effect has been attributed by the Group II and Group III mGluR at the pre presynaptic terminal [3, 28].

## 7. CONCLUSIONS

1. The pharmacological manipulation of presynaptic mGluRs may control temporal coherence of network synchrony.
2. The selective group III mGluR agonist ACPT-1 and antagonist UBP-1112 also modulate the frequency at which the oscillations are generated. The ACPT-1 has decreased the frequency and UBP-1112 has decreased it more in the superficial layer with both the frequency changes corresponding to the gamma (30-80 Hz) frequency range. In contrast with above ACPT-1 increase the frequency and UBP-1112 boosted it more with both changes in the frequency corresponding to the gamma frequency range in the deep layer of the mEC.
3. The oscillatory activity is modulated by Group III selective agonist (ACPT-1) and antagonist (UBP-1112) in both low (theta, 4-12 Hz) and high (gamma, 30-80 Hz) frequency range.

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