Modelling Homeostatic Plasticity In The CA3-CA1 Synapse In The Hippocampus

A Thesis

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by

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From May 2022 to Mar 2023

INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH PUNE

Certificate

This is to certify that this dissertation entitled 'Modelling Homeostatic Plasticity In The CA3-CA1 Synapse In The Hippocampus' towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by Varun G Mallya at Indian Institute of Science Education and Research under the supervision of Dr. Suhita Nadkarni, Associate Professor, Department of Biology, during the academic year 2022-2023.

Nadkarni

Dr. Suhita Nadkarni

Biology

Dr. Suhita Nadkarni

Dr Chaitanya Athale

This thesis is dedicated to my parents and everyone who believed in me

Declaration

I hereby declare that the matter embodied in the **Modelling Homeostatic Plasticity In The CA3-CA1 Synapse In The Hippocampus** are the results of the work carried out by me at the Department of Biology, Indian Institute of Science Education and Research, Pune, under the supervision of Dr. Suhita Nadkarni and the same has not been submitted elsewhere for any other degree.

Varun

Varun G Mallya

Date: April 10,2023

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Acknowledgments

I would like to take this opportunity to express my sincere gratitude to my thesis supervisor, Dr. Suhita Nadkarni, for her invaluable guidance and support throughout my research. Her mentorship, insights, and expertise have been instrumental in shaping my scientific development, and I am incredibly grateful for the time and effort she has invested in my growth as a researcher.

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Contributions

Contributor Name	Contributor Role
Dr. Suhita Nadkarni,Varun G Mallya	Conceptualization Ideas
Dr. Suhita Nadkarni,Varun G Mallya,Nishant Singh	Methodology
	Software
Dr. Suhita Nadkarni,Varun G Mallya	Validation
Dr. Suhita Nadkarni,Varun G Mallya,Nishant Singh	Formal analysis
Varun G Mallya	Investigation
Dr. Suhita Nadkarni	Resources
Varun G Mallya	Data Curation
Varun G Mallya	Writing - original draft preparation
Dr. Suhita Nadkarni	Writing - review and editing
Dr. Suhita Nadkarni,Varun G Mallya,Nishant Singh	Visualization
Dr. Suhita Nadkarni	Supervision
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Dr. Suhita Nadkarni	Funding acquisition

Abstract

Homeostatic synaptic scaling is a critical mechanism that allows neurons to maintain stable levels of synaptic activity. This process involves a variety of homeostatic changes, including the up or downregulation of AMPA receptor density in the postsynaptic PSD, changes in the density of glutamate transporters in astrocytic glia, and alterations in the kinetics of AMPA channels and release probability of glutamate. These changes are believed to be involved in the regulation of synaptic strength and plasticity, and their dysregulation has been implicated in several neurological disorders.

To better understand the impact of homeostatic changes on synaptic function and plasticity, we developed a computational model to simulate the rapid synaptic current generated by AMPA receptors.Using this model, we observed that increasing AMPA receptor density, release probability of glutamate, and the open probability of AMPA receptors led to an increase in miniature excitatory postsynaptic current (mEPSC) amplitude. A significant finding from our study was that all homeostatic upscaling changes gave rise to paired pulse depression, which can regulate the balance between excitation and inhibition in neuronal networks.

Finally, we incorporated all of these homeostatic changes into our model and observed an increase in mEPSC amplitude that was validated by experimental studies. These findings highlight the importance of homeostatic synaptic scaling in regulating synaptic function and plasticity, and may have important implications for the development of new treatments for neurological disorders that involve dysregulation of this critical process.

2.Introduction

Neurons are the basic unit of the brain.The human brain contains in the order of 10^{11} neurons.Although they can be classified into a thousand different types, neurons share the same basic architecture.The large number of these neurons connected in precise anatomical circuits is what leads to complex behaviours rather than individual specialisation.Nerve cells with very similar properties can produce very different actions, this is a key organisational principle of the brain.

The cell body, dendrites, axon, and presynaptic terminals are the typical four morphologically distinct sections of neurons. metabolic centre of the neuron which contains the nucleus is the soma.The soma gives rise to several short processes called dendrites which branch out in a tree like fashion.Dendrites are the main recipients of input signals from other neurons.On the other hand, an axon extends away from the soma and serve as the primary means of transmitting signals to other neurons.The neuron transmits signals from the enlarged endings of its axon branches, called the presynaptic terminal.These electrical signals are rapid, transient, all or none nerve impulses called action potentials.

The site of communication between neurons is called the synapse and is responsible for information transfer between neurons. The arrival of an action potential in the terminal of an axon leads to the discharge of neurotransmitters. These molecules diffuse across the synaptic gap and proceed to join onto receptors situated on the postsynaptic neuron, generating a change in its electric polarisation. This shift in polarity can have either excitatory or inhibitory effects.The strength of a synapse can change by a process called plasticity. (Kandel 2013)

Hippocampus

The hippocampus is located in the medial temporal lobe of the brain.It is primarily involved in the formation and retrieval of declarative memories eg.

facts and events.The hippocampus is composed of subfields-The dentate gyrus, CA1,CA2,CA3 and subiculum each having distinct functional and anatomical properties.

The hippocampus receives input from the neocortex,thalamus and amygdala and sends outputs to the entorhinal and prefontal cortex.These are the connections that allow for the formation of episodic memories and the sensory integration of information with contextual and emotional cues.

The CA3-CA1 synapse is important for information processing and storage in the hippocampus.This synapse is the major point of communication between the two regions and is essential for learning and memory.

The CA3 region is involved in the initial encoding and consolidation of new memories, while the CA1 region is important for the retrieval and recall of previously stored memories. The CA3 region receives input from various brain regions and forms associations between different pieces of information, while the CA1 region integrates this information and connects it to past experiences.

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Another noteworthy characteristic of the CA3-CA1 synapses is their capacity for long-term potentiation (LTP), a process by which synapses get stronger in response to repeated stimulation. Disruptions to LTP at this synapse have been linked to cognitive diseases including Alzheimer's disease. This process is thought to underpin the hippocampus's capacity to encode and retain memories(Andersen 2007)

Neuroplasticity

Neuroplasticity is the capacity of the brain to alter its structure and function over the course of a lifetime and in response to experience.The human brain's unique quality of neural plasticity facilitates our capacity for learning, memory consolidation and retrieval alongside recuperation following damage or diseases.This is done via forming new neural connections, pruning existing ones, strengthening or weakening of synapses.Various levels exist in this process stretching from molecular changes within individual cells right up to intricate interactions between diverse brain networks.(Merzenich 2013)

The two types of neural plasticity are :Structural and Functional.The former deals with physical changes in the brain structure.Some ways this occurs is through the formation of new synapses, change in shape and size of existing neurons.Functional plasticity on the other hand refers to the changes in the brains activity, such as the weakening or strengthening of synapses.

The most well known theory of synaptic plasticity is Hebbian Plasticity.This is based on the idea- “Neurons that fire together, wire together” i.e repeated correlated synaptic firing, leads to strengthening of the synapse and vice versa.Hebbian Plasticity underlies various forms of learning and associative memory.

Stability problem

Maintaining stability in neural circuits is a major challenge during circuit development and plasticity. For instance, achieving the right balance between excitation and inhibition to facilitate activity propagation in a network without causing it to die out or become uncontrollable is a complex task. There is another stability concern when it comes to circuits that contain adaptable synapses. These synapses can be modified as a result of learning, where neural networks recognize connections between environmental cues and make changes to synaptic strength and other cellular properties. These changes

include long-term potentiation (LTP) and long-term depression (LTD), which strengthen and weaken certain pathways in the brain, respectively. However, this process has a negative aspect. Synapses that become stronger tend to be more effective at depolarizing the postsynaptic neuron, resulting in an unchecked positive feedback cycle that causes neuronal activity to reach a saturation point. Additionally, the unchecked positive feedback loop can result in a loss of specificity among synapses. This means that even poorly correlated presynaptic inputs can cause postsynaptic firing and strengthen, without any associated environmental stimulus triggering the process. Consequently, nervous systems must possess a set of plasticity mechanisms that can counteract these destabilizing forces. Homeostatic plasticity is one such mechanism that allows neurons to maintain a stable level of neural activity despite changes in their synaptic strength.[Turrigiano 2008]

Homeostatic Plasticity

When faced with changes that might affect their excitability, homeostatic plasticity is a form of plasticity that aids in stabilising the activity of neurons or neural circuits. Changes in synapse number or strength, as well as cell size, may be among these changes. In order to maintain a constant level of activity despite disturbances, the homeostatic type of plasticity modifies the sensitivity of neurons to incoming signals or the strength of their synaptic connections.

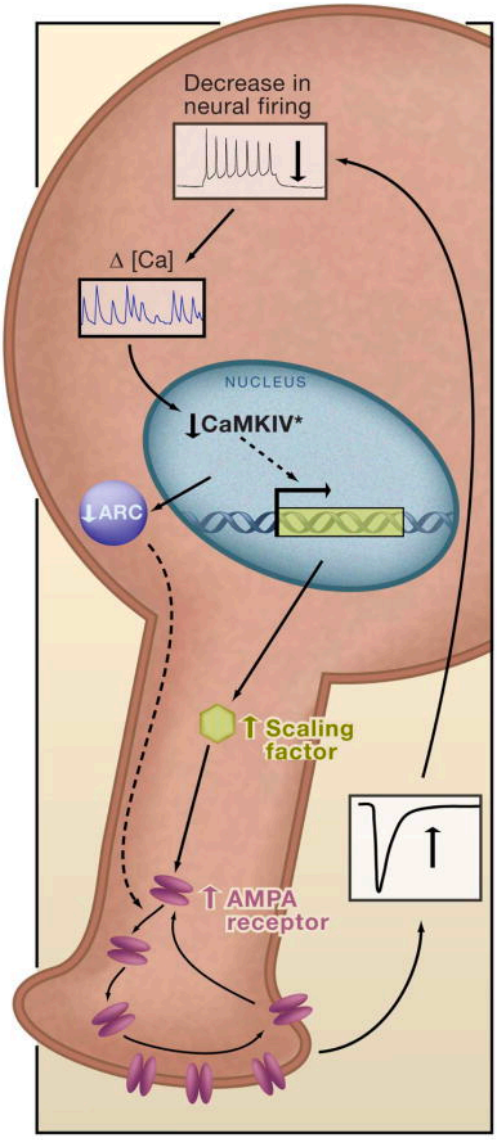


Figure1:A Feedback Mechanism for Regulating Synaptic Strength[Adapted from Turrigiano 2008]

When neuronal firing decreases, there is a corresponding decrease in somatic calcium levels. This, in turn, reduces the amount of activated CaMKIV (known as CaMKIV*) in the nucleus. Consequently, transcription of a "Scaling Factor" is increased to enhance the accumulation of AMPA receptors at synapses via an unknown mechanism. As a result, there is an increase in excitatory synaptic strength and firing rates, which helps bring the activity back to target levels.

. [Turrigiano 2008]

Synaptic Scaling

According to theoretical models, network activity will remain constant despite changes in synaptic strength or connectivity if neurons can monitor their own activity levels and modify their connections to maintain a set-point value. This process, known as synaptic scaling, has been seen in neocortical neurons and indicates that traditional homeostatic feedback regulation is a factor in regulating average neuronal activity. Neurons most likely need to perceive some component of activity, integrate it over a long time period, then modify synaptic characteristics to reduce the gap between this value and an activity set-point in order to establish this feedback system. Identifying the element of neuronal or network activity that is perceived, the signalling pathways that are activated, and the processes by which changes in synaptic strength are implemented are all part of our present understanding of homeostatic synaptic scaling.

Through homeostatic processes that regulate several elements of neuronal excitability, neurons have the capacity to modulate their firing rates. These processes could balance the firing properties-determining inward and outward voltage-dependent conductances, control the number or strength of inhibitory and/or excitatory synapses, or modify the ease with which other types of plasticity can be generated (known as "metaplasticity"). All of these systems may be involved in the homeostatic control of neuronal firing rates in central circuits, according to research. Yet, the kind of neuron and developmental stage may affect each mechanism's efficacy. [Turrigiano 2008]

AMPA receptor

The transmission of excitatory impulses between neurons depends heavily on the ionotropic glutamate receptor known as the AMPA receptor, which is present throughout the brain.

A neurotransmitter called glutamate binds to the receptor and changes its conformation, opening the channel and letting positively charged ions like sodium and calcium enter the cell. An influx of ions causes the neuron to become depolarized, which can cause an action potential and send the signal to other neurons.

The AMPA receptor is made up of four subunits, each of which has three major structural domains: a transmembrane domain, an intracellular carboxyl-terminal domain, and an external amino-terminal domain.

The AMPA receptor has a role in both synaptic transmission and synaptic plasticity, which is the ability of synapses to become stronger or weaker over time due to activity. AMPA receptor involvement has been identified in various forms of synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD). These mechanisms are believed to be essential for learning and memory and involve alterations in the number or function of AMPA receptors at synapses.

The density of AMPA receptors at the synapse can alter during homeostatic scaling, as can their conductance and/or kinetics. Particularly, neurons increase the number of AMPA receptors at their synapses and increase the conductance of individual AMPA receptors in response to chronic changes in neuronal activity, such as reduced activity levels, whereas neurons decrease the number

of AMPA receptors at their synapses and increase the conductance of individual AMPA receptors in response to chronic increases in activity. It is believed that these alterations in AMPA receptor characteristics will modify the total intensity of synaptic transmission in order to return the network to a homeostatic set-point.

In response to variations in network activity, synaptic strength can scale up or down as part of the homeostatic scaling process. In neocortical neurons, selective inhibition of postsynaptic firing initiates a signalling cascade that necessitates a decrease in somatic calcium influx, a reduced level of CaMKIV activation, and transcription. This increases micro amplitude and improves evoked transmission by increasing the concentration of AMPA receptors in the postsynaptic membrane at all excitatory synapses. According to studies, scaling up is accompanied by a coordinated rise in the AMPAR GluA1 and GluA2 subunits. Moreover, a number of chemicals have been linked to synaptic scaling, including BDNF, Arc, TNF, MHC1, 3 integrins, PICK1, and PSD-95.

It is yet unknown how exactly these molecules work together to control AMPAR abundance while scaling up. On the other hand, scaling down targets the GluA2 subunit and includes increased calcium influx, gene transcription, the CaMKK/CaMKIV signalling pathway, and other factors.(Kandel 2013)

Glia

In the central nervous system (CNS), glia are a class of non-neuronal cells that nourish and maintain neurons. Neuroglia or just glia are other names for glia cells. The most prevalent glial cells in the central nervous system (CNS) are astrocytes, a kind of star-shaped glial cell. Astrocytes have a variety of functions in the brain, including modulating synaptic transmission, maintaining the blood-brain barrier, and controlling extracellular ion and neurotransmitter concentrations. Astrocytes play a role in the growth and plasticity of neurons as well as in the creation and upkeep of synapses.

In addition to neurons, glial cells, particularly astrocytes, are also impacted by homeostatic scaling. Astrocytes control the extracellular concentrations of neurotransmitters and ions as well as the activity of neurons, both of which are necessary for preserving synaptic homeostasis.

Astrocytes undergo changes in their morphology, gene expression, and signalling capacities during homeostatic scaling in order to adjust to variations in neuronal activity. One of the key alterations that happens in astrocytes during homeostatic scaling is an increase in the expression of glutamate transporters, which helps to maintain the balance of extracellular glutamate levels.(Allen 2021)

Hypothesis

Aim: To demonstrate how homeostatic changes in the CA3-CA1 synapse affect synaptic transmission and plasticity.

Homeostatic scaling involves changes in astrocytic glutamate transporters density, AMPA receptor density,AMPA receptor open probability. The thesis investigates how the nanoscale changes of critical molecular machinery in the synapse orchestrates synaptic function and how plasticity is modified as a consequence of Homeostatic Synaptic Plasticity.

3.Materials and Methods

The MCell software, version 3.2, was used to run Monte Carlo simulations. MCell stochastically executes user-specified chemical processes while simulating the diffusion of individual molecules (each with a unique diffusion constant) existing on a surface or in a restricted volume. Using physiologic spatial distributions and concentrations of pertinent chemicals, we generated a dendritic spine terminal. To determine spatiotemporal trajectories, these simulations keep tabs on each molecule and the pertinent processes. An HP PROLIANT SL230s Gen8 high-performance computing cluster with 1464 processing units located at IISER Pune was utilised to run the simulations. Each compute node in the cluster has two CPUs with ten cores each.

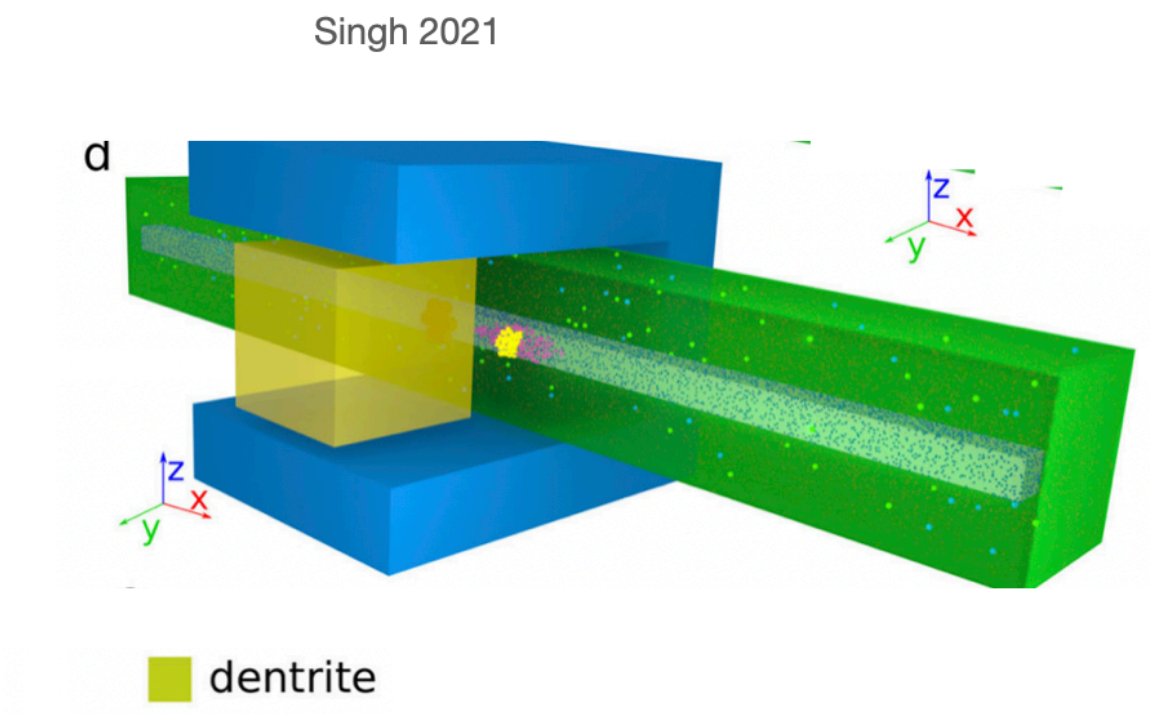


Figure2:Geometry of Model used (adapted by Nadkarni 2021)

Prior studies have defined and confirmed Monte Carlo techniques for simulating synaptic transmission. A three-dimensional random walk was employed to describe the diffusion of molecular glutamate. Throughout this investigation, a fixed time step of 1 micro second was employed.The probabilistic treatment of individual unimolecular or bimolecular kinetic interactions was done based on macroscopic rate constants.The primary output of the simulation was the time series of receptor states, which includes the open-conducting state. It is believed the number of open AMPARs scales linearly with the AMPA postsynaptic current. We use the assumption that all simulations would take place at room temperature because rate constants were determined from experiments done there. (Bartol et al., 1991; Stiles and Bartol, 2001; Stiles et al., 2001)

Model Geometry

Simulations were conducted on a postsynaptic terminal with a volume of 0.5 × 0.5 × 0.5 μm and a surface area of 0.25 μm2. This size is representative of a typical CA1 postsynaptic terminal with simplified cube geometry. The tripartite synapse canonical model comprises three primary geometrical components, which are a cuboidal presynaptic, a cubical postsynaptic terminal, and a U-shaped astrocyte that surrounds the synapse.

Model Components

Glutamate

Based on experimental data from several research, the time constant of glutamate clearance is around 1.2 milliseconds. The time constant measures how quickly a system reacts to changes in input, and in this example, it measures how long it takes for the concentration of glutamate to drop to 37% of its original level in the synaptic cleft after it has been released from the presynaptic neuron.With a diffusion coefficient of around 0.2-microns-squared

per millisecond, glutamate diffuses rather slowly when compared to how quickly it is released and cleared from the body.Its slow diffusion prevents glutamate from spreading to adjacent synapses and causing synaptic crosstalk. (Clements 1992)

The remarkable temporal accuracy of synaptic transmission is largely attributed to the quick release of glutamate and its effective clearance. When it comes to the encoding and processing of information in the brain, the exact temporal regulation of synaptic transmission is made possible by the time course of glutamate release and clearance.

In our model 2000 molecules of glutamate were released at the time of synaptic transmission.The site of release was a-pore-at (0,0,1e-3).Various glutamate release patterns were used in the study:

a) Single Pulse: a single quantal release of glutamate molecules at t=0 for single pulse simulations

b) Paired Pulse: Two quantal release of glutamate molecules with inter-spike interval of 20ms,50ms,100ms.

c) Train: Quantal release of glutamate molecules every 100ms,50ms,20ms corresponding to a spike train of 10Hz,20Hz,50Hz respectively.

Astrocytic Glutamate Transporters

Proteins known as astrocyte surface-expressed glutamate transporters are present in astrocytes. These transporters play a key role in maintaining healthy neurotransmission and avoiding neurotoxicity by controlling the concentration of the neurotransmitter glutamate in the synaptic cleft.

At densities between 1000 and 2000/ um2, astrocyte-derived glutamate transporters (GluTs) are evenly and broadly dispersed throughout the neuropil. (Takahashi et al., 1996; Lehre and Danbolt, 1998).

We encoded transporters in our model using a mathematical models as described in Geiger et al. 1999 that explains how astrocytic glutamate transporters take in and release glutamate. The model accounts for the kinetics

of glutamate transporters, glutamate diffusion in extracellular space, and astrocytes' ability to act as a buffer.

According to the model, glutamate can exist in two different states: unbound and bonded to a transporter. From the extracellular environment, glutamate molecules can attach to the transporter. Once bound, they can move across the cell membrane and be released into the cytoplasm. Glutamate can then either be digested or stored in vesicles for later release..

Each transporter in the simulation was modeled with three states: T0 and T1 represent the transporter in its unbound and bound states, respectively, while T2 serves as a transitional stage when the bound glutamate is removed from the simulation. The kinetic rates were as follows: KT0T1, 1.80 × 107m−1sec−1;KT1T0, 180 sec−1;KT1T2, 180 sec−1; and KT2T0, 25.7 sec−1 (Geiger et al., 1999).

Under normal conditions astrocytes were evenly placed on the astrocytic surface with a density of 1000/um2.This number was systematically increased to 5000,10000,15000/um2 to emulate homeostatic upscaling.A point ode model was run to figure out the occupancy of these transporters in each state when the last glutamate molecule from the cleft was absorbed.The transporters were defined with the ratio of states derived from the ode model.

AMPA Receptor

AMPA receptors were integrated into our model using the Milstein Nicolle scheme and the Jonas Sakmann Scheme.

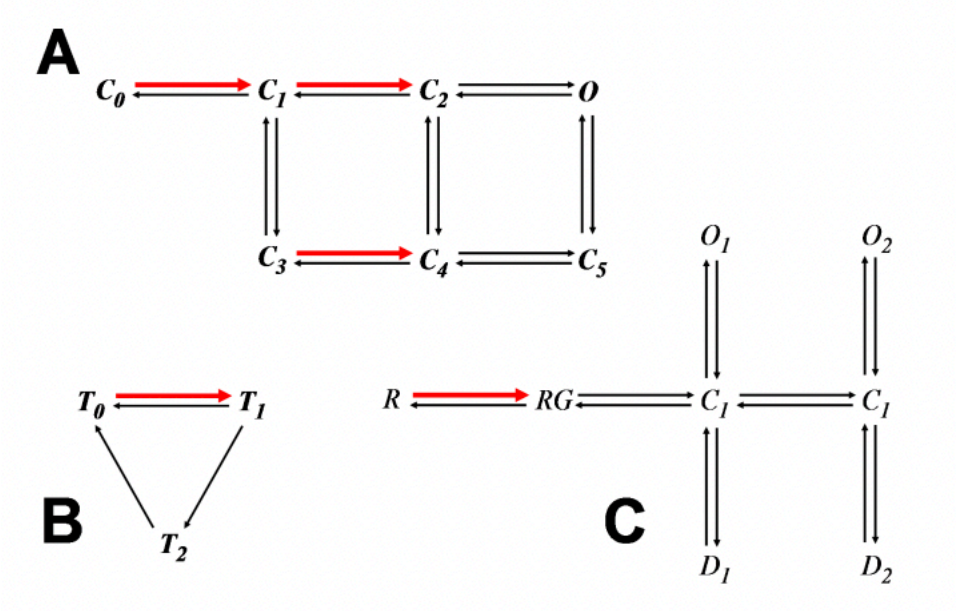
The Milstein Nicolle Scheme is a model that is used to explain how AMPA receptors are activated and desensitised. There are three primary states of AMPA receptors: resting, active, and desensitised. The receptor is closed and incapable of allowing ions to flow through it in the resting state. Glutamate causes a conformational change in the receptor when it binds to it, opening the channel and letting ions pass through.This is known as the active state.

Ions can pass through the channel once it is open for a short while until the receptor reaches a desensitised state, when it is closed once again and unable to react to glutamate. This desensitised state is regarded to be crucial for limiting overexcitation of neurons and regulating the synaptic transmission's temporal characteristics.

The JS Scheme models the two-step process of glutamate binding to the receptor. Initially, a glutamate molecule binds to the receptor, changing its shape and raising the receptor's affinity for a subsequent glutamate molecule. A further conformational shift in the receptor occurs when the second glutamate molecule binds, opening the ion channel pore and allowing ions to pass through.

Figure3:Kinetic Scheme used for encoding AMPA receptors using A)Jonas Sakmann Scheme B)Two state Transporter Scheme C)Milstein Nicolle Scheme[adapted by Holcman 2011]

JS scheme		MN scheme	
$k_{C_0C_1}$	$4.59\,10^6 M^{-1}s^{-1}$	$k_{R,RG}$	$1\,10^7 M^{-1}s^{-1}$
$k_{C_1C_0}$	$4.26\,10^3 s^{-1}$	$k_{RG,R}$	$5\,10^4 s^{-1}$
$k_{C_1C_2}$	$2.84\,10^7 M^{-1}s^{-1}$	k_{RG,C_1}	$3.65\,10^4 s^{-1}$
$k_{C_2C_1}$	$3.26\,10^3 s^{-1}$	$k_{C_1,RG}$	$4.55\,10^3 s^{-1}$
k_{C_2O}	$4.24\,10^3 s^{-1}$	$k_{C_1C_2}$	$3\,10^2 s^{-1}$
k_{OC_2}	$9.00\,10^2 s^{-1}$	$k_{C_2C_1}$	$1\,10^4 s^{-1}$
$k_{C_1C_3}$	$2.89\,10^3 s^{-1}$	$k_{C_1O_1}$	$1\,10^4 s^{-1}$
$k_{C_3C_1}$	$3.92\,10^1 s^{-1}$	$k_{O_1C_1}$	$6\,10^3 s^{-1}$
$k_{C_2C_4}$	$1.72\,10^2 s^{-1}$	$k_{C_2O_2}$	$1\,10^4 s^{-1}$
$k_{C_4C_2}$	$0.727\,s^{-1}$	$k_{O_2C_2}$	$6\,10^3 s^{-1}$
k_{OC_5}	$1.77\,10^1 s^{-1}$	$k_{C_1D_1}$	$1.1\,10^3 s^{-1}$
k_{C_5O}	$4.00\,s^{-1}$	$k_{D_1C_1}$	$1\,s^{-1}$
$k_{C_3C_4}$	$1.27\,10^6 M^{-1}s^{-1}$	$k_{C_2D_2}$	$3\,10^2 s^{-1}$
$k_{C_4C_3}$	$4.57\,10^1 s^{-1}$	$k_{D_2C_2}$	$10\,s^{-1}$
$k_{C_4C_5}$	$1.68\,10^1 s^{-1}$		
$k_{C_5C_4}$	$190.4\,s^{-1}$		



Channel kinetic parameters. The AMPA receptors were spread evenly across the PSD at predetermined densities. The PSD, which is a disk-shaped structure measuring 300 nanometers in diameter located on the synaptic face of the postsynaptic spine, was used for this purpose. The Jonas et al. (1993) reaction scheme and kinetic rate constants were used(as shown above) to model the AMPA receptors.

AMPA receptor density was 1280 /um2.The PSD where majority(90%) of the AMPA was present was a disc of diameter 300nm present at the centre of the postsynaptic surface.Under normal conditions our CA1 terminal had 130 AMPA receptors present on the postsynaptic surface.When AMPA receptor count was increased, only those is the PSD where changed.

Statistics and reproducibility.

The simulation was run 500 times for each scenario, and data analysis was carried out using the python programming language. The model code and python scripts are both available, and the plots were created using the matplotlib library in python.

Using mEPSC as our main output

In research on homeostatic synaptic scaling, miniature excitatory postsynaptic currents (mEPSCs) are frequently utilised as a proxy for synaptic strength. mEPSC amplitude is a useful postsynaptic output for investigating homeostatic scaling for a number of reasons:

Synaptic strength quantified: The strength of excitatory synaptic transmission is quantified by the mEPSC amplitude.

Reflects variations in synaptic effectiveness: During homeostatic scaling, the balance of synaptic activity is maintained by the mEPSC amplitude, which captures changes in synaptic efficacy at the level of the individual synapse. Researchers can evaluate how well homeostatic mechanisms keep the overall balance of synaptic activity by monitoring variations in mEPSC amplitude.

mEPSC plots were calculated using the conductance model of AMPA receptor as described in [].Using the open ampa time series we calculated mEPSC waveform generated.

4.Results and Discussion

Homeostatic Synaptic Scaling is the process by which neurons adjust their synaptic strengths in order to maintain network stability.As mentioned earlier ,the critical ways by which this is done are:

a)Change in Transporter Count in the Astrocytic glia

b)Change in AMPA receptor count in the postsynaptic PSD

c)Change in kinetics of the AMPA receptor(Open probability)

d)Change in release probability

We now look at how each of these homeostatic changes in the synapse affect synaptic function and transmission.

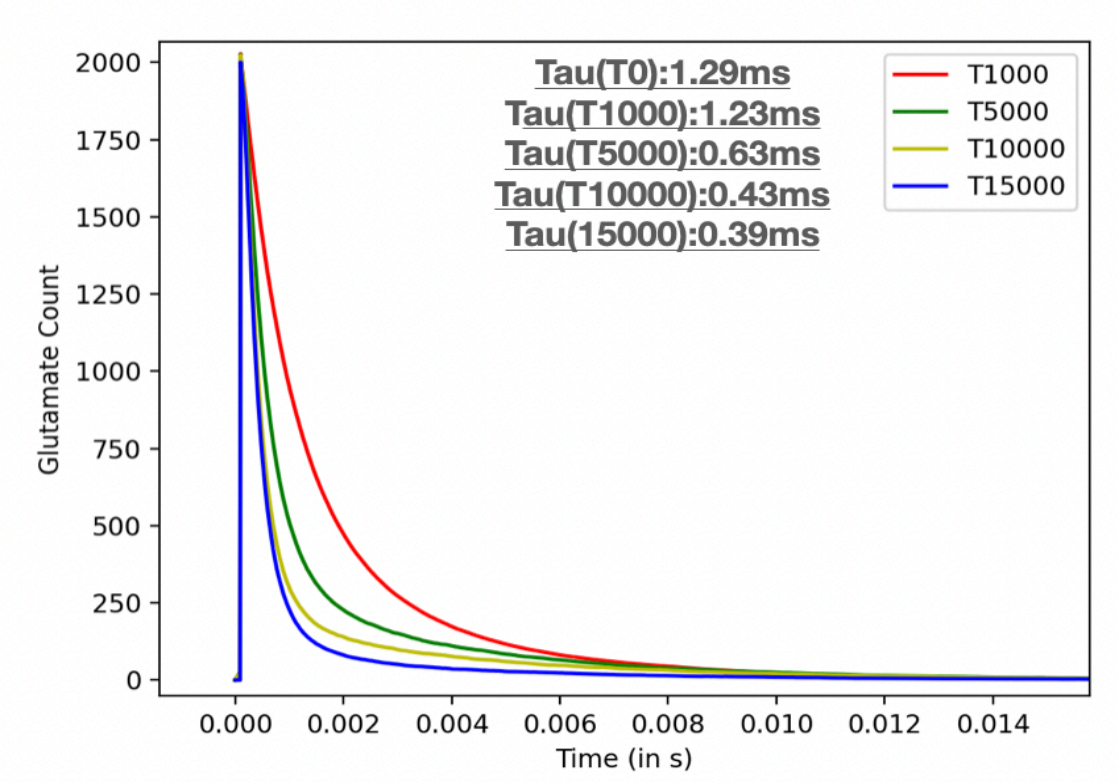
Disclaimer:For all plots units for mEPSC amplitude are in picoAmpere

Effect of Homeostatic Scaling of Transporter Density on Synaptic Function

a)Effect on glutamate temporal dynamics

We first looked at how changing the Transporter density on the Astrocytic Glia affected the temporal dynamics of Glutamate in the synaptic cleft.The results of the change in time constant(Tau) of glutamate clearance in the synaptic cleft can be seen in the figure 4 shown below.As mentioned earlier the time constant measures how long it takes for the concentration of glutamate to drop to 37% of its original level in the synaptic cleft after it has been released from the presynaptic neuron.The time constant of glutamate in the cleft changes by a factor 3.3 on changing the Transporter density.This is validated as shown in experimental studies by Jahr et.al 2005 which found that increasing the expression of glutamate transporters in the postsynaptic cell reduced the time constant of glutamate clearance

Figure 4:Glutamate count in the synaptic cleft plotted v/s time passed(in sec)



During homeostatic changes there is a change in the Release probability of vesicles,Quantal size of vesicle(Goel et al 2011) which can lead to an increase in the availability of glutamate in the synaptic cleft.Hence an increase in Transporter count during homeostatic scaling can act as a mechanism to prevent excessive accumulation of glutamate, which can be toxic to neurons.

b)Effect on mEPSC evoked

We then looked at how change in Transporter density would impact the evoked mEPSC in the postsynaptic CA1 terminal.On increasing Transporter density systematically from to 0 to 15,000 as shown in the figure below, we observed a 3.3% reduction in mEPSC amplitude as we increased the transporter count.This was much lesser than we initially expected as the glutamate clearance reduced by a factor of 3.3.We then counted the number of glutamate receptors that are actually in contact with the postsynaptic density for it to activate the AMPA receptors.As you can see in Fig 6 the number of glutamate

molecules that are actually “hit” the postsynaptic density are very similar.Hence despite the change in transporter density, the astrocytic glia transporters don’t have much of an influence on the evoked mEPSC during a single pulse stimulus.

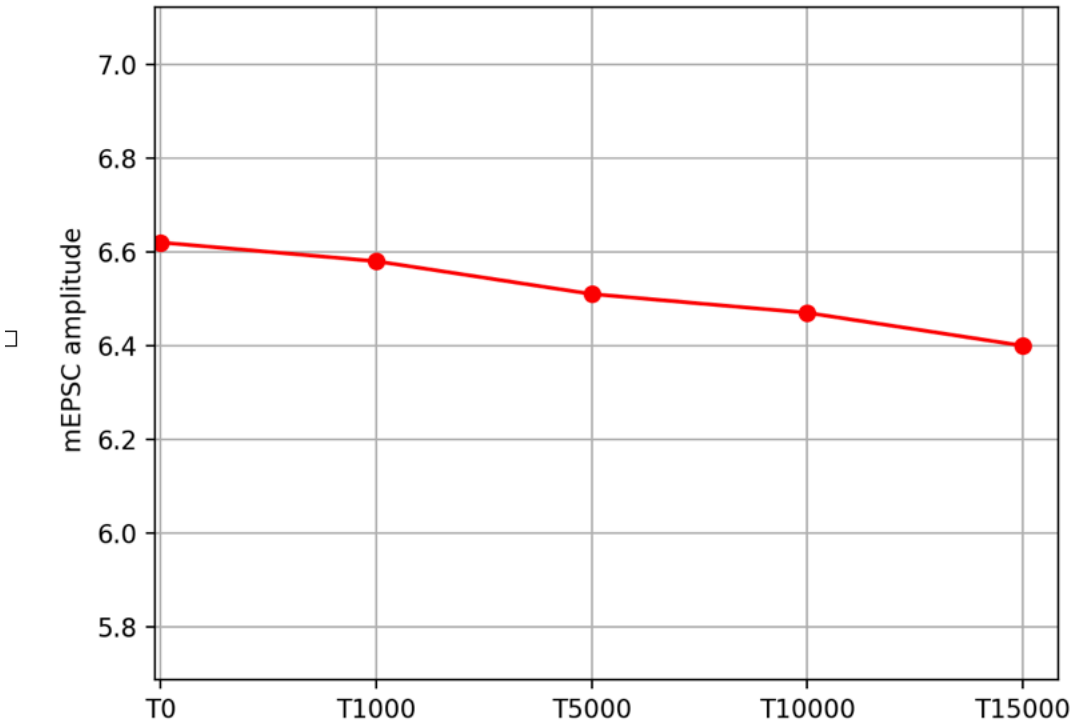
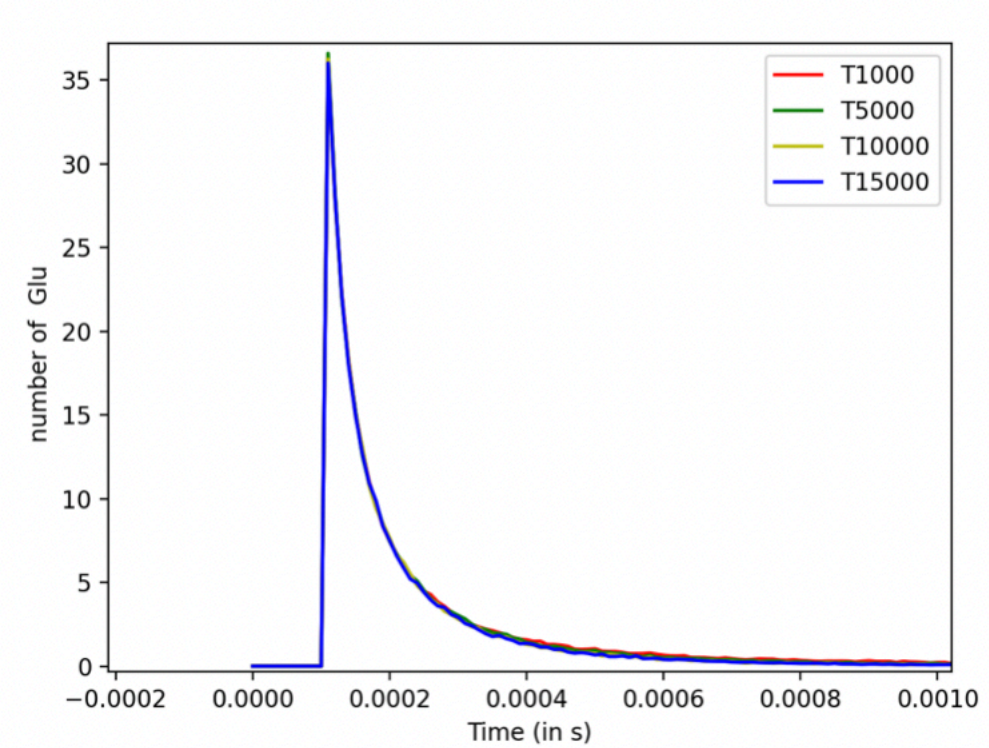


Figure 5:mean mEPSC amplitude plotted for different Transporter densities

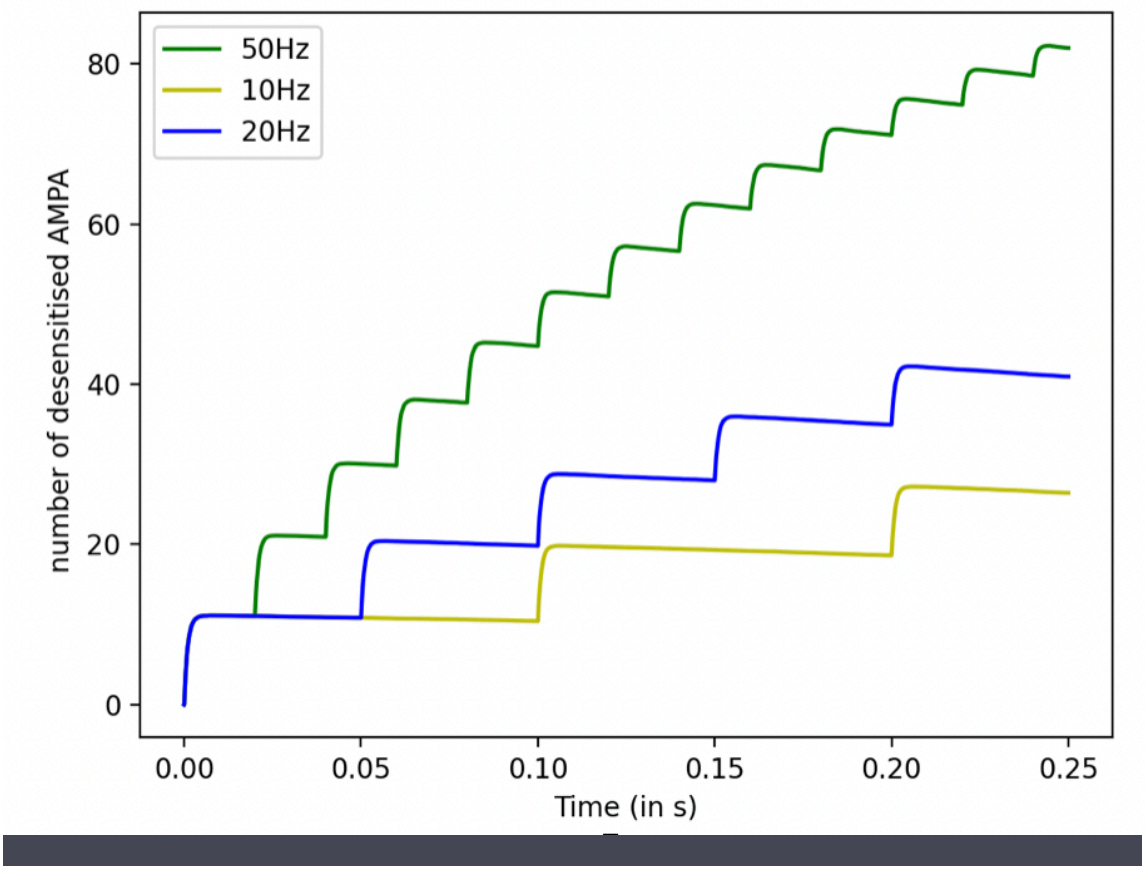
Figure 6:Glutamate count that diffused through the cleft and came in contact with postsynaptic PSD v/s time passed(in sec)



In accordance with experimental findings (Isaacson and Nicoll, 1993; Sarantis et al., 1993), we have demonstrated that AMPAR activation is indifferent to the amount of uptake by Glutamate transporters. (Franks et al., 2002).Glutamate once released into the cleft binds to AMPA receptors irrespective of whether it was taken in by astrocytic GluT.Hence transporters dont directly affect AMPAR activation. However,the amount of glutamate available in the synapse reduces with an increase in Transporter density which could lead to indirect effects especially in the regime when AMPA receptors get close to saturation, with an accumulation in desensitised AMPA as shown in Fig.7

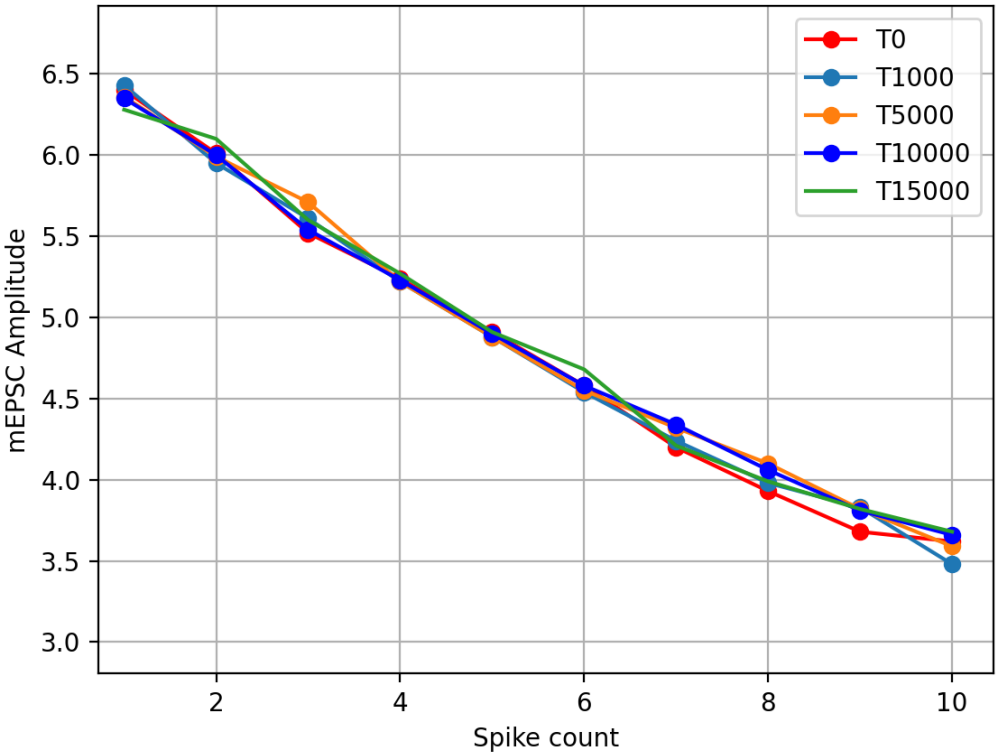
c)Effect of transporter count in the saturated regime

Figure 7:Number of desensitised AMPA plotted for different frequency train stimulus(10Hz,20Hz,50Hz) v/s time passed (in sec)



To check for any indirect effect of Astrocytic Glutamate transporters, we decided to give it a 50Hz stimulus train for 250ms and the corresponding mEPSC values for different transporter density have been shown.Again there is no real change in mEPSC amplitude seen.We believe this is because we havent varied the distance of the glia from the PSD.Studies as done by Ransom (2013) have shown that distance between the Astrocytic glia and PSD play an important role in postsynaptic activation.Since there is no evidence of change in distance during homeostatic synaptic scaling we have not crossed that path.

Figure 8:mean mEPSC amplitude values plotted when given a 50Hz spike train stimulus plotted v/s the spike count(ten spikes were considered



Effect of Homeostatic Scaling of AMPA receptors on Synaptic Function

a)Effect of AMPA receptor upscaling on mEPSC amplitude for a single stimulus

Typically an increase of 20-30% of AMPA receptors is seen post homeostatic upscaling[Gainey et all 2009].

AMPA receptor count was systematically increase by 10%,20%,30% and a 33.58% increase in mEPSC amplitude was seen as shown in Fig 9

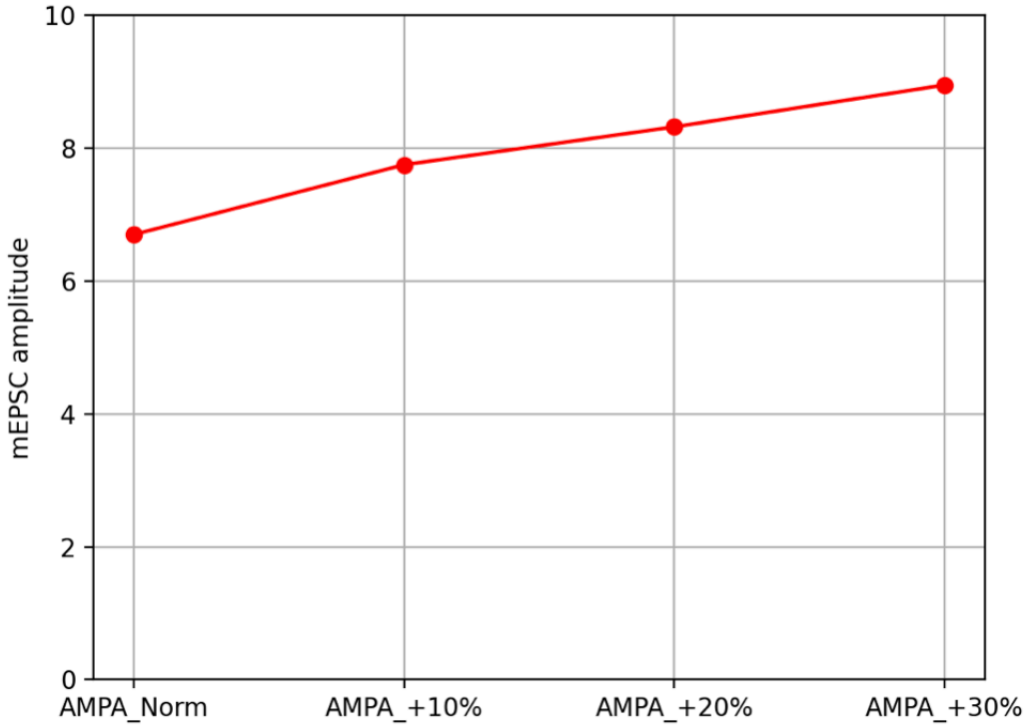


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Figure 9:mean mEPSC amplitude plotted ,as AMPA receptor count in the

These results are validated as shown in experimental studies done in cortical neurons show a 40% increase in mEPSC amplitude[Goel and Lee2007] and a two)fold increase in mEPSC amplitude post homeostatic scaling in cultured hippocampal neurons[turrigiano1998].Hence an increase in AMPA receptor count increases the strength of the synapse

Since there are more AMPA receptors at the synapse the likelihood that at least one AMPA receptor channel is open for a given amount of time is increased. This increases the mEPSC amplitude which increases the possibility of postsynaptic depolarisation and the formation of an action potential. This increase in strength of the synapse can also affect the synapse's capacity to experience other types of plasticity, including as long-term potentiation (LTP) or

long-term depression (LTD).

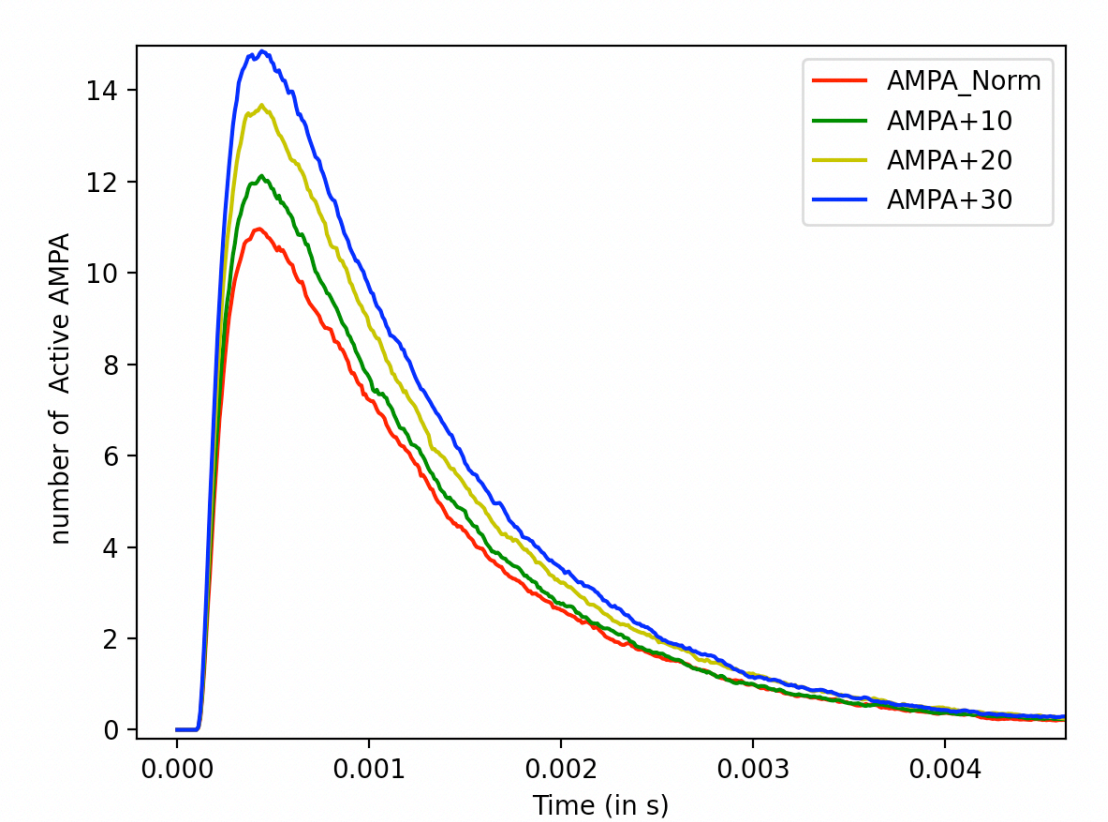


Figure 10:Total number of active AMPA receptors for different AMPA receptor counts plotted v/s time(in sec)

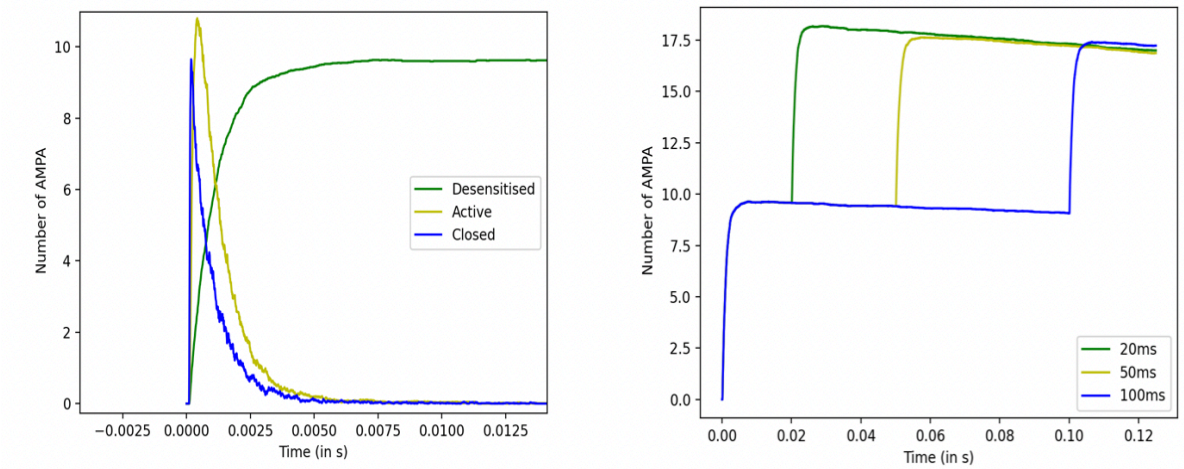


Figure 11:a)The timescale dynamics of Active v/s closed v/s desensitised AMPA receptors b)Number of desensitised AMPA for interspike interval of 20ms,50ms,100ms

B)Effect of AMPA receptor upscaling on Short Term Plasticity

We used the standard STP PPF Protocol. The PPF protocol involves delivering two quick electrical stimulations with a brief interspike interval of 20 ms, 50 ms, or 100 ms that was systematically altered. The test pulse is the second stimulus after the conditioning pulse, which is the initial stimulus. The difference between the postsynaptic response to the conditioning pulse and the response to the test pulse is then used to calculate changes in synaptic strength.

The ppr ratios were all <1 with and a general trend of reduction in ppr values for inter spike interval of 100ms as compared to 20ms was observed as shown in Fig12 .PPR ratio of less than 1 signifies that the synapse has undergone paired pulse depression.PPD can help regulate the balance of excitation and inhibition in neural circuits by reducing the strength of excitatory synapses. This can help prevent overexcitation and maintain an optimal level of neural activity.The trend of reduction in ppr as interspike interval is increased can possibly a role in temporal encoding of information in the synapse by modulating the strength of synaptic transmission based on the timing of incoming stimuli.

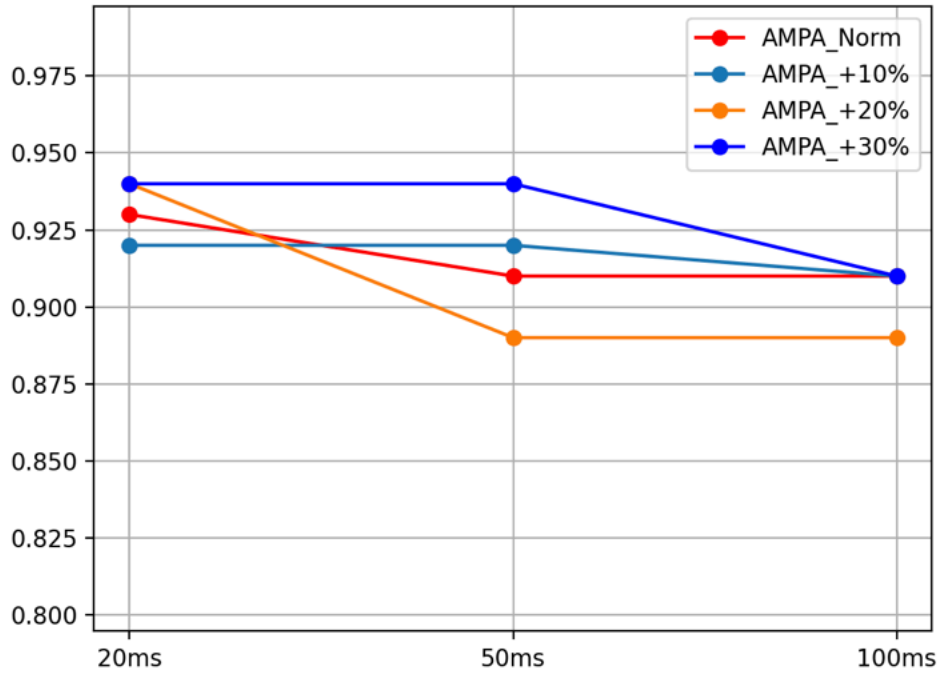


Figure 12:ppr ratios calculated for different AMPA receptor count for interspike intervals of 20ms,50ms and 100ms

The ppf values being less than one can be explained from the above graphs.Desensitisation Tau is 2 orders of magnitude higher than than the active and closed state and hence during the second pulse there are lesser AMPAs available to bind to glutamate.

Effect of Homeostatic upscaling of AMPA receptor kinetics on synaptic function

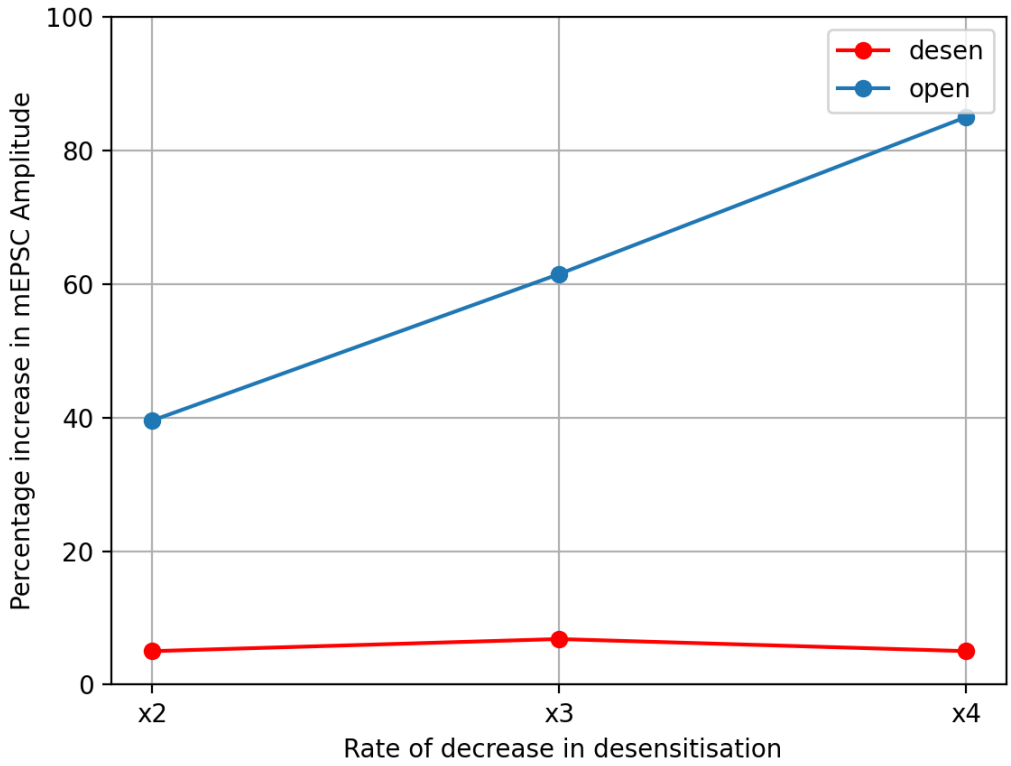


Figure 13:Percentage increase in mean mEPSC amplitude plotted for an increase in open rate and also a reduction in desensitised(essentially increasing open probability by 2,3,4

The open probability of an AMPA receptor refers to the likelihood that the receptor channel will be in an open state.This was achieved by increasing the rate at which the closed ampa receptor transitions into its open state.The rates were systematically increased two, three, four fold.

Previous studies on neocortical neurons have shown a two fold increase in open probability post homeostatic upscaling[turrrigiano1998].In cultured rat

hippocampal neurons the open probability of AMPA receptors was increased by approximately 1.5 fold.[ibata 2008].On increasing open probability of AMPA receptor a 85% increase in mEPSC amplitude was observed as seen in Fig 13

We also increased the open probability of AMPA receptors by reducing the rate at which the closed receptor state transitions into the desensitised state by systematically reducing it by two, three ,four fold. On reducing the desensitisation rates of AMPA receptor the mEPSC amplitude showed only a 6.83% increase.This results shows us that desensitisation rates don't play an important role for a single pulse stimulus.

Effect of Changing Presynaptic Release Probability on Synaptic Function

A)Effect of Change in Release probability for Single Pulse stimulus

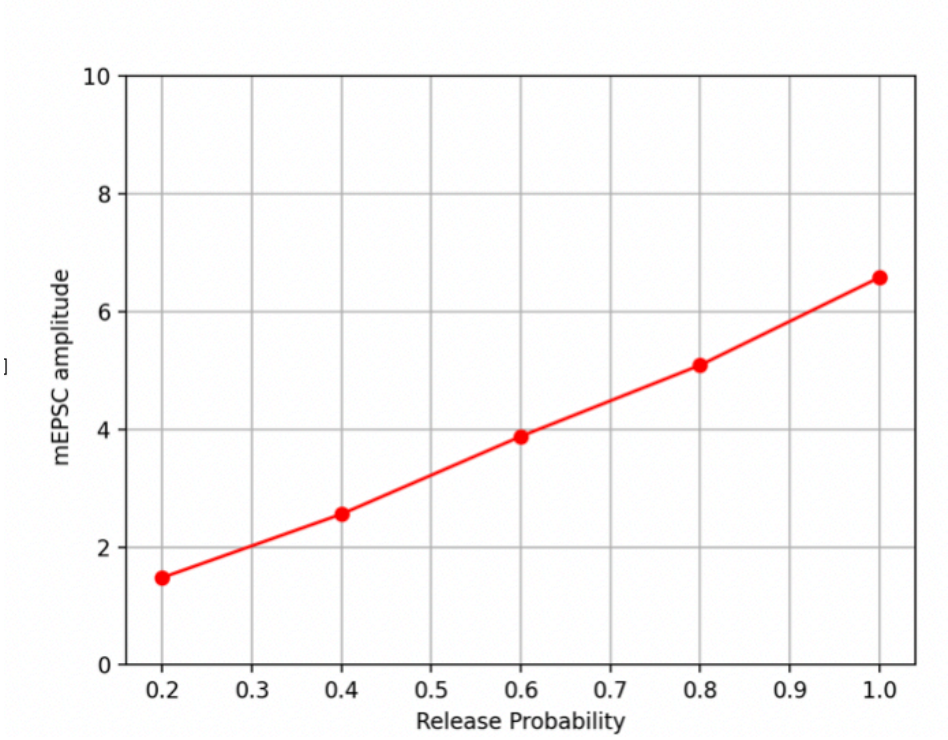


Figure 14:mean mEPSC amplitude plotted for different release probabilities of

Presynaptic release probability refers to the likelihood that a neurotransmitter will be released from a presynaptic neuron in response to an action potential. Studies have reported changes in presynaptic release probability during homeostatic synaptic scaling[Wierenga CJ, Ibata K, Turrigiano GG 2005] with the precise mechanism being unknown.We systematically increased the release probability from 0.2 to 1 and saw a linear increase in mEPSC amplitude as shown in Fig. X .As the release probability increases, more vesicles of neurotransmitter may be released, resulting in a more efficient transfer of information across the synapse.

B)Effect of Change in Release Probability on Synaptic Function in a Paired Pulse Protocol

We then proceeded to use the standard STP protocol as described earlier for different release probabilities and calculated the ppr values for different interspike intervals-20ms,50ms,100ms.Interestingly for release probability of 0.4 ppr values are >1 for all interspike intervals.The release probability for a standard CA3 presynaptic neuron is 0.2.As shown in figure 15 there is a complete transfer from paired pulse depression to paired pulse facilitation when the standard release probability is doubled.This could be a mechanism for maintaining the balance between excitation and inhibition in neural networks and may play a role in learning and memory processes. The transition from PPD to PPF may also have an impact on the network's calculations and behaviour in terms of the temporal dynamics of information processing.

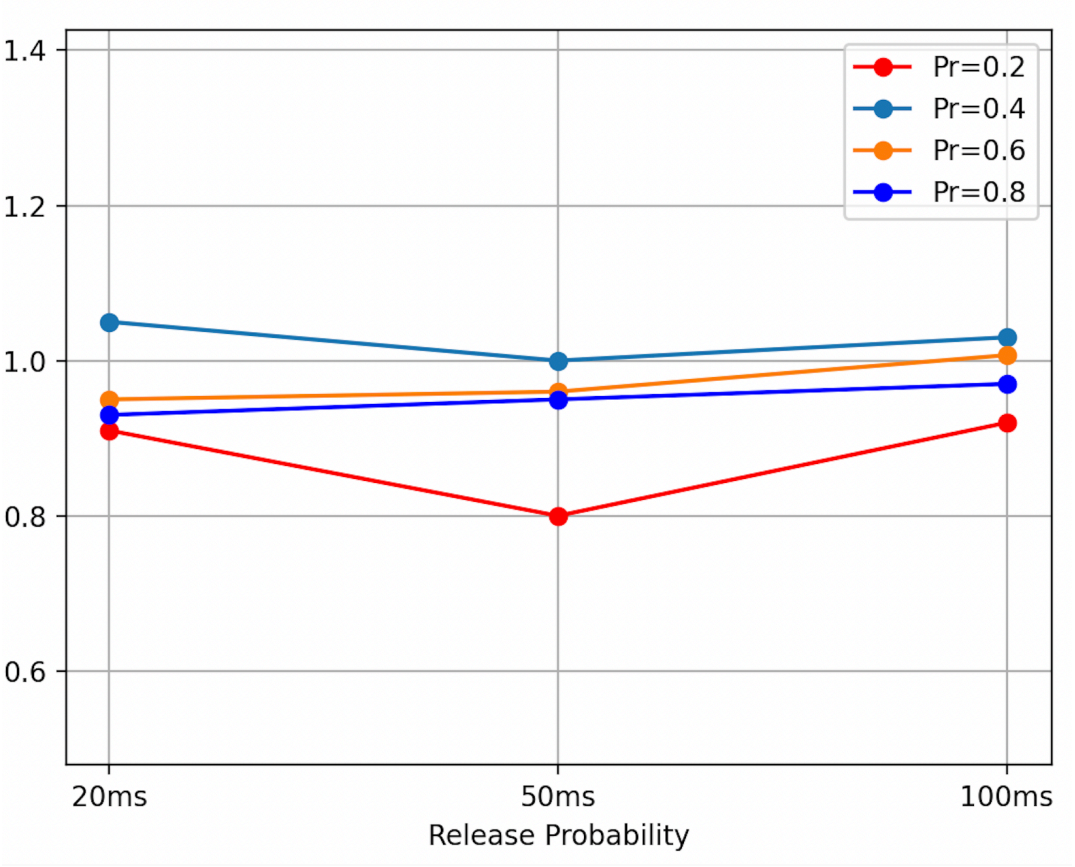


Figure 15:ppr ratios calculated for different release probability for interspike intervals of 20ms,50ms and 100ms for standard AMPA number

Comparison of MN and JS Model

The complexity of the two schemes is different. The JS Scheme is more straightforward and concentrated on the basic characteristics of AMPA receptor function, whereas the MN Scheme is more complex and has more states and parameters. As shown in the figure16, the decay time of the Active AMPA is larger while the desensitisation decay time is lesser. We tested the JS Model for

upscaling of AMPA receptor count by systematically increasing AMPA count by 10%,20%,30% and then compared it with the results of the MN Scheme.As expected an increase in mEPSC amplitude very similar to the one obtained using the MN model was obtained.

We then tested the model for a pulse train of 50 Hz and compared it to the results

obtained from the MN model.Interestingly, mEPSC amplitude starts to saturate

after the fifth spike, while it continues to reduce with the MN model.The best use of

these models would be to use both of them at the same time.Since AMPARs undergo both short(few milliseconds)

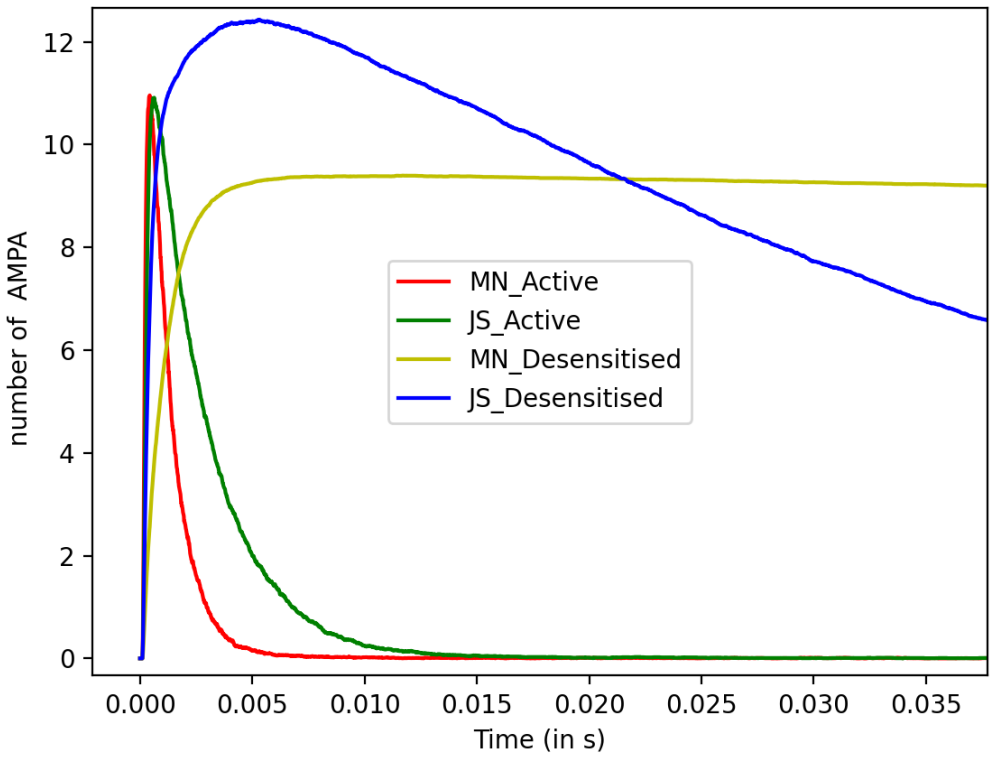
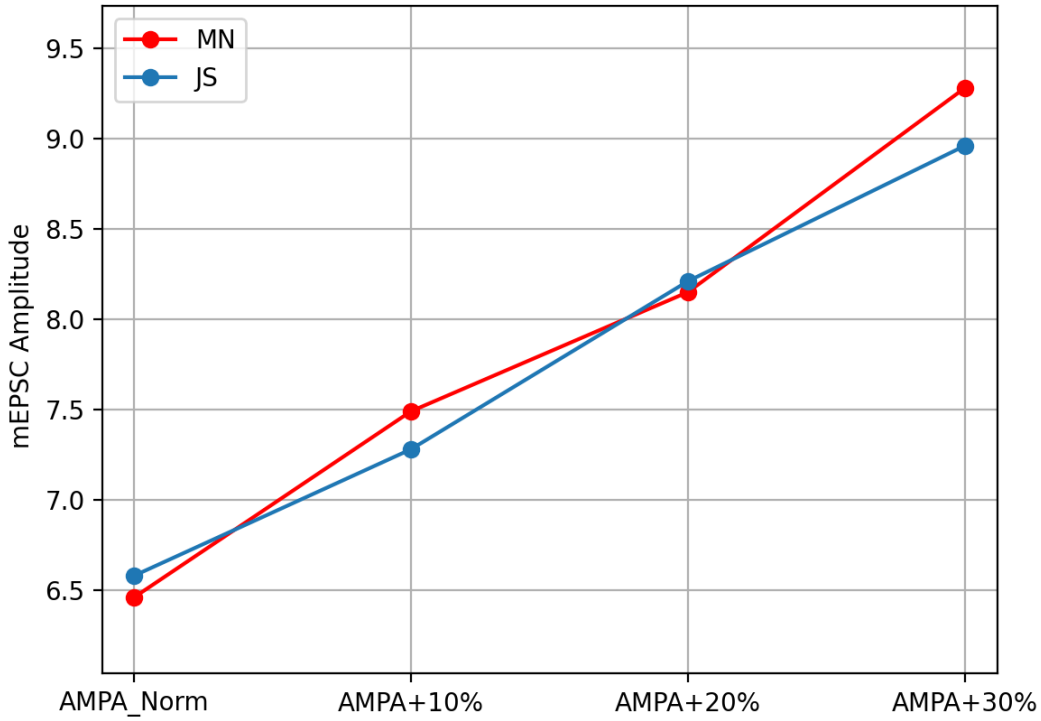


Figure 16:Comparison of the open and desensitised state timescale dynamics for the MN and JS Scheme

Figure 17:mean mEPSC amplitude plotted, as AMPA receptor count in the postsynaptic PSD was varied systematically by 10%,20%,30%.AMPA was modelled using both the JS and Mn scheme and compared.



and long desensitisation(seconds to minutes) using a mixture of both models would likely lead to a more realistic output, with long desensitisation being relatively less often except during a high frequency train stimulus as given above. [McBain CJ, et al

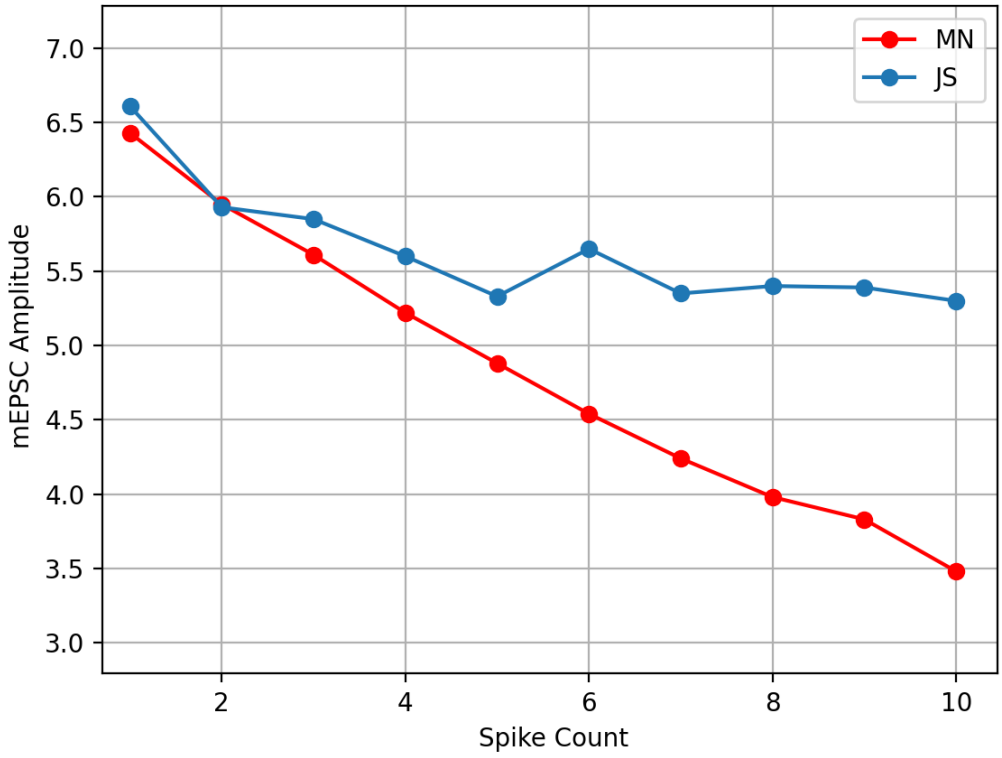
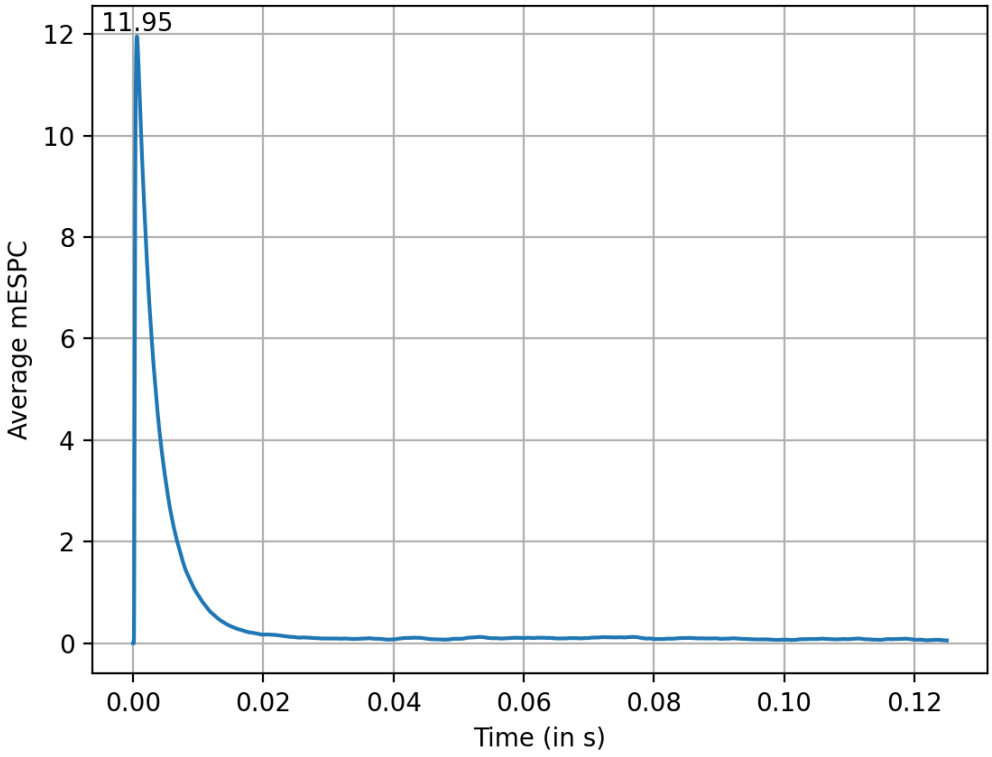


Figure 18:mean mEPSC amplitude values plotted when given a

Cumulative effect of Transporter Density,AMPA receptor upscaling and AMPA receptor kinetic on synaptic function

Finally we incorporated all the homeostatic scaling changes together and the result obtained was very well validated with experimental data [Turrigiano 1998].The increase in mEPSC amplitude for an increase in AMPAR count by 30%,Doubling of open probability of AMPA receptor and 15000/um2 transporter density gave a 81% increase in mEPSC amplitude.This brings about an important question as to how energy-information tradeoff is achieved in the synapse during homeostatic synaptic

Figure 19:mEPSC waveform generated when cumulative homeostatic changes occur i.e +30% increase in AMPA receptor count,2x open probability and 15000/ um2 Glutamate Transporter density



plasticity.

The capacity of synapses to convey information can be improved by increasing the density of AMPA receptors, but doing so also increases the energy needed to sustain these receptors. This is due to the energy-intensive processes involved in AMPA receptor trafficking and synaptic maintenance, which also include the synthesis of new proteins, the remodelling of synaptic architecture, and the activation of several signalling pathways.

The capacity of synapses to control the concentration of neurotransmitters in the synaptic cleft may also be improved by increasing the density of transporters, such as glutamate transporters, which can influence synaptic transmission and plasticity. Yet, as the transport of neurotransmitters against

their concentration gradient necessitates ATP-dependent activities, this also necessitates an increase in energy expenditure.

In order to guarantee effective information transmission without overtaxing the cell, the increase in AMPA receptor density and transporter density during homeostatic scaling must be balanced against the energy requirements of the neuron. The selective strengthening or weakening of certain synapses, the control over presynaptic neurotransmitter release, and the modification of postsynaptic receptor and transporter characteristics are just a few of the techniques that may be used to accomplish this.

In order to ensure effective neural communication while preserving cellular homeostasis, the energy-information tradeoff in synapses during homeostatic scaling is complicated and involves the balancing of a number of factors, including changes in the density and properties of AMPA receptors and transporters.[Turrigiano GG 2004,2012]

5.Conclusion and Outlook

In this thesis we investigated how homeostatic upscaling of AMPA receptor density,Astrocytic Glutamate Transporter density and kinetics of AMPA receptor affected synaptic function and transmission.We observed an increase in mEPSC amplitude on increasing AMPA receptor density, release probability of glutamate and increasing the open probability of AMPA receptors. An increase in mEPSC amplitude is synonymous to an increase in the strength of the synapse which has implications in the synapses capacity to undergo other forms of plasticity like LTP and LTD.All results of our Pulse Paired Protocol gave values <1 implying a depression in our synapse during homeostatic upscaling which is a point to ponder about.This is most likely to regulate the balance between excitation and inhibition in neuronal networks i.e to prevent over-excitation and maintain an optimum level of neural activity.

Homeostatic synaptic plasticity is a fundamental mechanism by which neuronal networks maintain stability.Studies (Heller JP 2020) have shown that alterations in AMPA receptor and astrocytic GluT homeostatic scaling can contribute to the pathogenesis of various neurological disorders like Alzheimers,epilepsy and schizophrenia.Understanding the mechanisms underlying these changes can potentially help identify targets for therapeutic interventions in these disorders.Research along these lines can also provide insights into the basic mechanisms behind synaptic plasticity and advance our understanding of how the brain processes and stores information.

Homeostatic synaptic plasticity is a process that regulates synaptic function, but the dynamic regulation of spine function through this process is not fully understood. The molecular pathways involved in various forms of homeostatic plasticity are currently being studied, and while many molecular pathways have been implicated in homeostatic plasticity, there is still no clear understanding of how homeostatic feedback is structured at the molecular level.

Building a robust model incorporating the key homeostatic molecular players would prove pioneering.This would be done by incorporating calcium dynamics into the model.Calcium plays a critical role in homeostatic synaptic scaling.As a first step we would integrate NMDA receptors, Ca buffers and VDCCs into the model.Homeostatic scaling has similar affects on the NMDA receptor as it does on AMPA[Turrigiano 2012].

CaMKII is a key player in the regulation of AMPAR function and is activated by calcium influx into the postsynaptic neuron. CaMKII has the ability to phosphorylate the AMPAR GluA1 subunit, which increases the likelihood that the receptor channel will open and causes the insertion of additional AMPARs into the postsynaptic membrane.

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