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Cholinergic Modulation of CA1 Pyramidal Cells via M1 Muscarinic Receptor Activation: A Computational Study at Physiological and Supraphysiological Levels

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Abstract

The hippocampus receives extensive cholinergic modulation from the basal forebrain, which has been shown to have a prominent role in attention, learning, and synaptic plasticity. Disruptions of this modulation have been linked to a variety of neural disorders including Alzheimer's Disease. Pyramidal cells of the CA1 region of the hippocampus express several cholinergic receptor types in different locations throughout the cells' morphology. Developing a computational model of these cells and their modulation provides a unique opportunity to explore how each receptor type alters the overall computational role of the cell. To this end we implemented a kinetic model of the most widely distributed receptor type, the M1 muscarinic receptor and examined its role on excitation of a compartmental model of a CA1 pyramidal cell. We demonstrate that the proposed model replicates the increased pyramidal cell excitability seen in experimental results. We then used the model to replicate the effect of organophosphates, a class of pesticides and chemical weapons, whose effects consist in inhibiting the hydrolysis of acetylcholine; we demonstrated the effect of increasing concentrations of acetylcholine on the pyramidal cell's excitability. The cell model we implemented and its associated modulation constitute a basis for exploring the effects of cholinergic modulation in a large scale network model of the hippocampus both under physiological and supraphysiological levels.

I. INTRODUCTION

The hippocampus receives cholinergic modulation from the basal forebrain, primarily from the medial septum and the diagonal band of broca. Axonal projections from these regions target both pyramidal cells and several types of interneurons as evidenced by the presence of various cholinergic receptor types [1]. Higher concentrations of acetylcholine (ACh) in the hippocampus have been associated with learning spatial tasks [2], while lesions of cholinergic cells have been linked with deficits of contextual spatial memory [3]. Different

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tonic concentrations of ACh have also been measured and linked to different states of consciousness [4].

With so many links between ACh and normal hippocampal function, it is not surprising that dysfunctional cholinergic activity is linked to several neural disorders in humans. Post-mortem examination of the hippocampi of Alzheimer's disease patients revealed decreased activity of acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) [5]. These two enzymes are responsible for the hyrdolysis and synthesis of ACh, with their absence suggesting an overall decrease in cholinergic modulation. Cholinergic dysfunction has also been linked to schizophrenia and depression (see [6] for a review).

Among the various types of cholinergic receptors found in the hippocampus, the most widely distributed is the M1 muscarinic acetylcholine receptor (mAChR). The M1 mAChRs act through a Gq protein cascade which can lead to a variety of downstream interactions with other mechanisms. Among the known interactions is the inhibition of Kv7 voltage gated potassium channels. Kv7 channels are primarily expressed in the soma and axonal initial segment of pyramidal cells [7]. Remaining open at resting potentials, these channels oppose depolarization and play an important role in spike frequency adaptation. Blocking these channels using selective antagonists has been shown to induce spontaneous spiking in CA1 pyramidal cells [7]. Having such a strong ability to modulate the pyramidal cell spiking behavior, it appears critical to include this interaction between M1 mAChRs and Kv7 channels to accurately simulate the functional roles of pyramidal neurons in response to cholinergic modulation.

II. METHODS

A. M1 mAChR Kinetic Model

To properly model the dynamics of muscarinic receptors requires a model of the G protein chain that the receptors are coupled to. In a series of papers Bertil Hille and co-authors generated a collection of kinetic models linking M1 mAChR activation to the inhibition of Kv7 channels [8]–[11]. The final paper in this series, [11], analyzed the kinetics of Kv7 inhibition in sympathetic ganglion neurons. We implemented these models in the NEURON simulation environment to ease future inclusion in cell network models [16]. We first tested our implementation to ensure its behavior matched the reported behavior in [11].

All kinetic parameters for the M1 receptors were based upon experiments using oxotremorine M (Oxo-M), a very selective receptor agonist. While it is understandable to use a very selective agonist to explore the effect of a receptor's activation, we first focused on recalibrating the parameters to account for the binding of the more biologically relevant endogenous agonist acetylcholine. After confirmation that the model was functional with Oxo-M, we therefore sought to recalibrate a subset of parameters to simulate exposure to ACh.

B. Calibrating Acetylcholine Parameters

For this calibration we assumed that the agonist identity was only affecting the binding rate and not altering any of the other kinetics. Under this assumption there are only two reactions

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that would require new parameters. In [8], the forward and reverse rate constants for these two reactions (ligand binding to the receptor with and without a G protein already attached) are based upon fluorescence resonance energy transfer (FRET) measurements. A very similar technique was performed in a study to compare how M1 receptors were activated by several different agonists, including ACh and Oxo-M [12]. These studies provided a quantitative way of comparing the relative effectiveness of these two agonists based on their relative concentrations.

After isolating the ligand binding reaction, the rate parameters were tuned to produce the difference in the EC50s seen in the fluorescence measurements of ACh and Oxo-M. These revised parameters were then used in the full kinetic model to demonstrate how altered M1 parameters altered the steady state percent Kv7 inhibition achieved by a concentration of agonist. The resulting curves can be seen in Results subsection A.

C. Integration in a Compartmental Model

Shah and coauthors explored the effect of inhibiting Kv7 channels using the selective blocker XE991 [7]. Along with the experimental results, the authors simulated the results in a compartmental model of the CA1 pyramidal cell. As this model had explored the effect of inhibiting Kv7 channels, it seemed a fitting basis for simulating the effect of M1 mAChR activity in a CA1 pyramidal cell. We first confirmed that varying the maximum conductance of the Kv7 mechanism produced results consistent with those reported in [7]. Upon satisfactory validation, we tied the value of the Kv7 inhibition in the Kruse model to the value of the maximum conductance for Kv7 channels in the CA1 pyramidal cell compartmental model. Having combined these components into a new model, we validated its behavior against experimental activation of M1 receptors in CA1 pyramidal cells.

D. Model Validation

Exposure of CA1 pyramidal cells to ACh in vitro has been shown to cause a variety of functional outcomes, depending on the application, whether it is phasic (short duration) or tonic (long duration). Transient exposure leads to hyperpolarization from resting potential during which action potentials are suppressed, followed by a period of accelerated action potential generation [13]. Meanwhile, tonic exposure results in a depolarization from resting potentials, decreased afterhyperpolarizations, and decreased spike-frequency adaptation [13].

To validate our model, we proposed to recreate the experimental procedures of [13] in silico. In [13] phasic responses were tested by focal ACh (100 μM) application while at resting membrane potential or during the induction of action potentials. Since this experiment was done in vitro, the elimination of ACh was due to AChE activity. While AChE hydrolysis of ACh is known to be rapid, the precise time course of ACh concentration in this experiment is unknown and thus difficult to replicate. To overcome this, we first tuned a simulated current clamp’s amplitude to provide the target initial spiking frequency (6 Hz). We then ran a series of simulations varying the length of ACh exposure to recreate the increase in spiking frequency seen in the experimental results. This same time period was then used in

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simulations without any current sources to replicate the phasic exposure experiments at resting membrane potential.

E. Cell Hyperexcitability

AChE inhibitors are currently the primary method of treatment for patients with Alzheimer's disease. These medications act by slowing down the hydrolysis of ACh to compensate for the weakened sources of ACh. Additionally, AChE is also the target of several classes of pesticides and chemical weapons which act as irreversible AChE inhibitors. The result of exposure to these chemicals is a chronic overabundance of ACh leading to a variety of negative and potentially life-threatening outcomes.

To simulate how our pyramidal cell model behaves when exposed to chronically high levels of ACh, we used our model to simulate long term exposure to a range of concentrations to measure the altered behavior.

III. RESULTS

A. ACh vs Oxo-M Inhibition

Fig. 1 shows the maximum levels of receptors bound to ligands (RL) in simulated exposure to different concentrations of agonists. This concentration profile indicates that the tuned parameters for the receptor's affinity lead to an increased affinity for ACh . Fig. 2 displays how the increased affinity for ACh shifted the concentration required to achieve a percent inhibition of Kv7 channel activity. In this figure we can see that the inhibition of Kv7 occurs at much lower concentrations for ACh than Oxo-M. This brings the effective concentration range of inhibition closer to the range measured in microdialysis experiments [2]

B. Model Validation

The model replicated increased firing frequency after phasic exposure of acetylcholine during action potential generation as seen in Fig. 3 B. However, while , the model did not reproduce the transient period of hyperpolarization and halted action potential generation seen in the CA1 results (see Fig. 3 A). The simulated results demonstrated more in common with the CA3 pyramidal cell results, which lacked the period of hyperpolarization. This suggests that manipulating Kv7 channels only explains part of the behavior seen in CA1 pyramidal cells and additional mechanisms are required to fully capture the transient hyperpolarization. However, for cases where the model has reached steady state due to long exposure to ACh concentration, our model properly simulates the behavior of the cells.

C. Pyramidal Cell Response to Supraphysiological Cholinergic Modulation

To study the effects of organophosphates such as certain pesticides, herbicides and nerve gas, which irreversibly block ACh degradation, we simulated higher ACh concentrations and measured the resulting spiking rate of our pyramidal cell model. As can be seen in Fig. 4, higher levels of acetylcholine caused a depolarization from rest similar to results seen in [13]. However, at concentrations greater than 0.3 μM, the cell started exhibiting spontaneous spiking activity. This is consistent with the results of [7] after heavy blockade of Kv7

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channels. The frequency of this spontaneous spiking increased asymptotically, reaching a maximum of around 26 Hz at the highest concentrations of ACh.

IV. CONCLUSION

The model proposed constitutes the fundamental building block to studying cholinergic modulation in the hippocampus at physiological and supraphysiological levels. It successfully recreates the tonic depolarization and increased excitability observed experimentally after cholinergic agonist application. Notably, it does not demonstrate the hyperpolarization and inactivation demonstrated in CA1 pyramidal cell after acetylcholine exposure [13]. Instead, it behaves more like a CA3 pyramidal neuron, which lacks this transient hyperpolarization. This behavior is likely due to the CA1 compartmental model we used that is lacking mechanisms to simulate SK-type calcium-activated potassium channels. These channels are likely the hyperpolarization's source as the transient hyperpolarization can be eliminated by application of apamin, a selective SK channel blocker [13]. Consequently, further work will examine the inclusion of SK channel mechanisms or the use of a different CA1 pyramidal model containing such mechanisms such as the one developed in [14]. This is useful for cell network simulations where the increased excitability afforded by Kv7 inhibition will alter the necessary amount of excitatory input required for signal propagation. Further work will also go to simulating the way that cholinergic agonists suppress synaptic transmission at excitatory synapses onto CA1 pyramidal cells from sources in the CA3 [15]. The combination of increased excitability with decreased excitatory drive suggests that increasing acetylcholine concentrations alters how CA1 pyramidal cells integrate excitatory inputs.

Simulating the behavior of pyramidal neurons in varying concentrations of ACh may shed some light on the role acetylcholine plays in shaping the functions of the hippocampus, and help us identify efficient electrotherapy or pharmacotherapy to restore this function after disruption of the cholinergic system. The model presented constitutes a useful in-silico testbed for simulating the effects of hyperexcitability following exposure to irreversible AChE inhibitors for which experimental results are prohibitively hard to obtain due to the numerous restrictions linked to manipulation of these dangerous substances.

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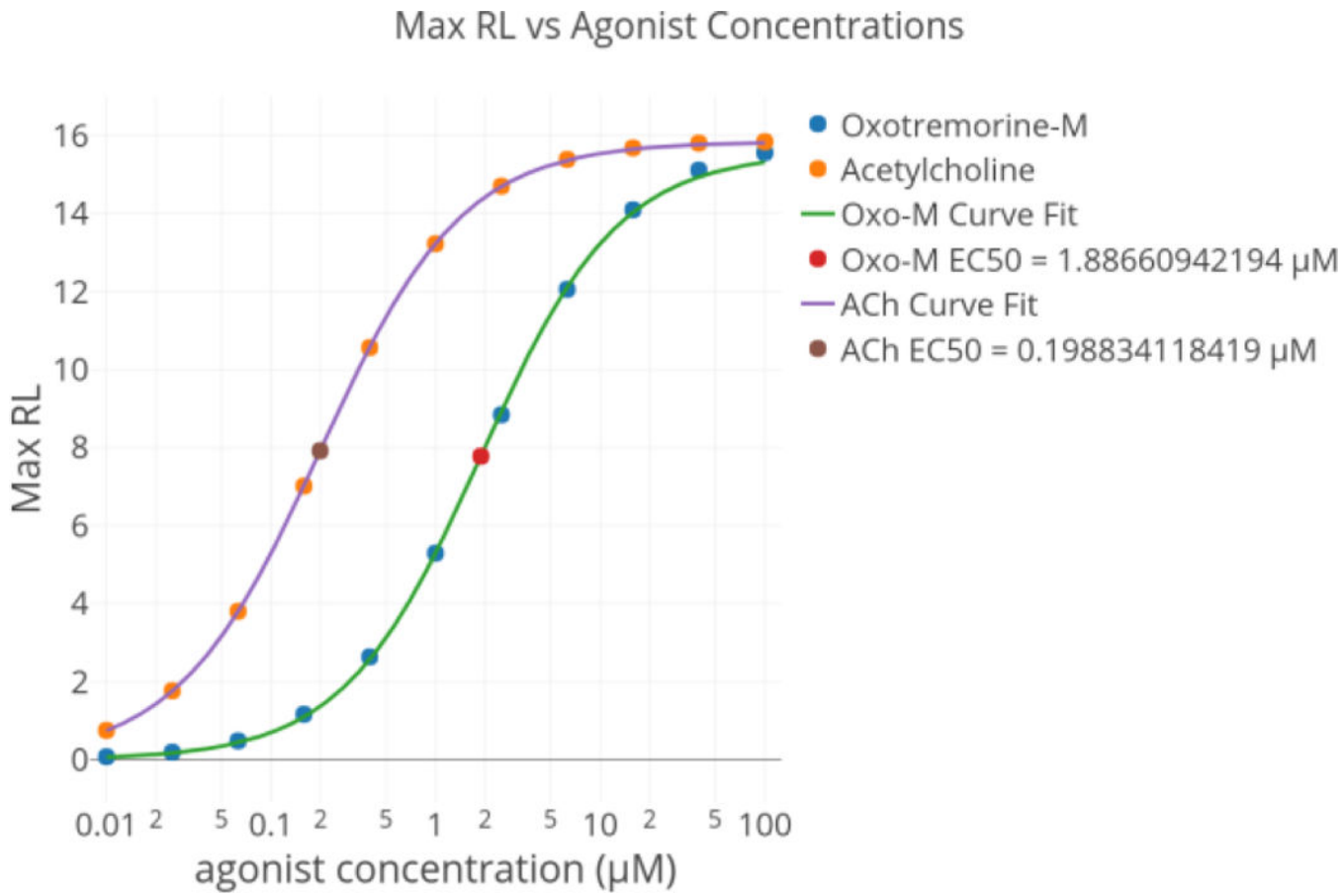

Figure 1:

Maximum steady state Receptor-Ligand binding versus concentration of agonist. EC_{50} refers to the concentration that elicits 50% of the maximum effect. [8, 17]

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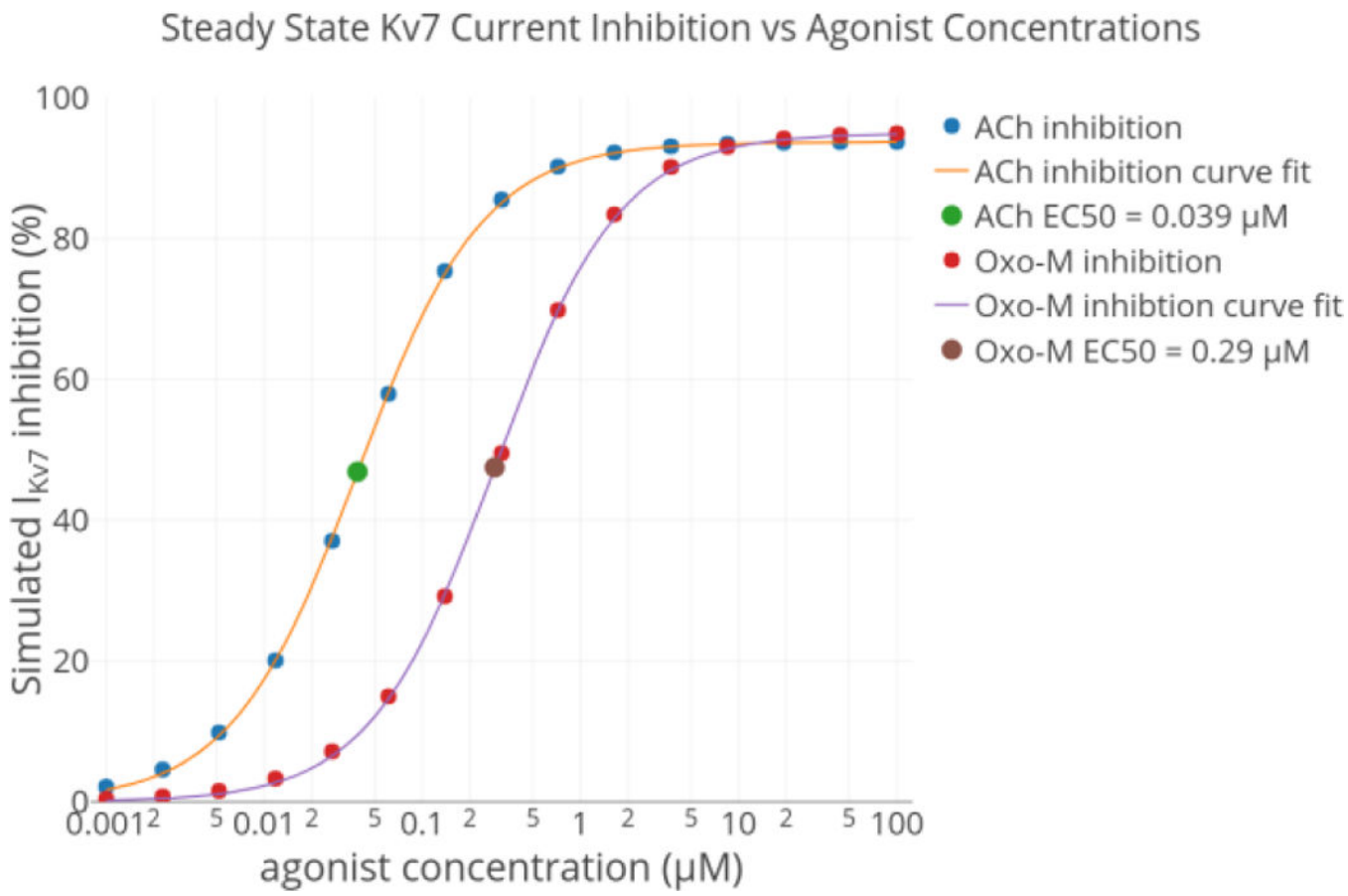

Figure 2:
Kv7 Inhibition vs Agonist Concentration [8]

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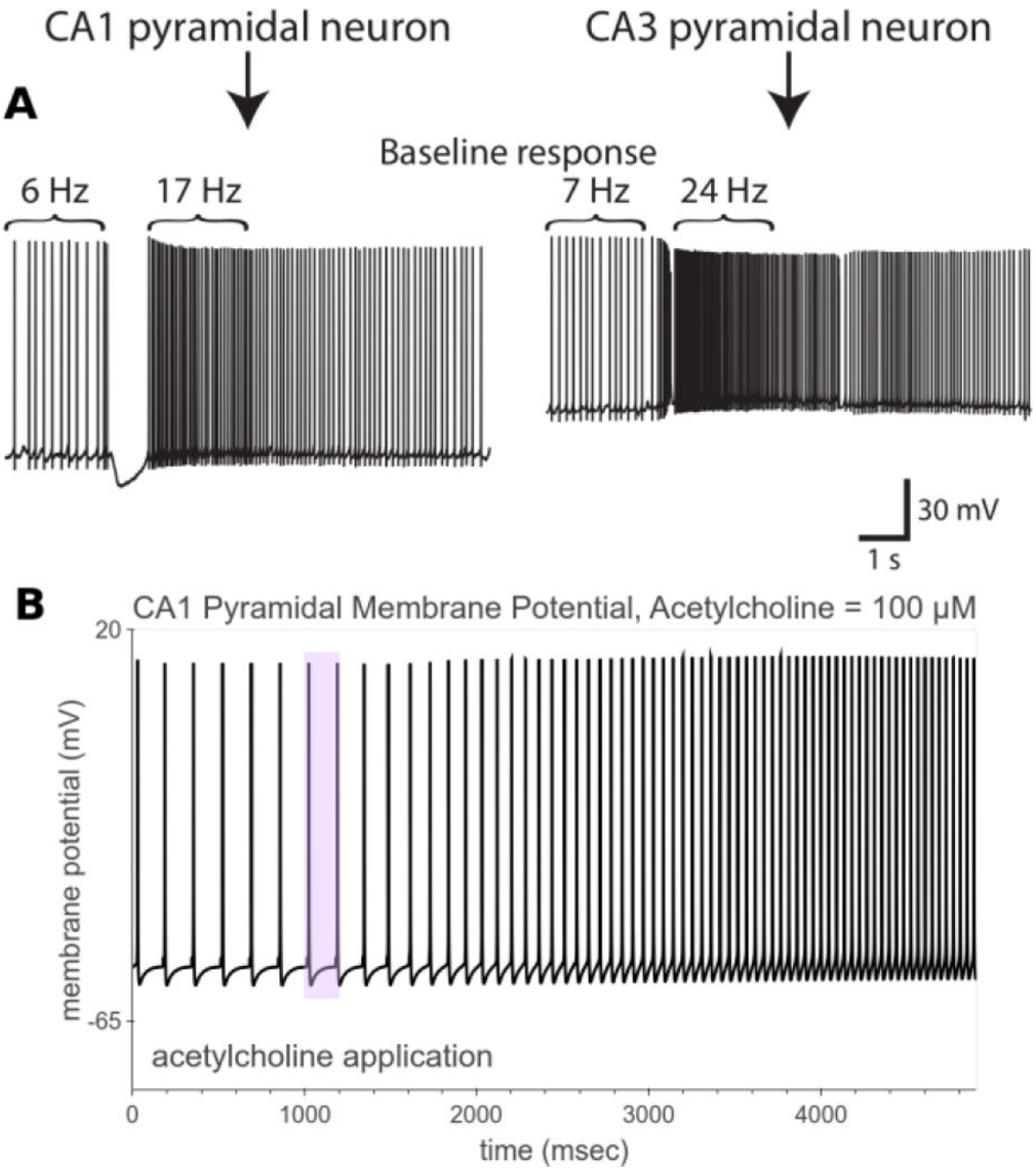

Figure 3:
Transient acetylcholine exposure. A) Experimental response of pyramidal cells to 100 µM of ACh. Source: [13] Fig. 1 B) Simulated response to 200 msec of 100 µM ACh (lavender window).

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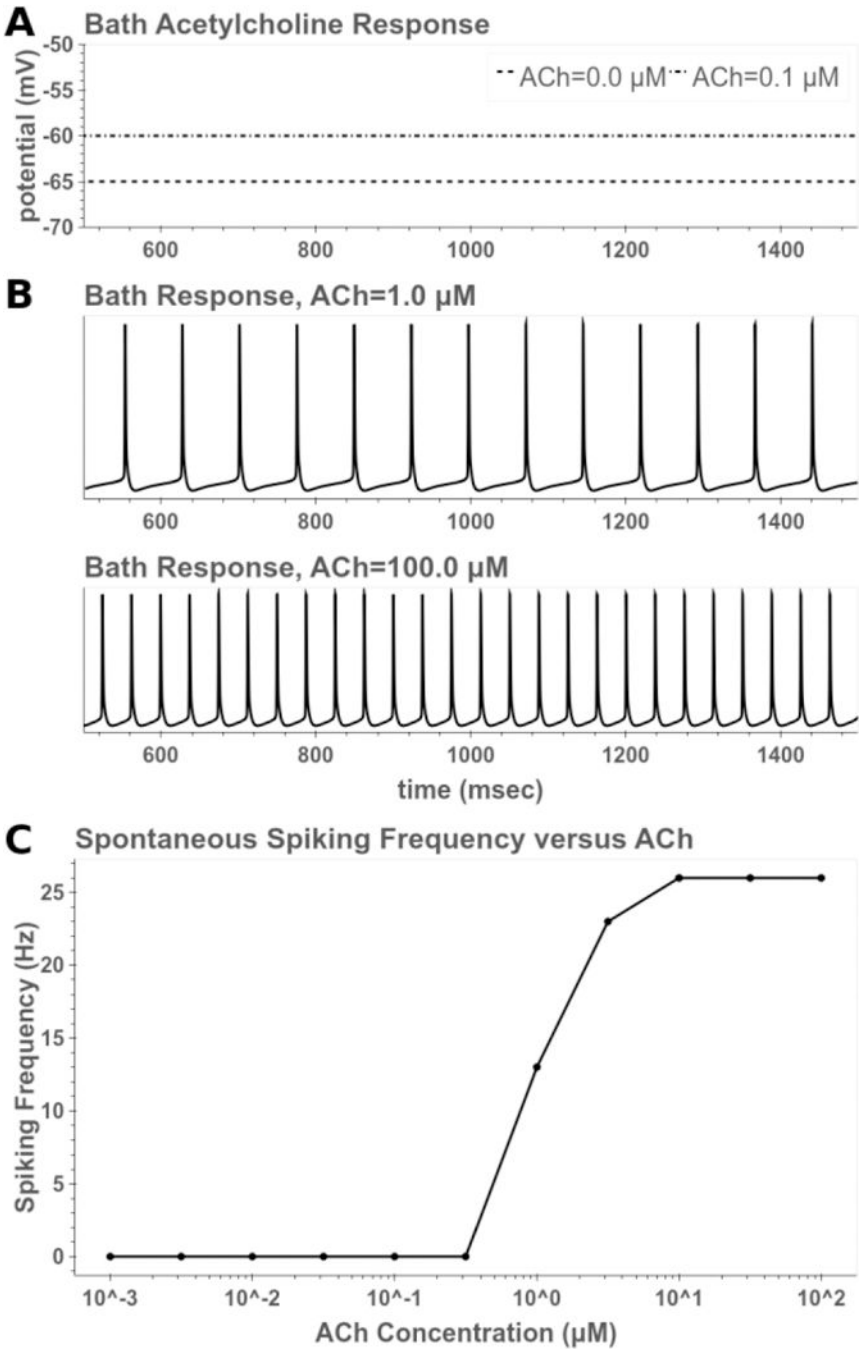

Figure 4:

Varying responses to acetylcholine concentration. A) depolarized resting potentials B) spontaneous spiking at higher concentrations of ACh C) Frequency of spontaneous spiking increase asymptotically with higher concentrations of acetylcholine