CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY

Name(s)
Mingmei Niu

Project Number
S1412

Project Title
Slow and Steady Won the Race: Surprising Findings about GABAA-mediated Synaptic Inhibition in Brain Slices

Abstract

Objectives/Goals
Neuronal processing critically depends on the timing of synaptic inhibition. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in mammalian brain circuits. In the hippocampus, several types of GABA-mediated synaptic inhibition appear to play different roles in inhibiting pyramidal cells in the CA1 region. Furosemide, a GABAA fast antagonist, was used to investigate the time course of GABAA fast inhibition, which provides insight into the cell#s total inhibition and into the hippocampus#s roles in learning, memory formation, and maintaining consciousness. This is the first study to measure the duration of depression resulting from different forms of inhibition in the CA1 circuit.

Methods/Materials
The brain slices used in these experiments came from adult Long-Evans rats weighing from 150-220 grams and were cut either coronally or horizontally into 450 µm slices and kept ontop of filter papers inside a humidified O2/CO2 (95%/5%) chamber along with ACSF at 22°C. Bipolar tungsten microelectrodes placed in the stratum radiatum layer of the hippocampus electrically activated Schaffer-collateral fibers. Glass microelectrodes filled with ACSF placed in stratum pyramidal/oriens border recorded evoked population spikes. Latency experiments (using Igor Pro 6 software) measured population spike amplitudes from peak negativity to positivity. The duration of inhibition is the time the wave of the greatest magnitude took to return to 80-90% of the magnitude of the control wave.

Results
Control: the average period of inhibition was 25 ms for coronal slices, and 40 ms for horizontal slices. Since furosemide blocks GABAA fast inhibition, a second population spike was visible within 10 ms of the first one in all of these experiments. Blocking GABAA fast inhibition with furosemide (1 mM) produced a small increase in CA1 neuron discharge visible for only a short time period (<20 ms). A minimal change in population spike amplitude occurred when furosemide was present.

Conclusions/Discussion
The experiment showed that GABAA fast contributes only briefly to overall circuit inhibition. GABAA slow inhibition lasts up to 200 ms after the initial stimulus and thus plays the major role in regulating circuit level signaling. In addition, blocking GABAA fast inputs appeared to increase the amount of inhibition indirectly by uninhibiting GABAA slow neurons that synapse onto pyramidal cells.

Summary Statement
This project focused on finding the duration of inhibition using furosemide to block GABAA-fast inhibition and the relative importance of GABAA-slow over GABAA-fast, contributing to a more complete understanding of the hippocampus.

Help Received
Used lab equipment at Stanford University under the supervision of Dr. M Bruce MacIver