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# Effects of LTP Induction on Hippocampal Cellular Excitability in the Freely Behaving Developing Rat Brain

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*Abstract*— **Our aim is to assess and quantify the exact effects of the induction of long term potentiation (LTP) on tonic inhibition and facilitation in a neural circuit within the hippocampal formation of neonatal rats. The neural circuit of interest in this paper is the perforant pathway-dentate gyrus synapse which serves as the first leg of the hippocampal trisynaptic circuit. A quantitative measure of the modulation of the perforant pathway/dentate gyrus circuit is the paired-pulse index which measures changes in the response of this circuit to a pair of pulses separated by varying interpulse intervals (IPI). It is known that such modulation influences the gating of neuronal transmission into and through the hippocampal formation; and, thereby, may play an important role in the development of learning and memory both in early life and throughout adulthood. Also important to this discussion is the finding that the dentate gyrus is one of the few areas of the rat brain which continues to generate new nerve cells well after birth. In an effort to quantify both age-related and LTP-dependent effects, LTP was induced using high frequency stimulation (HFS) of the perforant pathway-dentate gyrus synapse in freely behaving 10-12 day old, male Sprague-Dawley rats. Population spike amplitude measures which correspond to cellular discharge to a synaptic event were extracted from evoked field potentials recorded at the level of the molecular layer of the dentate granule cell population following induction of LTP in the same synapse. Preliminary results indicate the paired-pulse index was altered following induction of LTP.**

## I. Introduction

The brain, like the body, develops tremendously throughout the growth cycle of an organism. The hippocampus has been known to be important component in memory processing and consolidation in the brain. Studies investigating its participation in learning have identified hippocampal long-term potentiation (activity-dependent enhancement in synaptic strength) as a possible mechanism underlying learning and memory [2]. Changes in hippocampal cellular excitability can also be quantified using paired-pulse index (PPI) analysis techniques. For example, when paired-pulse stimuli are applied to the perforant path, the dentate granule cell population response to the second stimulus pulse is dependent both on the stimulation intensity and the interpulse interval (IPI). By comparing the population response of the second pulse to that obtained from the first, a measure (the PPI) of the level of inhibitory and facilitatory modulation of granule cell excitability can be established [1]. Also important to this discussion is the finding that the dentate gyrus is one of the few areas of the rat brain which continues to generate new nerve cells well after birth [3]. The goal of the present study is to quantify the effects of the induction of long term potentiation on local circuit modulation of cellular excitability of dentate neurons in the developing rat brain. The information gathered in this study will permit us to better understand the functional development of this neural circuit so important for learning and memory processes in the mammalian brain.

## II. Materials and Methods

### A. Research Animals

Young Sprague-Dawley rats between 10 to 12 days of age served as subjects. The Trinity College animal care protocol. Animals undergoing stereotaxic surgery were anesthetized (sodium pentobarbital, 35mg/kg). A small incision was made on the dorsal portion of the head allowing access to the surface of the skull. A concentric bipolar stimulating electrode was then lowered into the perforant pathway of the entorhinal cortex (AP= -5.4 mm, LAT= 4.0 mm from Bregma) and a monopolar recording electrode was lowered into the ipsilateral dentate gyrus of the hippocampus (AP= -3.0 mm, LAT= 2.0 mm from Bregma). Two reference electrodes were then positioned onto the contralateral cortical surface. Once the dentate gyrus signal was obtained and determined to be consistent the electrodes were cemented in place using dental cement. Throughout surgery the body temperature of the animal was maintained at 32.0 °C using heating lamps or a heating pad. The animals were allowed to recover for 24 hours during which time they were placed with two littermates in a cage where the temperature was maintained at 32.0 °C.

### B. Electrophysiology

After recovery the animals were moved to a recording chamber where they were allowed to acclimate for 1-2 hours. After acclimation was complete the electrodes were connected to the recording apparatus and an initial input/output (I/O) curve was generated by administering 10 single-pulse currents ranging from 300-1400 μA. The current intensity evoking 50% maximum population spike amplitude (PSA) was determined

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from the I/O curve and used during the remainder of the experiment.

During the paired-pulse index analysis a pair of pulses of equal magnitude were administered at different inter-pulse intervals (IPI) ranging from 20-1000 ms. The PPI is calculated using the resulting evoked responses (PSA1 and PSA2) in the following equation: $((PSA_2\text{-}PSA_1)/PSA_1*100)$ (See Fig. 1). PPI indices were collected at 30 min and one hour post-LTP induction and compared to a baseline PPI obtained prior to LTP onset. LTP was induced using theta burst stimulation consisting of 10 bursts of 10 biphasic pulses (pulse width = 0.25 us, 50 % duty cycle) with a pulse frequency of 400 Hz and interburst frequency of 5 Hz.

## III. RESULTS AND DISCUSSION

As is shown in Figure 2, the infant PPI vs. IPI graph yields a tri-phasic profile with short and long IPIs yielding paired-pulse depression (PPD) and intermediate IPIs moderate paired-pulse facilitation (PPF). In the baseline readings the first part of this tri-phasic curve (20-50ms) shows that there was inhibition, the second phase (50-150ms) shows facilitation and the third phase (+150ms) again shows inhibition. It is clear that there was a drastic change in facilitation during the 1 hour recording indicating that LTP does in fact induce a change in the local circuit modulation of cellular excitability.

The most intriguing finding of this research is seen in Figure 2 where we observed a large increase in facilitation during the facilitatory phase (intermediate IPI's) at one hour post-LTP. A possible mechanism responsible for this observation is the activity of the $GABA_A$ auto-receptor which is thought to be responsible for the relative facilitatory phase. It is possible that tetanization caused the auto-receptor to be hypersensitive to the neurotransmitter GABA which would shut off presynaptic release and thereby inhibit an inhibitor causing facilitation [4-5].

The data presented here are preliminary in that we have thus far only been successful with 2 animals due to the complexity of survival surgery and electrophysiological experimentation in freely behaving rats at such a young age (10-12 day old). Further studies are being conducted to increase the numbers of animals to a level of statistical significance.

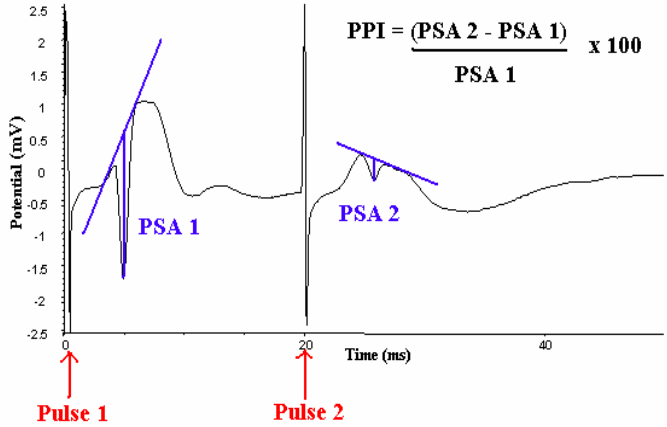


Fig. 1. Calculation of PPI using Population Spike Amplitude.

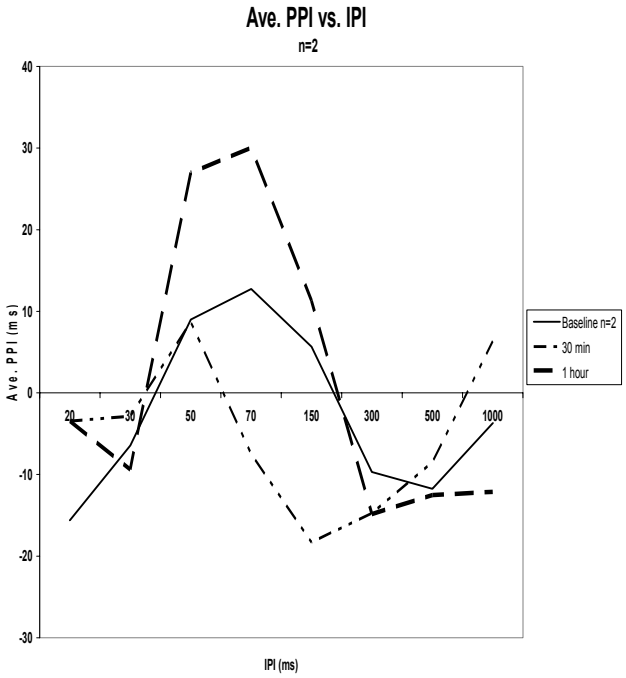


Fig. 2. Average PPI vs IPI plot showing the effect of LTP induction on the paired-pulse index in immature freely behaving rats (10-12 days of age, n = 2)

## IV. ACKNOWLEDGEMENT

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