# Plasticity in excitability of CA1 and CA3 pyramidal cells after inhibitory avoidance learning

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Figure 1. Inhibitory avoidance modulates the AHPs of CA1 pyramidal cells of both trained and context-only rats (A). AHP amplitude (B & C), duration (D), and integrated area (E) are reduced in trained and context-only rats compared to naive controlls.

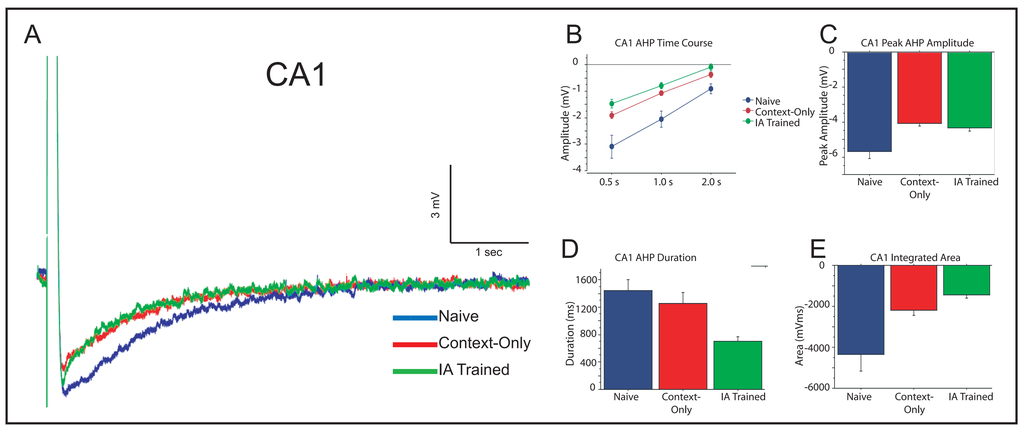



Figure 2. Inhibitory avoidance modulates the AHPs of CA3 pyramidal cells of trained (A). AHP amplitude (B & C), duration (D), and integrated area (E) are reduced in trained rats compared to naive controlls and context-only rats.

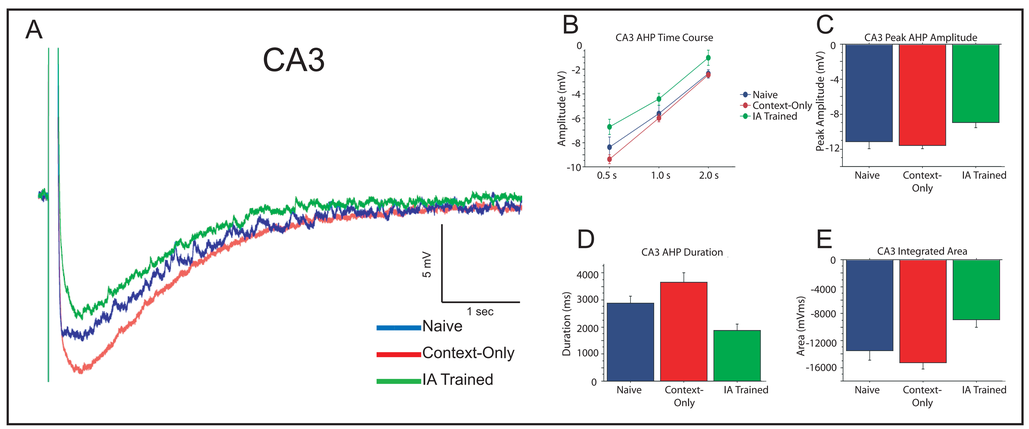
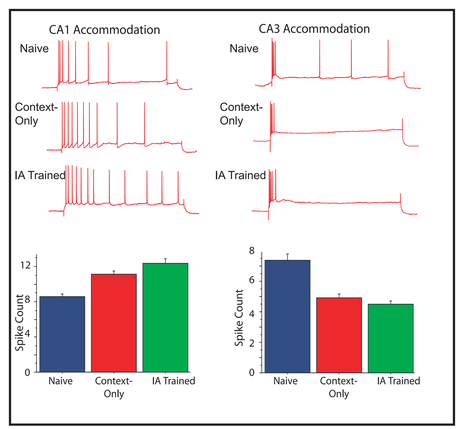



Figure 3. Reductions in AHPs are associated with increases in excitability in CA1 pyramidal cells. In CA3 pyramidal cells, reduction in AHPs are associated with a decrease in excitability.




## Background

Hippocampally-dependent trace eyeblink conditioning, win-shift spatial learning on the radial-arm maze, and spatial learning in the Morris water maze enhance postsynaptic excitability of hippocampal CA1 and CA3 pyramidal neurons but not granule cells in several species (Thompson et al., 1996; Moyer et al., 2000; Gant & Thompson, 2000; Oh et al., 2003; Tombaugh et al., 2005). Learning-dependent changes in excitability within the hippocampus have been characterized by temporally restricted reductions in slow-components of the postburst afterhyperpolarization (AHP) and in spike-frequency adaptation (accommodation). Reductions in slow AHPs permit pyramidal cells to more rapidly return to the resting potential, increasing excitability. Although increases in pyramidal neuron excitability have also been reported in olfactory cortex and hippocampus after olfactory discrimination learning (cf . Barkai & Saar, 2001), other standard learning paradigms have not been systematically tested to see if enhanced pyramidal neuron excitability is a generalized phenomenon in these tasks. The present study used another declarative memory task, inhibitory avoidance (IA) learning (cf. McIntyre et al., 2002), to assess learning-dependent changes in hippocampal excitability.

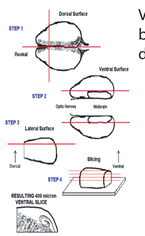
Figure 4. Illustration of inhibitory avoidance shuttle box.

## Methods

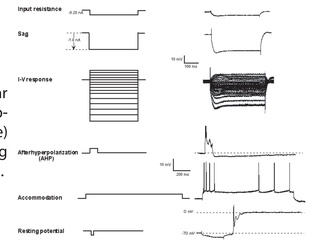
Subjects: Experiments were performed using a total of 31 male rats (2 - 4 mo). Rats were commercially obtained from Harlan (Indianapolis, IN), and maintained in our animal facility under conditions approved by the UT Dallas ACUC on a 12 hr/12 hr light/dark schedule prior to testing. Rats were handled 1 time daily for 5 min for 2 days prior to training.

IA Behavioral Training: Rats were placed in the light compartment of a rectangular shuttle box (90 cm long x 15 cm deep with a metal grid floor; see Figure 4 at left). Rats were allowed to cross to the larger dark compartment. Once rats reached the end of the dark compartment and turned around, a door was immediately lowered locking the rat in the dark compartment and a single footshock (0.5 mA, 1 s) was given. Rats were retained in the dark compartment for an additional 15 s after the footshock. Rats were then anesthetized and decapitated ~ 20 min after training, and ventral brain slices were prepared (see below). Exposure-only rats were exposed to the shuttle box in the same manner as IA trained rats but received no footshock. Naïve rats were taken directly from their housing cages for slice preparation and received no exposure to the shuttle box.

Slice preparation: Rats were anesthetized with isoflurane and decapitated ~ 20 min after training. The brain was quickly hemisected (center panel below) and immersed in cooled s-aCSF [in mM: 124 sucrose; 3 KCl; 1.3$MgSO_4$ ; 1.24 $NaH_2PO_4$ ; 2.4 $CaCl_2$ ; 26 $NaHCO_3$ ; 10 d-glucose, pH 7.4]. After the brain chilled for 3-4 min, it was blocked and 400 µm slices cut using vibratomes then placed in room temperature (25°C) aCSF [in mM, 124 NaCl; 3 KCl; 1.3 $MgSO_4$ ; 1.24 $NaH_2PO_4$ ; 2.4 $CaCl_2$ ; 26 $NaHCO_3$ ; 10 d-glucose, pH 7.4]. Both aCSFs were continuously oxygenated (95% O : 5% CO ). Sharp electrodes were prepared from borosilicate glass filled with 3 M KCl (30—80 MΩ), and intracellular recordings made (using AxoClamp 2b amplifiers and National Instrument LabView interfaces) from submerged slices (31°C) using the protocol illustrated below, at right




Ventral brain slice dissection.




Intracellular (sharp-electrode) recording protocol.

## Results

Experience-dependent plasticity in the post-burst AHP was observed in CA1 pyramidal neurons from young rats after exposure to the inhibitory avoidance apparatus (see Figure 1). Significant reductions ($p < 0.01$) in AHP amplitude and integrated area was seen in cells from both IA trained and context-only rats when compared to naïve cage controls. AHP amplitude and integrated area for cells from IA trained rats and context-only rats were not significantly different when compared to one another ($p > 0.05$), nor were AP duration nor sag. The AHP duration of cells from IA trained rats was reduced compared to both context-only and naïve rats ($p < 0.01$). Plasticity in spike-frequency accommodation was strongly correlated with AHP plasticity in cells from both IA trained and exposure-only rats (see Figure 3).

Plasticity in the AHP was observed in CA3 pyramidal neurons only from young IA trained rats (See Figure 2). Significant reductions ($p < 0.01$) in AHP amplitude, duration and integrated area were apparent for cells from IA trained rats when compared to cells from naïve cage controls and from context-only rats ($p < 0.05$). AHP amplitude, duration, and integrated area were not significantly different from each other in cells from naïve and context-only rats ($p > 0.05$). Plasticity in spike-frequency accommodation (see Figure 3) revealed a negative correlation with AHP plasticity in cells from trained and exposure-only rats ($p < 0.01$).

## Discussion

- CA1 pyramidal cells exhibit reductions in AHP peak amplitude, duration, and integrated area and a decrease in accommodation after rats undergo IA training.
- CA1 pyramidal cells also exhibit reductions in AHP peak amplitude but NOT in AHP duration after rats are exposed to the training context.
- CA3 pyramidal cells show a reduction in AHP amplitude, duration, and integrated area in rats trained in the inhibitory avoidance task.
- The reduction in the AHP amplitude of CA3 pyramidal cells is not associated with a decrease in accommodation.

## Acknowledgements

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