AHP plasticity in the hippocampus following temporary amygdala inactivation

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Behavioral Measures

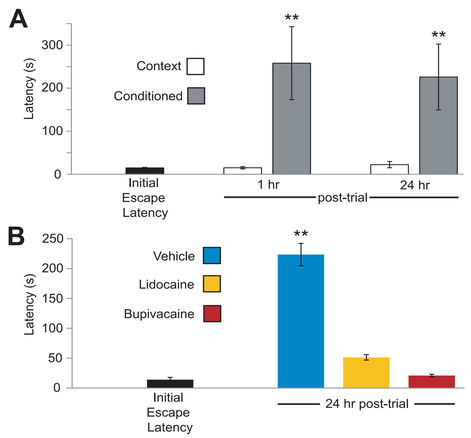


Figure 1: A) Rats exhibit increased escape latencies 1 hr and 24 hr following IA training but not following context exposure (no footshock). B) BLA inactivation blocks the increase in retention latencies 24 hr following IA training. Values represent means ± SEM, ** = p < 0.01.

Ventral CA1

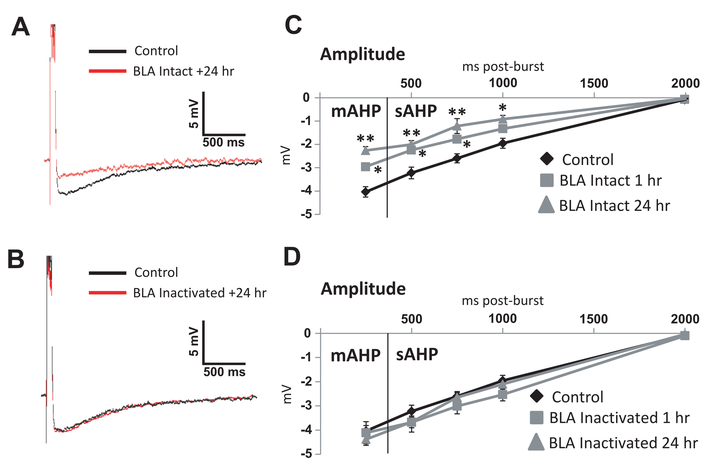


Figure 2: Ventral CA1 pyramidal neurons showed learning-dependent AHP plasticity in (A) BLA intact but not (B) BLA inactivated rats following inhibitory avoidance learning. (C) BLA intact rats exhibited significant learning-dependent reductions in both mAHP and sAHP components of the post-burst afterhyperpolarization. (D) Temporary BLA inactivation blocked the learning-dependent reductions in mAHP and sAHP both 1 hr and 24 hr following IA training. Values reported are means ± SEM; * = p<0.05, ** = p<0.01.

Dorsal CA1

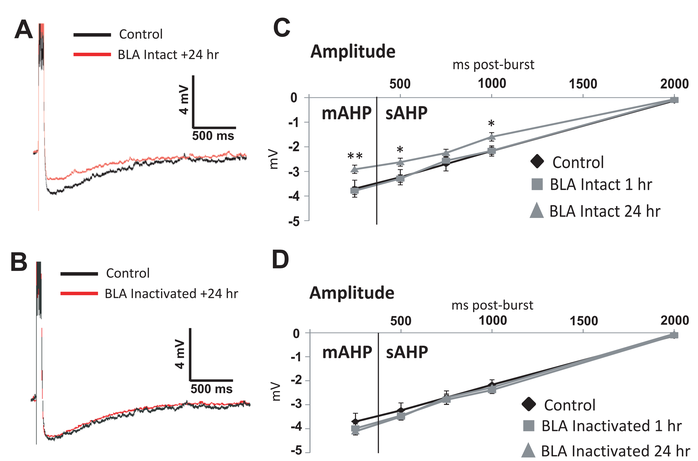


Figure 3: Dorsal CA1 pyramidal neurons showed learning-dependent AHP plasticity 24 hr post-trial in (A) BLA intact but not (B) BLA inactivated rats following inhibitory avoidance learning. (C) BLA intact rats exhibit significant learning-dependent reductions in both mAHP and sAHP components of the post-burst afterhyperpolarization 24 hr following IA training. (D) Temporary BLA inactivation blocks the learning-dependent reductions in the mAHP and sAHP observed 24 hr following IA training. Values reported are means ± SEM; * = p<0.05, ** = p<0.01.

Ventral CA1

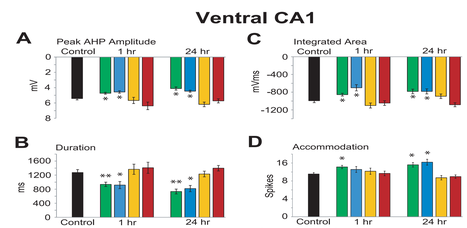


Figure 4: BLA intact rats exhibit learning-dependent reductions in A) peak AHP amplitude, B) duration, C) integrated area, and D) accommodation in ventral CA1 pyramidal neurons. BLA inactivation blocks this learning-dependent plasticity. Values represent means ± SEM, * = p < 0.05, ** = p < 0.01.

Control
Trained
Vehicle
Lidocaine
Bupivacaine

Dorsal CA1

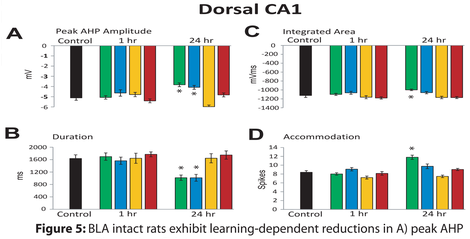


Figure 5: BLA intact rats exhibit learning-dependent reductions in A) peak AHP amplitude, B) duration, C) integrated area, and D) accommodation in dorsal CA1 pyramidal neurons. BLA inactivation blocks this learning-dependent plasticity. Values represent means ± SEM, * = p < 0.05.

Background

Hippocampal pyramidal neurons in vitro exhibit transient learning-dependent reductions in the amplitude and duration of calcium-dependent post-burst afterhyperpolarizations (AHPs), accompanied by other increases in excitability (i.e. increased firing rate, or reduced spike-frequency accommodation) after trace eyeblink conditioning or spatial learning, with a time-course appropriate to support consolidation of the learned tasks. Both these tasks require multiple days of training for acquisition. The hippocampus also plays a role in acquisition of single trial inhibitory avoidance (IA) learning. We have previously reported that pyramidal neurons of both the hippocampus (Farmer & Thompson, 2011) and the basolateral nucleus of the amygdala (Farmer *et. al.*, 2011) exhibit learning-dependent reductions in AHPs following single-trial IA learning. However, it is not known if an intact basolateral amygdala is required for the learning-dependent reductions in hippocampal AHPs.

	(n)		(M)	(mV)			
	Rats	Cells	Input Resistance	Sag	Resting Potential	Spike Width	AP Amplitude
CA1v							
Control	19	29	35.5 ± 1.9	6.2 ± 0.4	-66.8 ± 0.9	1.3 ± 0.02	86.0 ± 1.2
Trained 1 hr	22	37	41.0 ± 1.9	6.2 ± 0.4	-65.4 ± 0.9	1.3 ± 0.02	84.5 ± 0.8
Saline 1 hr	2	6	53.4 ± 4.0	8.0 ± 1.4	-69.6 ± 1.2	1.4 ± 0.04	89.3 ± 0.8
Lidocaine 1 hr	2	5	38.9 ± 4.7	8.5 ± 0.2	-67.2 ± 3.5	1.3 ± 0.03	86.7 ± 1.5
Bupivacaine 1 hr	3	8	46.6 ± 13.4	8.2 ± 1.9	-69.6 ± 1.9	1.3 ± 0.03	88.1 ± 1.0
Trained 24 hr	5	14	41.8 ± 1.6	5.7 ± 0.6	-66.7 ± 1.2	1.4 ± 0.02	84.4 ± .08
Saline 24 hr	3	8	52.5 ± 2.5	9.5 ± 0.4	-67.8 ± 1.3	1.5 ± 0.05	83.5 ± 0.9
Lidocaine 24 hr	4	7	41.8 ± 2.4	9.0 ± 0.6	-67.4 ± 3.5	1.2 ± 0.01	86.5 ± 0.7
Bupivacaine 24 hr	7	17	40.4 ± 2.7	9.0 ± 0.4	-67.5 ± 1.3	1.3 ± 0.02	88.0 ± 0.8
CA1d							
Control	8	12	40.9 ± 1.5	8.3 ± 0.5	-65.6 ± 1.6	1.2 ± 0.02	83.0 ± 2.5
Trained 1 hr	9	15	37.5 ± 2.2	8.3 ± 0.6	-65.9 ± 1.2	1.3 ± 0.02	86.0 ± 1.0
Saline 1 hr	3	6	42.6 ± 0.0	8.0 ± 0.7	-67.7 ± 0.7	1.3 ± 0.03	87.3 ± 1.3
Lidocaine 1 hr	2	6	36.3 ± 5.1	6.4 ± 0.7	-67.6 ± 3.1	1.3 ± 0.02	86.7 ± 1.5
Bupivacaine 1 hr	4	12	42.4 ± 0.0	6.6 ± 0.6	-67.4 ± 2.9	1.3 ± 0.01	85.1 ± 1.3
Trained 24 hr	6	14	36.7 ± 2.8	9.4 ± 0.7	-66.7 ± 1.0	1.3 ± 0.01	88.2 ± 0.8
Saline 24 hr	5	15	42.3 ± 2.7	7.9 ± 0.7	-68.9 ± 1.0	1.3 ± 0.01	85.3 ± 0.8
Lidocaine 24 hr	5	18	43.5 ± 3.8	7.1 ± 0.4	-64.2 ± 2.5	1.4 ± 0.02	88.6 ± 0.6
Bupivacaine 24 hr	7	18	39.1 ± 2.9	8.2 ± 0.4	-69.2 ± 1.6	1.2 ± 0.02	87.6 ± 0.5

Table 1: No differences in membrane properties of ventral CA1 and dorsal CA1 pyramidal neurons were observed between controls and after BLA inactivation following inhibitory avoidance learning. Input resistance, sag, resting potential, spike width, and action potential amplitude values represent means ± SEM.

Methods

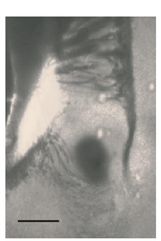
Subjects: Experiments were performed using a total of 91 Long Evans male rats (2-4mo). Rats were commercially obtained from Harlan (Indianapolis, IN), and maintained in our animal facility under conditions approved by the UT Dallas ACUC on a 12hr/12hr light/dark schedule. IA rats were handled once daily for 5 min for 2 days prior to training.

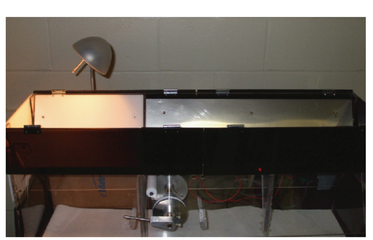
Surgery: Rats were anesthetized with isoflurane, and body temperature and respiration monitored and maintained at physiological levels. Rats were stereotaxically implanted with bilateral guide cannulae into the BLA under aseptic conditions, as follows. Two skull screw anchors were fixed to the skull, and cannulae (23 ga. stainless steel) were implanted (coordinates -- from bregma P -3.0 mm, L 4.9 mm; from cortical surface -7 mm) and secured in place with dental acrylic. Rats were given a single injection of tobramycin (1mg/kg) and allowed 72 hr to recover, with ad libitum access to food and water.

IA Behavioral Training: Rats were placed in the light compartment of a rectangular shuttle box. Rats were allowed to cross to the larger dark compartment where they received a single footshock (0.5mA, 1s). Rats remained in the dark compartment for an additional 15 s after the footshock.

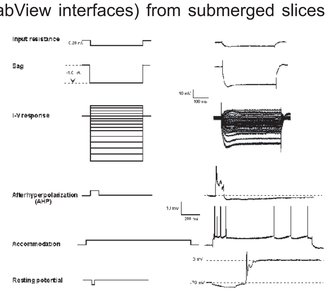
Infusions: 2% lidocaine or 1% bupivacaine in 0.1 M phosphate buffered saline (PBS) or the PBS vehicle alone either immediately prior to training trials (lidocaine) or immediately post-trial (bupivacaine). All infusions were 0.35 µL per hemisphere (0.7 µL total) administered over a period of 1 min. Injection cannulae were left in place for an additional 1 min following the completion of the infusions to allow intracranial pressure to equilibrate. Injection cannulae were then withdrawn and capped.

Slice preparation: Rats were anesthetized with isoflurane and decapitated ~ 20 min or 24 hr after training. The brain was quickly hemisected and immersed in cooled s-aCSF [in mM: 124 sucrose; 3 KCL; 1.3 MgSO4; 1.24 NaH2PO4; 2.4 CaCl2; 26 NaHCO3; 10 d-glucose, pH 7.4]. After the brain chilled for 3-4 min, it was blocked and 400µm slices cut using vibratomes then placed in room temperature (25°C) aCSF [in mM, 124 NaCl; 3 KCl; 1.3 MgSO4; 1.24 NaH2PO4; 2.4 CaCl2; 26 NaHCO3; 10 d-glucose, pH 7.4]. Both aCSFs were continuously oxygenated (95% O2: 5% CO2). Sharp electrodes were prepared from borosilicate glass filled with 3 M KCl (30-80 MW), and intracellular recordings made (using AxoClamp-2B amplifiers and National Instrument LabView interfaces) from submerged slices (31°C) using the protocol illustrated below.





Intracellular (sharp-electrode) recording protocol.



Results

Rats that received pre-training infusions of lidocaine (n = 10) or post-training infusions of bupivacaine (n = 10) exhibited statistically identical retention latencies when retested 24 hr later and were collapsed into one group (BLA inactivated). Temporary pre- or post-trial BLA inactivation blocked the increase in escape latency observed in rats with intact BLA 24 hr following training (figure 1).

AHP reductions were seen in ventral CA1 neurons from BLA intact but not BLA inactivated rats in both mAHP and sAHP components (figure 2), with plasticity in peak AHP amplitude, duration, area, and in accommodation 1 hr and 24 hr post-training (figure 4A-D). Significant reductions were observed 1 hr and 24 hr post-trial in BLA intact but not BLA inactivated rats in peak AHP amplitude ($F(8,125) = 18.6$, $p < 0.0001$), duration ($F(8,125) = 15.5$, $p < 0.0001$), area ($F(8,125) = 6.9$, $p < 0.0001$), and in accommodation ($F(8,125) = 12.9$, $p > 0.0001$).

AHP reductions were also seen in dorsal CA1 neurons from BLA intact but not BLA inactivated rats in both mAHP and sAHP components (figure 3), with plasticity in peak AHP amplitude, duration, area, and in accommodation 24 hr post-trial (figure 5A-D). Significant reductions were observed 24 hr post-trial in BLA intact but not BLA inactivated rats in peak AHP amplitude ($F(8,106) = 14.5$, $p < 0.0001$), duration ($F(8,106) = 8.5$, $p < 0.0001$), area ($F(8,106) = 6.3$, $p < 0.001$), and in accommodation ($F(8,106) = 4.4$, $p > 0.01$). Inhibitory avoidance learning did not alter other membrane properties measured in BLA intact or BLA inactivated CA1 pyramidal neurons (table 1).

Summary

- Pre-trial lidocaine or post-trial bupivacaine inactivation of the BLA produce the same behavioral and physiological effects
- BLA inactivation blocks learned increases in escape latencies 24 hr following IA training.
- BLA inactivation blocks learning-dependent AHP and accommodation reductions in hippocampal pyramidal neurons.

Acknowledgements

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