Supporting Information

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SI Text

SI Discussion

Another possible means of modifying the fAHP is by coexpression of regulatory subunits. This study dealt with the type-I BK current, which participates in AP repolarization and the fAHP and is blocked by both paxilline and iberiotoxin. When the beta-4 subunit is coexpressed with the alpha subunit, the current takes on different properties, including insensitivity to iberiotoxin and slower activation kinetics, which do not allow the type-II current to participate in AP repolarization (1). Aldrich and colleagues (2) suggest that a shift from type-II to type-I current caused by knockout of beta-4 subunits increases excitability by inducing epilepsy. In this case, an apparent increase in type-I BK current accompanied increased excitability, although it was not clear whether the increase in excitability was because of altered function or a change in channel numbers. We report a decrease in the fAHP and a broadening of APs after learning, which did not induce detrimental hyperexcitability; however, it is unknown exactly how this modulation occurs. An alteration in subunit expression could restrict the BK current from participating in AP repolarization after learning, rather than reducing overall current.

SI Methods

Subjects. Animals were group housed in a climate-controlled facility with a 12-h light/dark cycle and *ad libitum* access to food and water.

EBC Training. Animals were connected to a short tether during conditioning. The tether served the dual purpose of allowing monitoring of the eyelid EMG and positioning the air puff delivery tube near the eye while the rat was freely moving. An eyelid closure was defined as "an increase in integrated EMG activity that was greater than the mean baseline amplitude plus four times the standard deviation of the baseline activity, for a minimum of 10 ms" (3). Conditioned animals received 30 CS-US pairings in each of five training sessions for a total of 150 CS-US pairings. Pseudoconditioned animals received the same number of stimuli over five training sessions in a random, unpaired manner. The average ITI for conditioning was 30 s and 15 s for pseudoconditioning.

In Vitro Electrophysiology. Transverse (300 μm) slices were made from the dorsal half of the hippocampus. Slicing solution was the same as recording solution. Immediately after slicing, slices were incubated in bubbled ACSF at 34°C for 30 min and at room temperature for 1 h before recording. Internal solution pH was adjusted with KOH to 7.45, and the osmolarity was between 280 and 290 Osm.

***In Vitro* Data Collection and Analyses.** To be included in the study, cells were required to have a resting membrane potential between −58 and −70 mV, an input resistance of >25 MΩ that remained stable throughout the recording, and an AP height of at least 70 mV above the holding potential. Cells were excluded only on these criteria. Input resistance was calculated by using the steady-state voltage induced by a 800-ms hyperpolarizing step [$R_n = (V_{hold} - V_{ss})/\Delta I$; where $V_{hold} = -65$ mV, V_{ss} = steady-state voltage, and $\Delta I = -50$ pA]. AP threshold was determined as the point where the first derivative (dV/dt) of the AP was equal to 20 mV/ms. V_{rest} was the membrane voltage with no current injection. During recordings, all cells were held at −65 mV with current injection.

***In Vivo* Electrophysiology.** Each tetrode consisted of four formvar-coated nichrome wires (25-μm diameter bare; 38-μm diameter coated) bonded tightly together at one end with epoxylite. The free end of each wire was soldered to a gold-plated amphenol pin. The tetrodes of each array were passed through a 5-mm length of polyamide tubing fixed to the base of the array and glued in place. Impedance testing immediately before implantation typically yielded values of 0.5–1.5 MΩ. When the target depth of the recording array had been achieved, the rat was prepared for drug infusion. Animals were infused while awake and freely moving in a small Plexiglas box, cleaned, and filled with fresh bedding daily.

Histology. After marking lesions were made, animals were killed with a lethal dose of sodium pentobarbital (97.5 mg/kg). After perfusion and post-fixing in 10% formalin, brains were sectioned (75 μm), mounted on gelatin-coated slides, and stained with Cresyl Violet.

1. Reinhart PH, Chung S, Levitan IB (1989) A family of calcium-dependent potassium channels from rat brain. *Neuron* 2:1031–1041.
2. Brenner R, *et al.* (2005) BK channel beta4 subunit reduces dentate gyrus excitability and protects against temporal lobe seizures. *Nat Neurosci* 8:1752–1759.
3. Weiss C, *et al.* (1999) Trace eyeblink conditioning in the freely moving rat: Optimizing the conditioning parameters. *Behav Neurosci* 113:1100–1105.

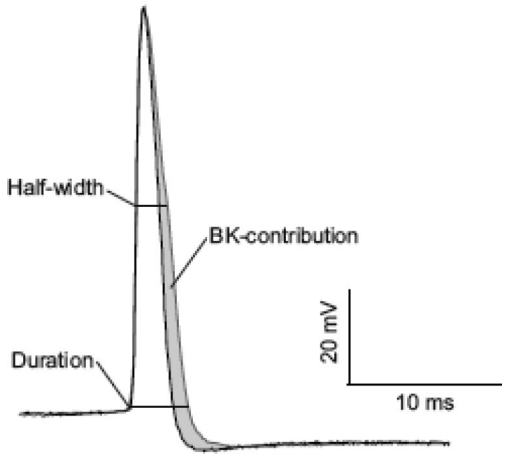

Fig. S1. BK contribution to AP half-width and duration. A prepaxilline (black) and postpaxilline (gray) action potential from the same cell are overlapped to show the contribution of the BK-mediated current (shaded). Because the BK current more strongly contributes to later repolarization, it has a greater influence on duration than on half-width.

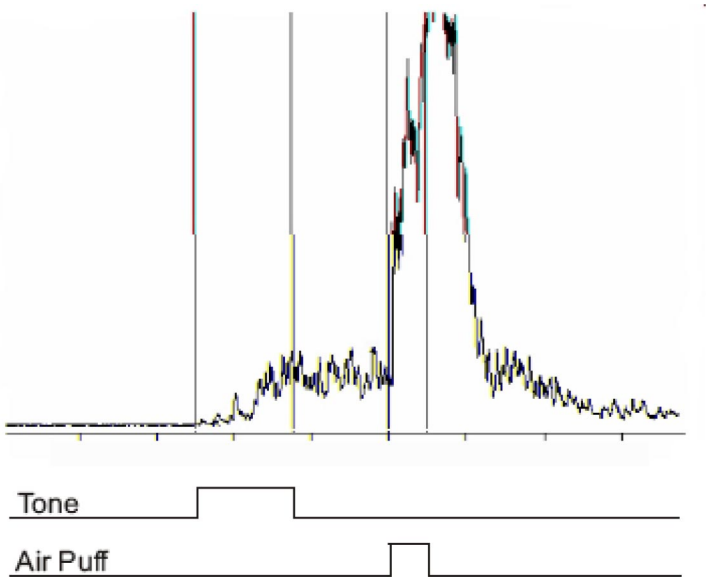

Fig. S2. EMG activity demonstrating a trained response. The integrated EMG trace shows a correctly-timed, adaptive closure of the eyelid after learning the pairing of a tone and an air puff. The trace interval between the offset of the CS and the onset of the US was 250 ms.

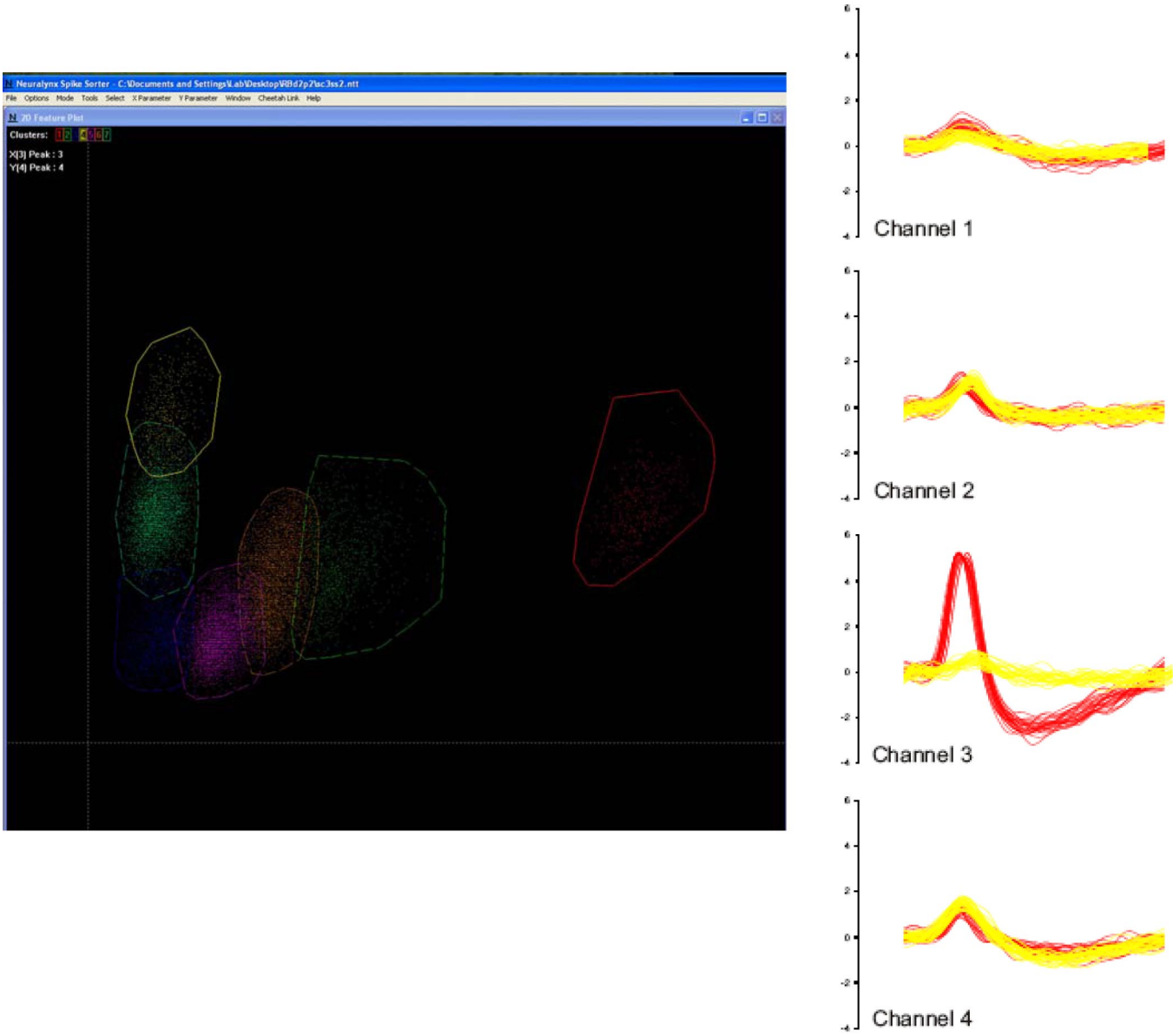

Fig. S3. Isolating single unit activity by using Neuralynx Spike Sorter. Spiking parameters (peak height, valley, and energy) were used to separate the activity of individual CA1 pyramidal neurons. These parameters were used to create 2D cluster plots (*Left*) composed of individual spike events exceeding the thresholds set during data acquisition. Tetrode recording techniques, such as those used in the present study, improve the reliability of single-unit isolation from a multiunit record by providing multiple 2D cluster plots for each record (4). The power of this technique is demonstrated as the two sets of waveforms illustrated to the right are clearly separable on channel 3, and not as clearly on channels 1, 2, and 4 (*y* axis: arbitrary units). Waveforms correspond to the ''red'' neuron and the ''yellow'' neuron. Only clusters yielding waveforms with a signal-to-noise ratio greater than 2.5:1 were included in the present analyses.

4. Gray CM, Maldonado PE, Wilson M, McNaughton B. (1995) Tetrodes markedly improve the reliability and yield of multiple single-unit isolation from multiunit recordings in cat striate cortex. *J Neurosci Methods* 63:43–54.

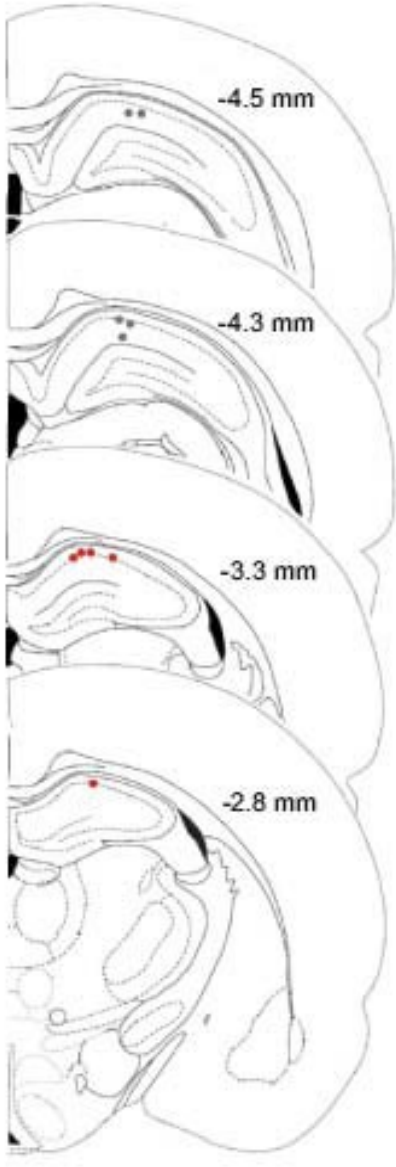

Fig. S4. Verification of single-unit electrode and cannula placements. Marking lesions verifying the location of the recording electrode in CA1 are illustrated on these coronal sections (red dots). The location of the infusion cannulae were verified at the same time and are shown (gray dots). Measurements are relative to bregma; adapted from ref. 5.

5. Paxinos G, Watson C (1998) *The Rat Brain in Stereotaxic Coordinates* (Elsevier, New York), 4th Ed.

Table S1. Action potential properties that were not altered by learning

	V_{thresh}, mV	Max rise slope, mV/ms	10–90% slope, mV/ms
Conditioned	−43.5 ± 0.8	230 ± 23	149 ± 17
Pseudoconditioned	−46.5 ± 1.4	262 ± 36	175 ± 29
Naïve	−46.6 ± 1.5	234 ± 44	163 ± 37

Maximum rise slopes were measured between threshold and the peak; 10–90% slope is the slope of the rising phase of the AP measured from 10% of the peak to 90% of the peak. The *P* values for these measures are as follows: $V_{thresh} - F_{(2,32)} = 2.375$; $P = 0.109$; Max slope $- F_{(2,32)} = 0.307$, $P = 0.783$; 10–90% slope $- F_{(2,32)} = 0.298$, $P = 0.745$.