The BK-mediated fAHP is modulated by learning a hippocampus-dependent task

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Intrinsic excitability is a plastic property of cells that, along with synaptic changes, can be modulated by learning. Action potential (AP) height, width, and frequency are intrinsically controlled properties which rely on the activation of Na^+, Ca^{2+}, and K^+ channels in the dendritic, somatic, and axonal membranes. The fast after-hyperpolarization (fAHP) after an AP is partially responsible for determining the half-width and duration of the AP and thus Ca^{2+} influx during the depolarization. In CA1 hippocampal pyramidal cells, the fAHP is carried by the voltage- and Ca^{2+}-dependent BK channel. In addition to modulating the duration of the AP, the BK-mediated potassium current exerts control over the frequency of AP generation in response to a depolarizing input. These facts position BK-mediated effects to not only modulate immediate intraneuronal communication, but also to control longer-term Ca^{2+}-dependent changes in the neuron, such as kinase activation, gene transcription, and synaptic plasticity. We examined how the BK-mediated fAHP was altered in hippocampal neurons after learning trace eyeblink conditioning. By using current clamp methods, it was found that the fAHP is reduced and the AP duration is increased in cells from conditioned animals. Additionally, *in vitro* and *in vivo* measures of firing frequency show that BK-channel blockade increases both evoked (*in vitro*) and spontaneous (*in vivo*) firing frequency of CA1 neurons, implicating the BK channel in the control of intrinsic excitability. These data indicate that the reduction of the BK-mediated fAHP is an essential part of the total increase of neuronal excitability known to accompany hippocampus-dependent learning.

after-hyperpolarization | eyeblink conditioning | excitability

A prevailing view of learning asserts that synaptic changes encode new information. Previous work from our laboratory and others has shown that intrinsic cellular excitability is also altered by learning. Increased excitability may be manifested by increased action potential (AP) frequency, lowered AP threshold, increased input resistance (R_N), or reduced after-hyperpolarization (AHP). These biophysical characteristics determine how a cell will respond to afferent drive and, in combination, may enhance or degrade the network's ability to form memories. Learning-related excitability changes in AP frequency accommodation and the AHP have been shown by using eyeblink conditioning, watermaze, and olfactory learning (1–3). This evidence indicates that the traditional view of synaptic information storage must be expanded to include intrinsic excitability as a plastic property of cells which can serve as a measure of cellular modification after learning (4).

Ca^{2+}-dependent potassium currents oppose neuronal activity and reduce cellular excitability. The currents underlying the slow and medium after-burst AHP are reduced in cortical (3) and hippocampal (5) neurons from animals that have successfully learned a task. The reduction in potassium current results in a global increase in cellular excitability as measured by the firing-frequency accommodation and the area and peak of the slow after-burst AHP. A fast AHP (fAHP), on the order of a few milliseconds, is seen after an AP in pyramidal neurons. This fAHP is largely mediated by the BK current, sometimes referred to as I_c (6). The BK current is Ca^{2+}- and voltage-dependent and plays a significant role in repolarizing the AP. The channel is blocked by iberiotoxin and charybdotoxin (7) and the small peptide paxilline (8) and it undergoes Ca^{2+}-dependent fast inactivation in the pyramidal cells of the hippocampus and cortex (9, 10). This inactivation results in the BK-mediated current contributing most strongly to repolarization of the APs at the beginning of a train, as the channel is inactivated during later APs (11). A reduction in the BK current results in a broader somatic AP, a reduced fAHP after the AP, and a quicker return to threshold, all of which can alter excitability. The role of the BK current in modulating cellular excitability and spike-frequency accommodation has been demonstrated in epilepsy (12), the vestibular-ocular reflex (13), circadian behavioral rhythms (14), olfactory encoding (15), extinction of fear conditioning (16), and invertebrate associative learning (17). We therefore sought to determine whether the BK-mediated fAHP was also changed after learning the hippocampus-dependent trace eyeblink task, with a concomitant increase in cellular excitability.

Results

R_N, AP height relative to holding, resting membrane potential (V_{rest}), and series resistance (R_S) were not different between training groups (Table 1). The after-burst AHP was somatically elicited with a train of 25 1.2-nA pulses (2 ms) given at 50 Hz. The AHP amplitude was measured as the negative-going peak after the offset of the last current step. Cells from trained animals that reached the learning criterion by the final training session showed increased excitability, as indicated by a reduced after-burst AHP peak voltage (Fig. 1) ($F_{(2, 48)} = 8.449$, $P = 0.0007$) and a reduced AHP area (data not shown) ($F_{(2, 48)} = 3.485$, $P = 0.039$). Firing-frequency accommodation was measured as the number of APs produced by an 800-ms step-current injection. The level of current was increased by 50-pA intervals to elicit firing between 1.25 and 12.5 Hz. Some cells could not be induced to fire above 8 Hz regardless of the current level injected; other cells readily fired at >12.5 Hz. Cells from conditioned animals demonstrated increased excitability by the decreased current level required to generate 5 APs in 800 ms ($F_{(2, 36)} = 4.135$, $P = 0.024$). Spike-frequency accommodation is because of active conductances and, as such, is independent of R_N, as measured in this study. Learning-related changes in intrinsic excitability, as measured by spike-frequency accommodation with no difference in R_N between groups, have been previously reported (18).

The fAHP was measured as the most negative potential after each AP in a train elicited by using a step sufficient to evoke at least 4 APs. Averaged fAHP measurements for the first through sixth APs in a train are shown in Fig. 2. Cells from conditioned

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Table 1. Intrinsic properties were not different between groups

	R_N, MΩ	AP, mV	V_{rest}, mV	R_S, MΩ
Conditioned	113 ± 9	106 ± 3	−64 ± 1	15 ± 3
Pseudoconditioned	113 ± 7	104 ± 3	−64 ± 2	13 ± 1
Naïve	118 ± 6	106 ± 3	−65 ± 1	13 ± 1

animals showed a significantly reduced fAHP for the first four APs in a train ($F_{(2, 43)} = 7.153, P = 0.002$). fAHPs after later APs in the train did not show a significant learning-related reduction, suggesting that the learning-related change was specific to an early current that was inactivated later in the train of APs.

Blocking the BK-channel with the blockers iberiotoxin (60 nM) or paxilline (10 μM) in cells from naïve rats resulted in a 37.5% ± 1.3% reduction in the negative-going peak of the fAHP and a 121.2% ± 2.8% increase in AP half-width (data not shown). These changes in AP shape confirm earlier evidence (11) that AP repolarization and the fAHP are largely mediated by the BK channel in the hippocampus. Paxilline did not affect the after-burst AHP in any training group ($F_{(1, 38)} = 0.008$; N.S., not significant).

As mentioned above, cells from conditioned animals exhibited more APs to a current-injection step compared with controls, with no difference in R_N. It has been previously reported that blocking the BK channel increases firing output to a given input current (13). By using an occlusion approach, the learning-related difference in firing frequency to a step input was shown to depend BK-channel activity. Paxilline (10 μM) or iberiotoxin (60 nM) were bath applied to the slice, and the firing-frequency increase was shown to be greater in cells from naïve or pseudoconditioned animals than in cells from the conditioned group. Cells exhibited an increase in firing frequency to a given current step in all training groups (Fig. 3). Comparisons of firing frequency (before/after drug) were always made between equivalent current inputs. The increase in firing frequency was much greater in cells from control groups ($F_{(2, 41)} = 10.874, P = 0.0002$).

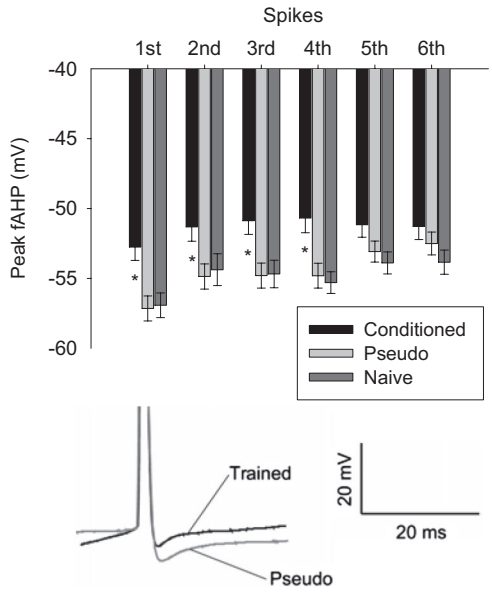

Fig. 2. The fAHP is reduced by learning. The peak fAHPs (*Lower*) of early spikes in a train were smaller in cells from conditioned animals compared with cells from control animals (first AP: conditioned, −53.17 ± 1.01 mV, $n = 17$; pseudo, −57.35 ± 0.91, $n = 18$; naïve, −57.53 ± 0.86, $n = 11$). fAHPs from later spikes were not different between training groups ($P = 0.09$). *, $P < 0.004$.

To examine the impact of blocking BK channels *in vivo*, recordings were made in awake, naïve animals. Waveforms from a total of 172 neurons were isolated. Of these, 37 neurons failed to meet the CA1 pyramidal-neuron recording criteria defined in *Materials and Methods* and were excluded from the study. Three concentrations of paxilline were used. All three concentrations significantly increased firing above baseline, so the data were pooled. The present analyses are based on spiking data from 85 neurons recorded during drug infusion and 50 neurons recorded

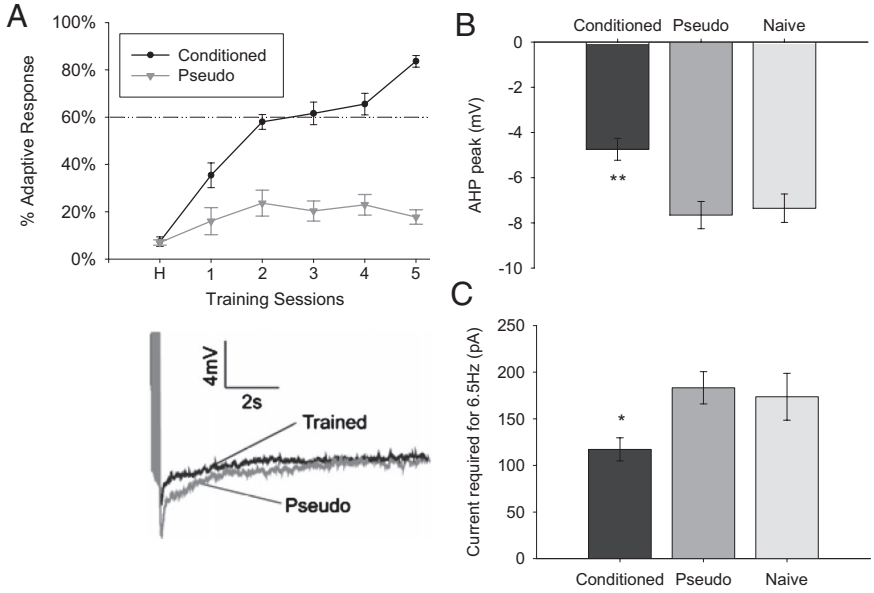

Fig. 1. Excitability changes after learning trace eyeblink conditioning. (*A*) Successful learning was defined as 60% or better adaptive response (dashed line) by the end of training. The random response rate for pseudoconditioned animals is also shown. (*B*) The peak AHP (*A, Lower*) was reduced as compared with controls (conditioned, −4.74 ± 0.48 mV, $n = 18$; pseudo, −7.65 ± 0.61 mV, $n = 17$; naïve, −7.33 ± 0.59 mV, $n = 15$). (*C*) Cells from conditioned animals showed increased excitability by requiring less current to fire five APs in 800 ms. (conditioned, 113.1 ± 12.7 pA, $n = 14$; pseudo, 183.2 ± 17.4 pA, $n = 14$; naïve, 177.0 ± 27.5 pA, $n = 11$). *, $P < 0.03$; **, $P < 0.002$.

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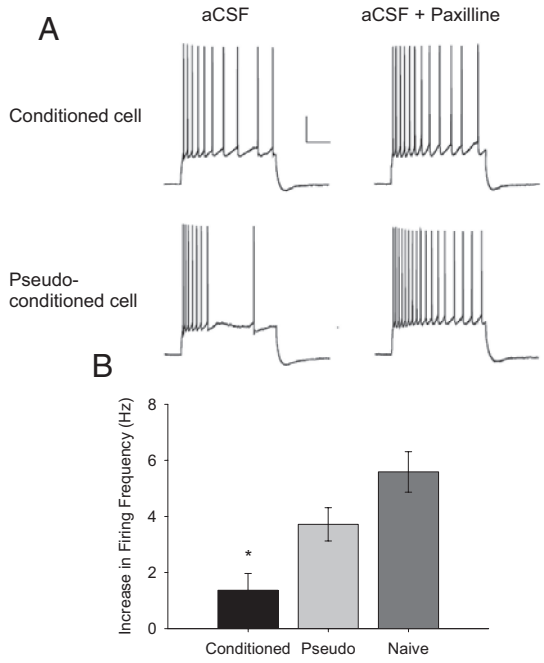

Fig. 3. *In vitro* block of BK channels increases firing frequency in a learning-related manner. (*A*) Cells from conditioned animals exhibited reduced accommodation compared with cells from control animals (*Left*). Blockade of the BK channel with Paxilline increased firing frequency and reduced accommodation (*Right*). (Scale bars, 20 mV and 200 ms.) (*B*) Cells from conditioned animals showed a smaller increase in firing frequency after BK-channel block than cells from controls (conditioned, 1.34 ± 0.59 Hz, $n = 16$; pseudo, 3.48 ± 0.60, $n = 14$; naïve, 5.59 ± 0.72, $n = 13$). *, $P < 0.05$.

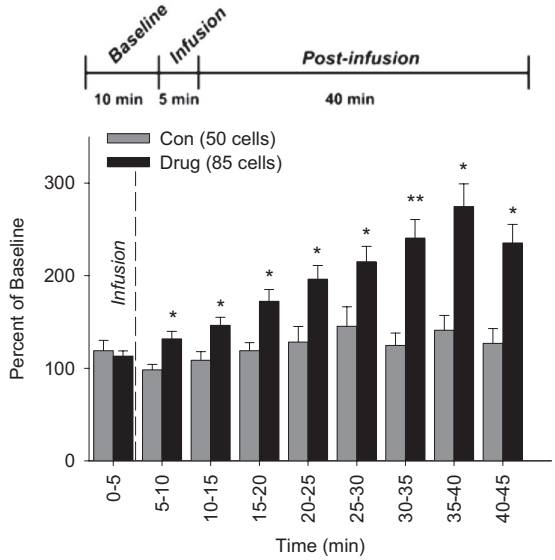

Fig. 4. *In vivo* block of the BK channel increases baseline firing frequency in naïve rats. A timeline for *in vivo* recordings is shown above the mean response magnitudes from CA1 neurons during the infusion and after-infusion interval, normalized to the preinfusion baseline and sorted into 5-min bins. No significant group differences were observed during the infusion interval. A significant difference was observed during the first five minutes of the after-infusion interval (drug: $n = 85$ cells; vehicle: $n = 50$ cells). The greatest group difference was observed during the 30- to 35-min after-infusion interval. *, $P < 0.001$; **, $P < 0.0001$.

during vehicle infusion. The analyses indicate that channel block with paxilline modulates *in vivo* CA1 pyramidal-neuron activity in a manner consistent with that observed during *in vitro* bath application. Infusion of paxilline resulted in an increase in firing rate across the infusion/after-infusion intervals compared with vehicle infusion (Fig. 4) ($F_{(8,1064)} = 9.184$, $P < 0.0001$). The difference in firing rate between groups within the first 5 min after the start of infusion was minimal. However, during the 5 min after the end of infusion, a group difference began to be observed ($F_{(1, 133)} = 10.61$, $P = 0.0014$), with the greatest difference between groups evident at the 30- to 35-min time point ($F_{(1, 133)} = 16.315$, $P < 0.0001$). Baseline neuronal firing rates did not differ by animal between the two recording sessions, indicating that neither drug nor vehicle infusions had a persistent effect on neuronal activity during the second infusion session 24 h later (data not shown).

The BK current contributes to the repolarization of the AP as well as the fAHP. Single APs were generated by an 800-ms somatic-current step, and the half-width and duration were measured [supporting information (SI) Fig. S1]. AP half-width was measured as the AP width at 50% AP height relative to threshold, and AP duration was measured as the AP width at threshold. Single APs from conditioned cells showed a trend toward an increased half-width as compared with APs from control cells (Fig. 5*A*) ($F_{(2, 41)} = 2.826$, $P = 0.07$). Importantly, single APs from conditioned cells showed a significantly increased duration compared with controls (Fig. 5*B*) ($F_{(1, 40)} = 5.991$, $P = 0.025$). Other AP properties were also measured (see Table 1 and *SI Text*). There was no significant difference in AP threshold, peak, or rise slope between the training groups (Table S1).

The change in the BK-mediated fAHP was not because of changes in recovery from inactivation. Pairs of APs were elicited by 2-ms, 1-nA steps while the interspike interval was increased. The ratio of the width of the second AP to the first AP was measured. The BK channel is known to inactivate in a use-dependent manner (19), causing an increase in the AP width

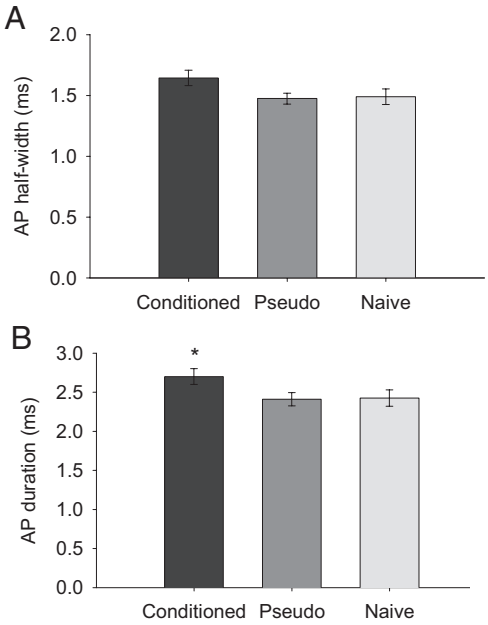

Fig. 5. AP half-width and duration are increased by learning. (*A*) AP half-width does not vary significantly between groups, but does show a trend toward increased widths in conditioned cells (conditioned, 1.56 ± 0.08 ms, $n = 16$; pseudo, 1.39 ± 0.04, $n = 15$; naïve, 1.48 ± 0.04, $n = 12$; $P = 0.07$, one-way ANOVA). (*B*) The AP duration measured at threshold is significantly greater in conditioned cells (conditioned, 2.70 ± 0.10 ms, $n = 16$; pseudo, 2.41 ± 0.08, $n = 15$; naïve, 2.43 ± 0.10, $n = 12$). *, $P < 0.05$.

when subsequent APs are closely spaced. The ratio of the AP half-widths between the two APs is therefore a measure of the channel's recovery from inactivation. When the channels have recovered from inactivation, the ratio of the two APs' half-widths will be 1. There was no difference between the groups in how quickly the channels recovered from inactivation, as measured by AP width ratios. In all groups, the AP width ratio returned to 1 when the interspike interval was >50 ms, which indicates that the learning-related differences in the fAHP, the BK-mediated component of accommodation, and the AP duration are not because of a change in the inactivation kinetics of the BK channel ($F_{(2, 44)} = 2.178$, $P = 0.13$; data not shown).

Discussion

A reduction in the fAHP after conditioning is a novel learning-specific excitability change, adding to those previously seen in the slow and medium AHP after successfully learning a hippocampal task (1, 5). The reduction of hyperpolarizing potassium currents increases the overall excitability of the neuron. Such excitability changes have been found to accompany many types of learning in vertebrates and invertebrates—olfactory discrimination in rats (3), water-maze learning in rats (2), eyeblink conditioning in rats (20) and rabbits (5), and associative learning in *Aplysia* (21) and *Hermissenda* (22)—suggesting that this is a highly conserved cellular mechanism involved in information storage. It has also been demonstrated that reducing a dendritic potassium current (A-type) increases the size of back-propagating APs and improves long-term potentiation (LTP) (23). Our data indicate that a decrease in the BK-mediated fAHP accompanies learning-related increases in cellular excitability. This conclusion is supported by reports of increased firing in response to BK blockers (13) and that blocking channels increases transmitter release and excitatory-postsynaptic-current amplitude at recurrent excitatory CA3-CA3 synapses (24). Consistent findings of learning-related increases in cellular intrinsic excitability by reducing potassium conductances suggest that this may be a fundamental functional mechanism of modulating information-processing capabilities, thereby supporting memory formation in combination with synaptic changes (4).

Although increased cellular excitability supports learning, continual unchecked increases in excitability eventually may result in epileptiform activity. Reductions in the slow AHP (25) as well as reductions in the expression of BK channels (26) have been reported to accompany epileptic activity in the hippocampus. To keep learning-related excitability changes from degenerating into epileptic activity, the changes must be transient and reversible. We did not examine how long the fAHP remained reduced after learning. However, the time course of learning-related synaptic and intrinsic excitability changes has been examined. LTP induced *in vivo* can last from less than a day up to several weeks (27). Learning eyeblink conditioning causes a change in the strength of the CA3-CA1 synapse (28); this enhancement decays back to baseline levels in ≈6 days (29). Likewise, the reduction of the slow AHP is maximal 24 h after acquisition of trace eyeblink conditioning and disappears 7–10 days later, even when the eyeblink behavior is maintained (5). In olfactory rule learning, the reversal of the after-burst AHP to pretraining levels occurs within 2 days of learning (30). Learning-related changes of the fAHP might be expected to be reset in a similar time course to the reversal of LTP and after-burst AHP.

The functional impact of reducing a short-lasting, very fast phenomenon such as the BK-mediated fAHP should be considered. The potential for an interaction between AP properties and synaptic plasticity presents a theoretically interesting function for the fAHP. Zhou *et al.* (31) reported that broadening APs caused spike-timing-dependent plasticity to shift toward long-term depression because of increased Ca^{2+} influx. The reduction of the fAHP during learning broadens APs and may change the window of potentiation at synapses. A similar role for the potassium currents governing AP half-width was suggested by a model of *Hermissenda* learning (32). We found that the AP duration was significantly greater in cells from conditioned animals, further supporting this idea. AP half-width was not significantly different, but the BK channel contributes to AP repolarization predominantly during the last half of the falling phase (Fig. 5) (33), so alterations in BK current are expected to affect AP duration more than half-width. Furthermore, the influx of Ca^{2+} during an AP is maximal during the repolarization phase, which allows both the opening of a large proportion of voltage-gated Ca^{2+} channels and an increased driving force for Ca^{2+} as the membrane repolarizes; therefore maximal Ca^{2+} influx would be seen with a long duration but relatively fast-falling AP (34). Interestingly, a decrease of AP duration in CA1 cells (without examining the fAHP) has been reported after olfactory-rule learning (30), raising the possibility that the learning-related increase in AP duration reported here is task specific. Several other currents contribute to AP repolarization such as A-type current (35), but the BK current is ideally situated to control the duration and shape of APs near the soma (6), thus determining the amount of Ca^{2+} influx and providing an interface between intrinsic excitability and synaptic plasticity.

It might seem that increased Ca^{2+} influx during longer duration APs from trained animals would counteract learning-related reductions in the after-burst AHP. As mentioned in *Results*, paxilline-induced AP broadening does not affect the post-burst AHP. Broadening all APs in a train by up to 120% with paxilline produced no effect on the after-burst AHP. The lack of interaction between AP width, increased Ca^{2+} influx, and the after-burst AHP is apparently because of the slow time course of the currents underlying the after-burst AHP (36). Recall that the BK current inactivates during a train of APs and the fAHP is only reduced during early APs in a train. The after-burst AHP is activated after trains of APs; however, by the fifth AP and beyond, the learning-related changes in the fAHP and AP width during early spikes are no longer apparent and thus do not influence after-burst AHP dynamics.

Another consequence of broadening APs might be to increase Na^+ channel inactivation. Such inactivation is seen in dendritic APs (37) and reduces the rate of AP rise and AP height and increases threshold (38), essentially decreasing cellular excitability. BK channels do not participate in the repolarization of dendritic APs (6), nor do the somatic measurements used in this study directly address dendritic potentials. It is unlikely that the learning-related increase in somatic AP duration reported here was caused by a build-up in inactivated Na^+ channels because threshold, AP height, and AP rise slope—all markers of Na^+ channel inactivation—were not different across training groups.

Exact cellular mechanisms underlying learning-related changes in the fAHP remain to be uncovered, although several possibilities are suggested by the literature. Alkon *et al.* (17) hypothesized that their observed reduction in a Ca^{2+}-dependent potassium current after associative learning in *Hermissenda* was because of increased inactivation of the channel after training. We found a similar reduction in the BK-mediated fAHP after trace eyeblink conditioning in rats, but our study does not support expanding their hypothesis to vertebrate learning. We indirectly assessed channel inactivation by examining the ratio of AP widths between two APs with an increasing interspike interval. There was no difference in the ratios between training groups, indicating that the learning-related decrease in the fAHP was not because of an alteration in inactivation kinetics.

The large Ca^{2+}-dependence of the BK channel provides another method for modifying the BK-mediated fAHP by either limiting Ca^{2+} entry or reducing internal Ca^{2+} with endogenous

Ca^{2+}-sequestering proteins. The first option, altered Ca^{2+} entry after learning, has been investigated by using pharmacology (39) without finding any significant difference in Ca^{2+} currents in neurons from trained animals, although this question should be revisited with more sensitive methods. The second option, decreased channel activation because of decreased local Ca^{2+} is unlikely because of the extremely fast dynamics of BK gating and the close positioning of the probable Ca^{2+} channel. Muller *et al.* (33) estimate that the Ca^{2+} entry point for somatic BK channels is ≈8–18 nm away, making it unlikely that an endogenous chelator such as calbindin would be fast enough to limit BK activity.

Another possible way to alter the fAHP is phosphorylation or dephosphorylation of the BK channel. The channel has been shown to coprecipitate with several kinases and phosphatases, including PKC (40), PKA (41), CAMKII (42), and calcineurin (43). The effects of these enzymes on the BK conductance are complex. Calcineurin works to inhibit BK activity, although not through dephosphorylation of the channel (43). PKA can either activate or inhibit BK activity depending on the previous phosphorylation status of the channel (40) and/or the splice variant of the channel (44). Even if these kinases and phosphatases do not directly alter BK-mediated current in the hippocampus, they could cause a reduction in the fAHP through an alteration in the number, function, or distribution of the channel protein similar to that found controlling the *Drosophila* circadian rhythm (14). The above hypotheses present interesting alternatives that may not only explain the learning-related alteration in the fAHP, but also may contribute to the changes in the Ca^{2+}-dependent medium and slow AHP observed after learning. Further experiments are required before any mechanism of the fAHP reduction can be conclusively determined.

The convergence of the *in vitro* and *in vivo* data reported here expands and supports the idea that changes in excitability serve to sustain successful learning. It has been reported that trace eyeblink conditioning results in increases in CA1 activity *in vivo*, correlating with the development of well-timed learned responses (45). The increases in activity are specifically related to the strength of the CS-US association and are limited to the period between CS and US presentation; baseline activity is not increased. Also, these increases in activity are not seen in pseudo-conditioned animals, indicating that the increased activity was specifically associated with learning (46). *In vitro* data, as reported here and elsewhere (5), from animals trained in the same task to similar learning criteria closely match the *in vivo* findings of increased activity (and decreased AHPs) after training. Learning-related increased firing is mimicked after the blockade of BK channels both in naïve animals *in vivo* and in naïve slices *in vitro*, lending support to the idea that the learning-related alterations in the fAHP are relevant to information processing in the intact hippocampus.

This report confirms that the after-burst AHP is reduced after learning and adds the finding that the fAHP is also similarly reduced. As previously reported (5), these changes are not seen in all cells, but represent a shift in the population of cells from trained animals toward increased excitability. It is still unknown which channels underlie the after-burst AHP, but the evidence is strong that the fAHP is carried predominantly by the BK channel. Because the BK channel has been sequenced and is well characterized functionally, it offers experimental advantages for resolving the mechanism of how the BK-mediated fAHP is altered after learning. In particular, the large conductance and Ca^{2+} dependence of the channel make it an ideal experimental target for single channel or imaging experiments to examine the mechanism of the learning-related changes in the BK-mediated current. This study provides a well characterized protein, the BK channel, as a potential therapeutic target for modulating the excitability of the hippocampus and thereby increasing learning capacity in aging or diseased brains.

Materials and Methods

Subjects were 3- to 4-month-old male F1 F344 X Brown Norway rats (13 trained, 9 pseudoconditioned, 8 naïve, and 5 *in vivo*). Procedures were approved by the Northwestern University Animal Care and Use Committee and conformed to National Institutes of Health standards. All efforts were made to minimize the number of animals and their discomfort. More detail about these methods is available in *SI Text*.

Animals were prepared for eyeblink training with implanted EMG wires according to Kuo *et al.* (20) and given 5 days to recover. For the *in vivo* studies, a guide cannula was implanted at coordinates (in mm): AP: −4.5, ML: 3.5, and DV: 1.9. A 4-tetrode movable recording array was implanted at (in mm) AP: −3.6 and ML: 2.5.

Trace eyeblink conditioning procedures and analysis as described by Weiss *et al.* (47) were followed. Subjects received one session of stimulus-free habituation to the chamber followed by five conditioning sessions. Conditioned animals received 30 trials per session, consisting of a tone (80 dB, 250 ms) paired with a corneal air puff (4 psi, 100 ms) with a 250-ms trace interval interposed. An eye closure, as measured by the integrated EMG, occurring within 100 ms preceding the puff was defined as an adaptive response (Fig. S2). Pseudoconditioned animals experienced 30 tone and 30 air-puff trials in an unpaired manner. Transverse hippocampal slices were made 24 h after completing training. Naïve animals were handled 18–24 h before being killed.

The researchers were blind to the training status of animals during all recordings and analyses. Oxygenated recording solution contained (in mM) 124 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 26 NaHCO3, 2.4 CaCl2, 2 $MgSO_4$, and 25 glucose. Borosilicate recording electrodes (3–5 MΩ) were filled with internal solution containing (in mM) 115 K-methylsulfate, 20 KCl, 10 Na-phosphocreatine, 10 Hepes, 2 Mg2ATP, and 0.3 Na3GTP (pH 7.45). All recordings were made at 34°C. CA1 pyramidal neurons were visually selected by using differential interference contrast optics. Recordings were made in current clamp mode by using a Dagan 3200C amplifier; electrode capacitance and R_S were compensated and systematically checked during the recording. The BK-channel blockers paxilline (10 μM) or iberiotoxin (60 nM) were bath applied in some experiments. No difference between the drugs was found, so paxilline was used in most experiments and data were pooled.

Inclusion criteria were the following: V_{rest} between −58 and −70 mV, an R_N of >25 MΩ, which remained stable during the recording, and an AP height of at least 70 mV above the holding potential. Cells were excluded only on these criteria. AP threshold was determined as the point where the first derivative of the AP was equal to 20 mV/ms. Recordings were acquired and analyzed by using pClamp 9.2 (Axon Instruments). Significant differences were evaluated by using one-way or repeated measures ANOVA with Fisher's probable least-squares difference post-hoc tests. AP durations were compared by using one-way ANOVA with between-group contrast analyses. All data are reported as (group: mean ± SEM, *n*).

The *in vivo* recording arrays were implanted at a depth of 1.7 mm and lowered 40–80 μm/day until APs exhibiting pyramidal-cell characteristics were identified (48), after which the electrodes were no longer moved. Screening and subsequent recording sessions were performed in awake rats confined in a plastic box (30 cm × 17.5 cm × 17.5 cm). Drug infusions were performed by using a 2-μl syringe connected by a length of flexible, oil-filled tubing to an infusion cannula. One microliter of paxilline (1, 10, or 100 μM) or vehicle was infused at a constant rate of 0.2 μl/min by using a syringe pump. The recording protocol (Fig. 4*A*) was performed on two consecutive days for each rat, injecting paxilline one day and vehicle on the other. The sequence of drug- and vehicle-infusion sessions was counterbalanced across animals. The experimenter was blind to the identity of the infused substance. Neuronal recordings were made by using the Neuralynx Cheetah system.

Individual units were isolated according to parameters of the spike waveform by using Neuralynx Spike Sorting and Separation software (Fig. S3). Spike width and signal-to-noise ratios were determined for all neurons, based on the tetrode channel with the largest amplitude waveforms for each isolated unit. Neurons exhibiting peak-to-valley spike widths >0.30 ms and firing rates <6 Hz were classified as pyramidal (49). Only cells classified as pyramidal and exhibiting signal-to-noise ratios greater than or equal to 2.5:1 were included. Spiking data were divided into 5-min bins for analysis. Spike counts from the infusion and after-infusion interval bins were normalized to the mean baseline spike count per bin. ANOVAs were performed to measure group differences; unpaired *t* tests were performed to compare baseline firing rates across pairs of days.

Marking lesions to identify tetrode location were made by passing direct current (+25 μA) for 10 s through a single channel of each tetrode. Subjects

were killed and perfused with 0.9% saline, followed by 10% formalin. Brains were extracted and placed in 10% formalin solution for 2 days, then quick-frozen and sectioned. Slices were mounted on slides, stained, and examined under the microscope at X25 magnification. Only single neuron activity recorded from arrays verified as centered on the CA1 band of the dorsal hippocampus was included (Fig. S4).

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