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Spike-and-wave discharge mediated reduction in hippocampal HCN1 channel function associates with learning deficits in a genetic mouse model of epilepsy

A. Marie Phillips [a,d], Taehwan Kim [a,b], Ernesto Vargas [a], Steven Petrou [a,b,c], Christopher A. Reid [a,*]

[a] Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville 3010, Australia
[b] Centre for Neural Engineering, The University of Melbourne, Parkville 3010, Melbourne, Australia
[c] Department of Anatomy and Neuroscience, The University of Melbourne, Parkville 3010, Melbourne, Australia
[d] Department of Genetics, University of Melbourne, Parkville 3010, Australia

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ABSTRACT

The $GABA_A\gamma2$(R43Q) mouse is an established model of absence epilepsy displaying spontaneous spike-and-wave discharges (SWD) and associated behavioral arrest. Absence epilepsy typically results from cortico-thalamic networks. Nevertheless, there is increasing evidence for changes in hippocampal metabolism and electrical behavior, consistent with a link between absence seizures and hippocampus-related co-morbidities. Hyperpolarization-activated-cyclic-nucleotide-gated (HCN) channels are known to be transcriptionally regulated in a number of seizure models. Here we investigate the expression and function of these channels in the hippocampus of the genetic epilepsy model. A reduction in HCN1, but not HCN2 transcript, was observed in $GABA_A\gamma2$(R43Q) mice relative to their littermate controls. In contrast, no change in HCN1 transcript was noted at an age prior to seizure expression or in a SWD-free model in which the R43Q mutation has been crossed into a seizure-resistant genetic background. Whole-cell recordings from CA1 pyramidal neurons confirm a reduction in I_h in the $GABA_A\gamma2$(R43Q) mouse. Further, a left-shift in half-activation of the I_h conductance–voltage relationship is consistent with a reduction in HCN1 with no change in HCN2 channel expression. Behavioral analysis using the Morris water maze indicates that $GABA_A\gamma2$(R43Q) mice are unable to learn as effectively as their wildtype littermates suggesting a deficit in hippocampal-based learning. SWD-free mice harboring the R43Q mutation had no learning deficit. We conclude that SWDs reduce hippocampal HCN1 expression and function, and that the reduction associates with a spatial learning deficit.

Introduction

Epilepsy is a common multi-factorial disease with both genetic and environmental pre-disposing factors. Childhood Absence Epilepsy (CAE) accounts for a large proportion of all childhood epilepsies and is characterized by spike-and-wave discharges (SWDs) associated with behavioral arrest. The disease is generally well treated and self-limiting and as such frequently considered benign. However, despite this perception studies consistently demonstrate that children with CAE under-perform in learning tasks (Caplan et al., 2008; Kernan et al., 2012; Killory et al., 2011; Pavone et al., 2001). CAE has a genetic etiology, raising the question as to whether the cause of altered learning ability is due to seizure expression or due to the same, or similar, genetic factors responsible for the epilepsy. This is a difficult question to address in heterogeneous patient populations. In this study we investigate this issue in a genetic mouse model of epilepsy based on a human mutation in *GABRG2* that recapitulates the CAE phenotype (Reid et al., 2013; Tan et al., 2007).

Learning and memory are complex biological behaviors that involve many brain networks. Although SWDs originate from cortico-thalamic networks there is good evidence to suggest that metabolic and electrical properties of the hippocampus are altered during an absence seizure (Nehlig et al., 1998; Velazquez et al., 2007). Spatial memory is a well recognized function of the hippocampal system (Buzsaki and Moser, 2013). The hyperpolarization-activated-cyclic-nucleotide-gated (HCN) channels activate at hyperpolarizing potential and have a range of cellular functions (Dyhrfjeld-Johnsen et al., 2009). Recent evidence has suggested that HCN channels, in particular the HCN1 subtype, are a central modulator of hippocampal-based memory (Giocomo et al., 2011; Hussaini et al., 2011; Nolan et al., 2003, 2004, 2007; Wang et al., 2007). HCN channels are transcriptionally plastic with mRNA and consequent protein expression changing in several biological and pathological settings. Of particular interest is that HCN channel

* Corresponding author at: Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Parkville, Victoria 3010, Australia. Fax: +61 3 9347 0446.
E-mail addresses: m.phillips@unimelb.edu.au (A.M. Phillips), kaarkim@gmail.com (T. Kim), e.vargas@commercial.unimelb.edu.au (E. Vargas), steven.petrou@florey.edu.au (S. Petrou), careid@unimelb.edu.au (C.A. Reid).

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expression is altered in a number of epileptic states (Reid et al., 2012). For example, HCN channels undergo changes in expression in acquired rodent models of epilepsy (Brewster et al., 2002; Chen et al., 2001; Jung et al., 2007; Powell et al., 2008). In the case of HCN1 these post-seizure changes in hippocampal expression are temporally robust (Brewster et al., 2002; Chen et al., 2001). Transcriptional changes also occur in rodent genetic models of generalized epilepsy including the WAG/Rij and GAERS rats where they are postulated to play a role in pathogenesis (Kuisle et al., 2006; Reid et al., 2012; Strauss et al., 2004).

In this study we find a decrease in hippocampal HCN1 transcript and CA1 pyramid I_h that associates with a spatial learning deficit in a genetic mouse model of epilepsy. We propose that a post-SWD change in HCN1 transcript levels is a useful biomarker of hippocampal plasticity, and that a reduction in I_h is well placed to be part of the mechanism underling the observed learning deficit.

Materials and methods

Animals

Experiments have been carried out in accordance with the EC directive for animal experimentation. All experiments were approved by the Animal Ethics Committee, Florey Institute for Neuroscience and Mental Health, FNI 06-093, FNI 08-09 and 12-049-FNI. Mice were toe-clipped at P7 and tail samples genotyped by PCR as described previously (Chiu et al., 2008; Tan et al., 2007). Mice were re-genotyped after tissue collection. Cohorts of DBA/2J and C57Bl/6J mice segregating for the $GABA_A\gamma2$(R43Q) mutation ($Gabrg2^{tm1Spet}$) had been inbred in the laboratory for more than 20 generations. To generate the mice with a mixed strain background, parental crosses between male DBA-$GABA_A\gamma2$(R43Q) carriers and female C57-$GABA_A\gamma2$(WT) mice were established and the F1 offspring interbred.

Electrocorticogram (ECoG) recordings

ECoG electrode implantation surgeries were performed as previously described (Tan et al., 2007). Mice were anesthetized with 1–3% isoflurane, and two epidural-silver 'ball'-electrodes implanted on each hemisphere of the skull. Electrodes were placed 3 mm lateral of the midline and 0.5 mm, caudal from the bregma. A ground electrode was placed 2.5 mm rostral from bregma and 0.5 mm lateral from the midline. Mice were allowed to recover for at least 24 h. Electrocorticogram recordings were recorded in freely moving mice during the daylight. Signals were band-pass filtered at 0.1 to 200 Hz and sampled at 1 kHz using Powerlab 16/30 (ADInstruments Pty. Ltd., Sydney, NSW, Australia). Spike-and-wave discharge (SWDs) events were analyzed for counts and durations for 6 h/day for two days.

Tissue collection

Littermate pairs of mice of the same gender were killed by cervical dislocation and brains rapidly removed. Hippocampal extraction was as published in Beaudoin et al. (2012), with few modifications. In our study the mice were either P16 or adult mice. The cerebella were removed using a small scalpel and the remaining brain cleaved to yield two hemispheres. These were then further dissected to provide cortex/hippocampus and a thalamus enriched fraction. The hippocampus was identified and isolated from the cortical fraction as a complete tissue using a soft fine paintbrush. Partial or damaged hippocampi were discarded. All samples collected were placed into RNA *later* immediately following dissection and stored at −80 °C.

cDNA preparation

Total RNA from the stored tissue was prepared by homogenization in TRIzol (Invitrogen), and purifying and concentrating the RNA using a commercial column (RNeasy, Qiagen) according to the manufacturer's instructions. The RNA was quantified and quality assessed by spectrophotometry. Any RNA sample with an OD260/280 ratio less than 1.98 was discarded (ratios were typically 2.00–2.04). A sub-set of samples was analyzed by gel electrophoresis to confirm integrity. cDNA was prepared from 1.0 mg total RNA (cortex and thalamus) or 500 ng total RNA (hippocampus) using a commercial transcription kit and random-hexamer primers (Roche High Fidelity Transcription Kit) according to the manufacturer's instructions. All cDNA samples analyzed in any one qPCR run were prepared at the same time and using the same master mix of reagents. cDNAs were stored at −20 °C short term (less than 48 h) and at −80 °C for long term storage.

Reference genes for qPCR

Standard curves for GADPH, 18sRNA and β-actin were compared to find suitable reference genes. Pooled RNA from 6 littermate pairs was converted to cDNA and dilutions analyzed in triplicate by qPCR on a Chromo4 (and later repeated on a Corbett 6000). Standard curves were linear and identical for DBA-$GABA_A\gamma2$(R43Q) mice and their wildtype littermates for GADPH and 18S RNA, but not for β-actin (data not shown). Samples were run using GADPH as the reference gene and results verified using 18sRNA.

qPCR

Hydrolysis probes for *Mus musculus* HCN1 (Mm00468832_m1), HCN2 (Mm00468538_m1), GADPH (Mm99999915_g1) and β actin (4352933E) were obtained from Applied Biosystems as validated Gene Expression probes. 20 μl qPCR samples containing cDNA from 5 ng or 10 ng of total RNA, depending on the tissue level of expression, 1 μl of commercial probe and 1× Universal Master Mix were assayed in triplicate. The program involved a 2 min 50 °C hold to inactivate uracil-N-glycosylase, UNG, followed by a 10 minute 95 °C hold, and 40 cycles of 95 °C 30 s and 60 °C 60 s using Chromo4 (BioRad) and Corbett 6000 (Qiagen) cyclers. Both machines used show amplification efficiencies for individual samples, allowing Pfaffl correction for minor differences in efficiency (Pfaffl, 2001: REST software, Qiagen). All samples with poor amplification efficiency or variable reproducibility within triplicates were re-run from fresh cDNA. Verification of data, using 18sRNA as the reference gene involved analysis with 1× Platinum Sybr Green, (Invitrogen) with 40 nM 5′-cggctaccacatccaaggaa-3′ as the forward primer and 20 nM 5′-gctggaattaccgcggct-3′ as the reverse primer as described previously (Chiu et al., 2008).

Transcript normalization

In the comparative studies involving age-matched inbred DBA or C57Bl6 mice, the HCN1 transcript for all $GABA_A\gamma2$ heterozygotes, was normalized relative to the transcript level of a wildtype littermate, given a value of 1. A minimum of 5 individual litters was sampled for each experiment. The F2 $GABA_A\gamma2$ heterozygotes produced from mating the F1 of a cross between C57-$GABA_A\gamma2$(WT) mothers and DBA-$GABA_A\gamma2$(R43Q) fathers were genetically different and normalization of HCN1 transcript was to the average expression of four mice with zero SWDs. The average was obtained from two qPCR assays in triplicate, in which all four identically prepared cDNAs were analyzed in both qPCR runs.

Electrophysiology recording from CA1 pyramidal cells

Slices were prepared from wildtype and DBA-$GABA_A\gamma2$(R43Q) mice aged P30–40. Animals were anesthetized with isoflurane and decapitated. The brain was removed and placed in ice-cold artificial cerebral spinal fluid (ACSF,126 mM NaCl, 3.5 mM KCl, 2 mM $CaCl_2$, 1.3 mM $MgCl_2$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, and 11 mM glucose, pH to

7.2 and saturated with 95% O_2 and 5% CO_2). Coronal slices, 300 μm thick, were cut using a Vibratome (Leica), transferred to room temperature ACSF and allowed to recover for 1 h prior to recording. Slices were transferred to a recording chamber and perfused with room temperature ASCF at 2 ml/min. Whole-cell voltage clamp recording was made from CA1 pyramidal neurons using an Axoclamp 2B amplifier (Molecular Devices, Sunnyvale, CA) and Axograph acquisition software (Axograph, Australia). Signals were sampled at 5 kHz and low pass filtered at 3 kHz. Pipettes were filled with (in mM): 125 KGlu, 4 KCl, 2 MgCl2, 10 HEPES, 10 EGTA, 4 ATP-Mg and 0.3 GTP-Na, adjusted to a final pH of 7.3 with KOH. D-mannitol was used to adjust osmolarity to 300 mOsm. Cells were held at −50 mV and a current–voltage (I–V) relationship was generated by incrementing voltage in 10 mV steps from −120 mV to −40 mV for 2 s with a 1 s test potential of −100 mV at the end of the pulse to measure tail current. To obtain a normalized conductance–voltage relationship, peak tail current amplitudes were divided by the largest peak tail current, plotted against voltage and fit with a Boltzmann curve (GraphPad Prism, CA, USA).

Morris water maze studies

Spatial learning ability was assessed using a standard Morris water maze that consisted of a 15 cm diameter platform submerged in opaque water (at 25 °C) in one quadrant of a 1.9 m diameter water-maze-pool. Each mouse was introduced to the platform and then the mice were placed in a random quadrant and given a maximum of 2 min to find the platform using external visual cues. Mice failing to find the platform in 2 min were introduced to it again prior to the next test. The routine was repeated 4 times/day for 5 days. The mice were tested in age-matched cohorts of 17/20 animals, 8 DBA-GABA$_A$γ2(WT) and 9 DBA-GABA$_A$γ2(R43Q); 10 C57-GABA$_A$γ2(WT) and 10 C57-GABA$_A$γ2(R43Q). The platform was moved to a different quadrant on day 6 to further probe any learning/memory differences in the DBA strain.

Statistical analysis

All data was analyzed using GraphPad Prism 5 software (GraphPad, San Diego CA).

As the qPCR data is reported relative to the wildtype littermate which was given a value of 1, this data was analyzed using the Wilcoxon-matched pairs signed-ranking test, 2 tailed. All other statistical differences were tested with Student's 2-tailed *t*-test. Statistical significance was set at $p < 0.05$. Correlation data was analyzed using non-Gaussian parameters-Spearman (GraphPad, San Diego CA). All bar graphs are displayed as mean ± SEM.

Results

HCN1 mRNA levels are reduced in the hippocampus of a genetic model of absence epilepsy

DBA-GABA$_A$γ2(R43Q) mice display spike-and-wave discharges (SWD) that associate with behavioral arrest, both hallmarks of absence epilepsy seen in patients with the mutation (Fig. 1A). DBA-GABA$_A$γ2(R43Q) mice have between 20 and 50 SWD events per hour compared to wildtype littermates that express approximately 2 SWD events per hour (Reid et al., 2013; Tan et al., 2007). Given the plastic nature of I_h in several epilepsy models we investigated transcript levels of HCN1 and HCN2 in the hippocampus of P50 DBA-GABA$_A$γ2(R43Q) mice. HCN1 mRNA levels were reduced by ~20% in the hippocampus of DBA-GABA$_A$γ2(R43Q) mice when compared with their littermate pair (Fig. 1B). In contrast, HCN2 transcript in the same cDNA sample was that of wildtype mice (Fig. 1C). The magnitude of the reduction in HCN1 was similar for both males and females (male n = 4 pairs, females, n = 5 pairs; p = 0.57, unpaired *t*-test, df = 7).

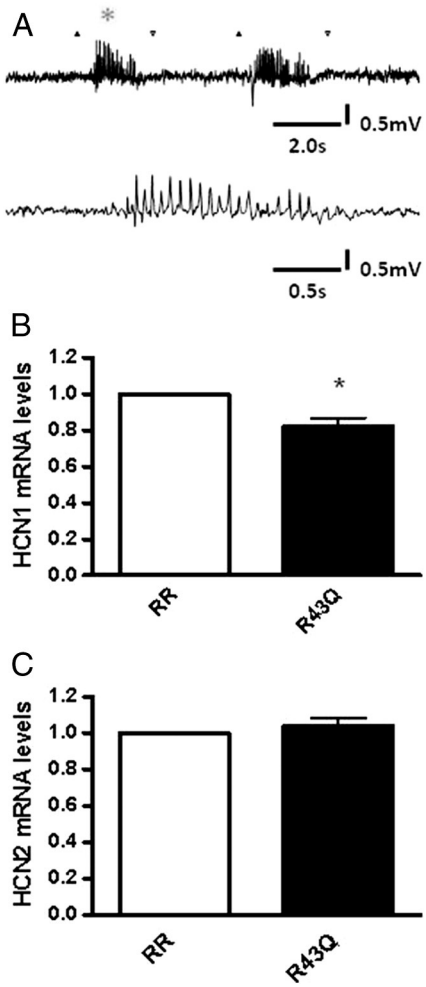

Fig. 1. P50 DBA-GABA$_A$γ2(R43Q) mice have reduced HCN1 transcript in whole hippocampus relative to their wildtype littermates. (A) A typical EEG recording from a DBA-GABA$_A$γ2(R43Q) mouse showing spontaneous SWDs. The arrowheads indicate the onset, and endpoint of immobility accompanying the SWD. The lower trace is an expansion of the marked trace (*) to more clearly show the typical SWD morphology. (B) Mean HCN1 transcript levels are reduced relative to wildtype (*p = 0.005, n = 11 littermate pairs). (C) Hippocampal HCN2 transcript levels are identical in DBA-GABA$_A$γ2(R43Q) mice compared to wildtype (p = 0.624, n = 11 littermate pairs).

To determine if the observed reduction in HCN1 transcript correlated with SWD incidence we used two different seizure-free controls. Firstly, as previous temporal mapping of SWD expression had shown that these only presented after P21 in the DBA-GABA$_A$γ2(R43Q) mouse (Tan et al., 2007), we used immature littermates as a control. Hippocampal HCN1 transcript levels in pre-seizure P16 mice were identical to that of wildtype (Fig. 2A). Secondly, the GABA$_A$γ2(R43Q) mutation on the SWD-resistant C57Bl6 strain (C57-GABA$_A$γ2(R43Q)) also provides a seizure-free model (Reid et al., 2013). Hippocampal HCN1 transcript levels measured from P50 C57-GABA$_A$γ2(R43Q) mice were not significantly different from wildtype littermates, with a trend of an increase rather than a decrease in expression (Fig. 2B). Together, these data support the idea that a reduction in hippocampal HCN1 transcription is due to SWD incidence and not simply a consequence of the presence of the GABA$_A$γ2(R43Q) susceptibility mutation.

Interestingly, if hippocampal HCN1 expression is analyzed in wildtype P50 DBA mice relative to age-matched wildtype C57Bl6 mice, the former have significantly greater transcript levels (DBA-GABA$_A$γ2(WT) 1.45 ± 0.08 vs. C57-GABA$_A$γ2(WT) 1.1 ± 0.03, p = 0.0015, n = 18/7; 2-tailed unpaired *t*-test with Welch's correction). This suggests that a lower HCN1 expression level determined by the DBA/2J background is not responsible for the increased seizure

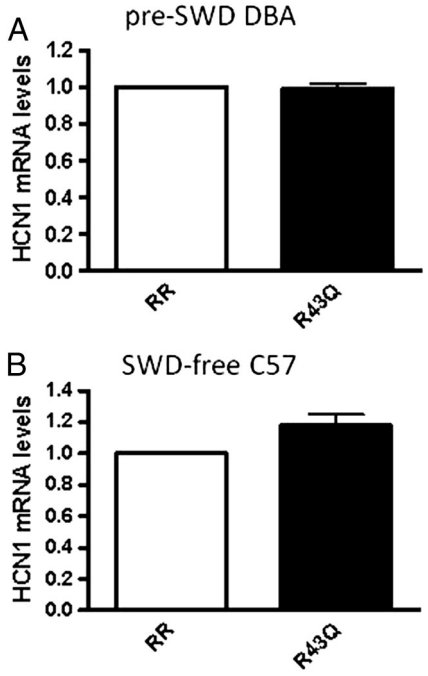

Fig. 2. There is no reduction in HCN1 transcript levels in the hippocampus in SWD-free mice. (A) Mean HCN1 transcript levels of P16 pre-SWD DBA-GABA$_A$γ2(R43Q) mice relative to wildtype were identical ($p = 0.83$, $n = 6$ littermate pairs). (B) P50 SWD-free C57-GABA$_A$γ2(R43Q) HCN1 transcript levels tended to be higher than their wildtype littermates ($p = 0.055$ $n = 9$ pairs).

susceptibility in this strain. However, changes in HCN1 channel expression may contribute to other behavioral differences between the DBA/2J and C57Bl/6J mice strains.

Reduction in HCN1 transcription is 'triggered' in mice having two or more SWDs

An F2 generation bred from DBA and C57Bl6 parents and harboring the GABA$_A$γ2(R43Q) mutation was used to map the impact of varying SWD occurrence on changes in hippocampal HCN1 transcript levels. F2 mice were monitored by EoCG to determine the number and duration of seizures in each mouse. In individual mice the average SWD/hour remained constant over time (data not shown), but varied from mouse-to-mouse consistent with differing genetic contributions from each parental strain (Fig. 3A). HCN1 transcript levels measured by qPCR were expressed relative to the mean expression in four F2 R43Q mice that were SWD-free over the time course of the EEG experiment. Mice averaging more than 2 SWDs/h, 2+ mice, showed a reduction in hippocampal HCN1 transcript levels (Figs. 3A,B). Interestingly, SWD occurrence did not regress with HCN1 transcript levels after the 'trigger' point of 2 SWDs/h (Fig. 3A). Seizure duration (Fig. 3C) showed no correlation with the level of HCN1 transcript decrease.

Functional reduction in I_h in the adult DBA-GABA$_A$γ2(R43Q) mouse

Whole-cell voltage-clamp recordings were made from CA1 pyramidal neurons where HCN1 is robustly expressed (Brewster et al., 2007) and I_h recorded (Fig. 4). A significant reduction in I_h amplitude was measured from neurons of the DBA-GABA$_A$γ2(R43Q) mouse (Fig. 4A). Interestingly, the conductance–voltage relationship revealed a left-shift in activation for mutant neurons (Fig. 4B). HCN1 has a more positive activation curve compared to HCN2 and this left-shift is consistent with a current more dominated by HCN2 channels, which are not transcriptionally altered in DBA-GABA$_A$γ2(R43Q) mice (Fig. 1C).

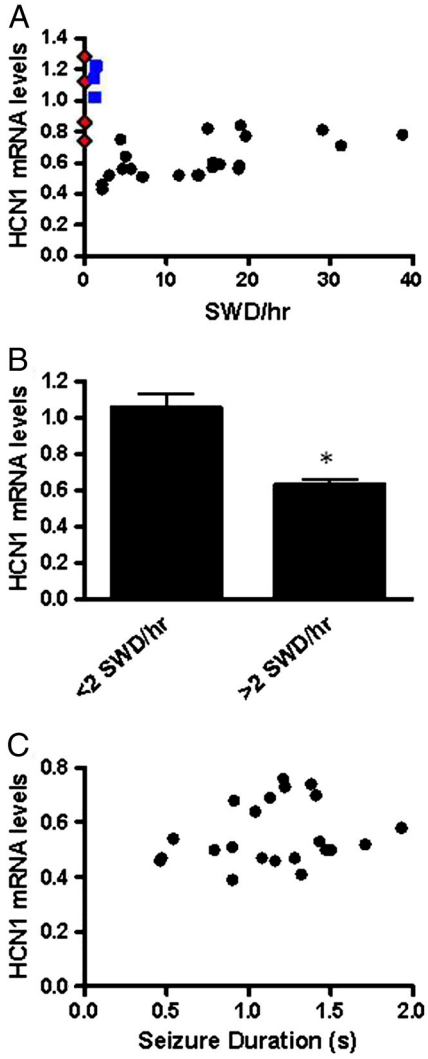

Fig. 3. SWD incidence above a threshold of 2 events per hour is necessary and sufficient for HCN1 transcript reduction. (A) HCN1 relative to SWD/hour in F2 GABA$_A$γ2(R43Q) mice with a mixed genetic C57/DBA background (red diamonds = no SWD, blue squares = <2 SWDs/h, black dots ≥ 2 SWDs/h). Increasing occurrence beyond 2 SWDs/h does not result in a greater HCN1 reduction. (B) Mice averaging greater than two SWDs/hour had significantly decreased HCN1transcript levels compared with littermates with 2 or fewer SWDs/hour, $p < 0.0001$. (C) SWD duration shows no correlation with HCN1 transcript reduction ($p = 0.14$, $n = 23$, Spearman r).

Hippocampal-based learning deficit in the DBA-GABA$_A$γ2(R43Q) mouse

HCN1 channels are known to modulate certain learning behaviors (Nolan et al., 2003, 2004). Here we test the spatial learning ability of mature, P50, DBA-GABA$_A$γ2(R43Q) and C57-GABA$_A$γ2(R43Q) mice relative to their respective wildtype littermates using the established Morris water maze test. On day one of testing naïve mutant and wildtype mice have identical latency to platform (Fig. 5A) and a similar mean length of swim (DBA-GABA$_A$γ2(R43Q); 2780 ± 0.98 cm vs wildtype DBA; 2479 ± 137.9 cm, $p = 0.076$, two tailed *t*-test) suggesting that that behavioral arrest was not confounding interpretation of the Morris water maze results.

In the DBA strain latency to platform was greater for the DBA-GABA$_A$γ2(R43Q) mice with the difference statistically significant by day 5 (Fig. 5A). At day 6 the platform was moved to a different quadrant. The latency to platform was significantly increased for the wildtype but unchanged in the DBA-GABA$_A$γ2(R43Q) mice (Fig. 5A). There was no difference in the time to platform between C57-GABA$_A$γ2(R43Q) and their wildtype littermates over the 5 day period (Fig. 5B). These data

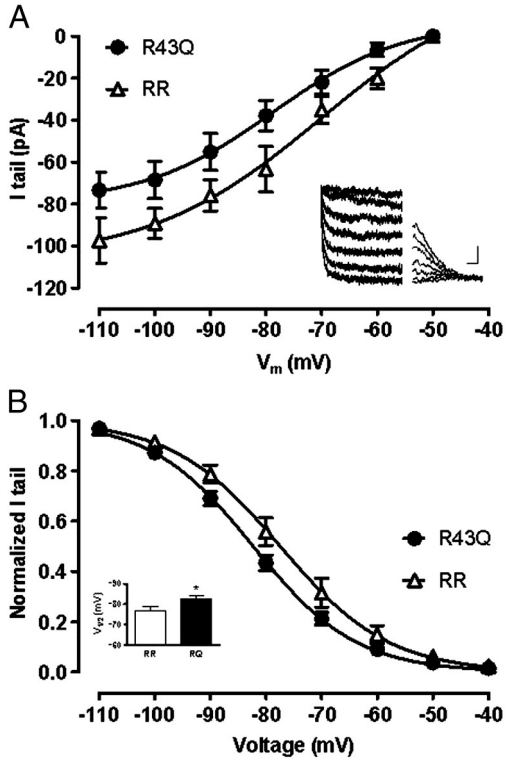

Fig. 4. Reduced I_h in DBA-GABA$_A$γ2(R43Q) CA1 pyramidal neurons. (A) Current–voltage relationship generated from tail currents recorded from DBA-GABA$_A$γ2(R43Q) and wildtype (RR) mice. Insert; steady state (left) and tail (right) I_h recorded in voltage clamp from a CA1 pyramid. Scale bars; vertical 20 pA, horizontal 300 ms for steady state current and 100 ms tail current. I_h is significantly smaller in DBA-GABA$_A$γ2(R43Q) CA1 pyramidal neurons (*$p < 0.05$, $n = 10$ and 15) (B) Boltzmann sigmoid fit to the normalized tail current–voltage relationship. A left-shift in the voltage-dependence of activation is seen for DBA-GABA$_A$γ2(R43Q) CA1 pyramids. Insert; bar graph of the average $V_{1/2}$ of each genotype (*$p = 0.036$, $n = 10$ and 15).

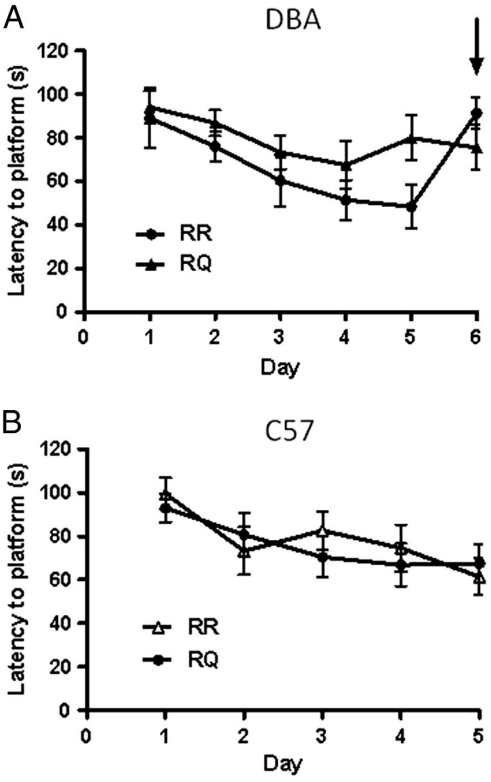

Fig. 5. DBA-GABA$_A$γ2(R43Q) mice have a spatial learning deficit. (A) Latency to platform is decreased in the DBA-GABA$_A$γ2(R43Q) mice relative to littermate controls. The difference is significant by day 5 ($p = 0.008$, $n = 8$ and 8, unpaired *t*-test). When the platform was moved to a different quadrant on day 6 (arrow), latency to platform increased in DBA-GABA$_A$γ2(RR) mice relative to day 5 times ($p = 0.023$, $n = 8$). In contrast, latency to platform relative to day 5 times did not change in DBA-GABA$_A$γ2(R43Q) mice when the platform was moved ($p = 0.82$, $n = 8$). (B) There is no difference in learning, as assessed by latency to platform, in C57-GABA$_A$γ2(R43Q) relative to C57-GABA$_A$γ2(RR) mice ($p = 0.76$, $n = 9$ and 9).

are indicative of a learning/memory deficit that correlates with observed reductions in HCN1 function and SWD occurrence.

Discussion

DBA-GABA$_A$γ2(R43Q) mice have a SWD-associated transcriptional down regulation of hippocampal HCN1 that results in a reduction in I_h measured from CA1 pyramidal neurons. This is accompanied by a hippocampal-based spatial learning deficit. Mice harboring the same mutation, but in the seizure resistant C57 background, show no change in HCN1 transcript and learn normally. These data strongly suggest that SWD-based seizures modify hippocampal properties and that these changes are well placed to underlie co-morbid states such as learning difficulties.

Absence epilepsy and learning difficulties

While severe cognitive defects have been associated with atypical absence seizures where SWDs are present in the hippocampus (Cortez et al., 2001; Farwell et al., 1985), there is increasing evidence for such defects in typical absence seizure (Caplan et al., 2008; Kernan et al., 2012; Killory et al., 2011; Pavone et al., 2001). Animal studies provide additional evidence for this idea demonstrating that an age-related defect in learning occurs in the WAGRj rat model of absence epilepsy (Karson et al., 2012). Our data strongly suggests that it is the SWD events that underlie changes in hippocampal function leading to learning deficits. Treatment with anti-epileptic drugs may therefore alleviate such learning difficulties. Recent evidence from GAERS, another rat model of absence epilepsy, supports this idea showing that treatment with an antiepileptic, ethosuximide, does reduce other co-morbid states in epilepsy (Dezsi et al., 2013). The F2 generation from the C57 × DBA cross produces individuals with varied genetic backgrounds and SWD incidence (0–40 SWDs per hour). This data indicates a threshold/switch effect with reductions in HCN1 transcript occurring when 2 or more SWDs occurred per hour. This finding may have clinical significance, with the suggestion that aggressive treatment of seizures, even infrequent seizures, is required in order to overcome potential cognitive deficits.

HCN1 channels and learning

HCN1 channels seem to have a role in modulating learning and memory. Global HCN1 knockout mice showed impaired motor learning and memory (Nolan et al., 2003). In contrast, forebrain-specific HCN1 knockout mice displayed improved short- and long-term spatial learning and memory (Nolan et al., 2007). The basis of this discrepancy is unclear. Our data shows that a reduced HCN1 expression correlates with a subtle learning deficit in the DBA-GABA$_A$γ2(R43Q) mice which is more consistent with total HCN1 knock-out data. Obviously, total knock-out of HCN1 from birth differs extensively from the milder transcriptional change noted in the genetic epilepsy model presented here. Although we cannot causatively link a reduction in CA1 pyramidal neuron HCN1 function to a memory deficit it remains a valid candidate. Further, HCN1 function in CA1 pyramidal neurons is known to modulate depressive and anxiety behaviors (Kim et al., 2012), and therefore may be part of the mechanism underlying other co-morbid states in generalized epilepsy.

How do SWDs engage the hippocampus?

The lack of a positive correlation between SWD events per hour and hippocampal HCN1 transcript reduction (F2 mice having >2 to 40 SWDs/h) argues strongly that these changes do not contribute to the neuronal excitability underlying SWDs. This is expected given the cortico-thalamic network origin of SWDs. Onat et al. (2013) canvass the involvement of limbic brain structures in absence with-and-without hippocampal SWDs, and find evidence for a hippocampal involvement in both, though via different neural networks. Therefore, while SWDs do not occur in the hippocampus in typical absence seizures the tissue is neither metabolically nor electrically isolated (Onat et al., 2013). We propose that this secondary engagement of the hippocampus is responsible for changes observed in this study and may underlie changes in learning behavior.

In summary, our data robustly shows that SWDs, that have a cortico-thalamic origin, can engage the hippocampus to cause changes in HCN1 transcription and channel function. Reductions in HCN1 function are a good candidate molecular mechanism for the cognitive deficits seen in absence epilepsy.

Acknowledgments

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