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Contribution of Kv4.2 to Neuronal Hyperexcitability in a Mouse Model of Alzheimer's Disease

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CONTRIBUTION OF KV4.2 TO NEURONAL HYPEREXCITABILITY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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ABSTRACT

The incidence of Alzheimer's disease (AD) is increasing with the aging population and an astonishing 5.2 million Americans are affected by AD, the most common cause of dementia. Cognitive impairment worsens with declining hippocampal function. Neuronal hyperexcitability occurs early in the pathogenesis of AD and contributes to network imbalance and the seizure activity seen in AD patients. In other disorders with neuronal hyperexcitability, dysfunction in the dendrites often contributes, but dendritic excitability has not been studied in AD models. We used patch-clamp recordings to directly examine dendritic excitability in the CA1 region of the hippocampus. We found that dendrites, but not the soma of hippocampal neurons, were hyperexcitable in the hippocampus of mice overexpressing human APP/Aβ. This dendritic hyperexcitability was associated with depletion of Kv4.2, a dendritically-localized potassium channel important in the regulation of dendritic excitability, synaptic plasticity, and learning and memory. We found that epileptiform activity drives the reduction in Kv4.2, and blocking epileptiform activity by tau reduction prevented both Kv4.2 depletion and dendritic hyperexcitability. The dendritic hyperexcitability induced by Kv4.2 depletion exacerbates behavioral deficits induced by Aβ and further increases dendritic excitability, creating a detrimental feedback loop. Therefore, we conclude that Kv4.2 is a dendritic effector downstream of Aβ and that increased dendritic excitability may contribute to neuronal dysfunction in early stages AD.

Keywords: Kv4.2, hyperexcitability, Alzheimer’s disease, mouse model, tau, dendrite

DEDICATION

This dissertation is dedicated to scientists who dream big and work hard, including my mother.

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INTRODUCTION

TAU-DEPENDENT KV4.2 DEPLETION AND DENDRTIC HYPEREXCITABILITY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

LIST OF ABBREVIATIONS

Aβ	amyloid-β
ACSF	artificial cerebrospinal fluid
AD	Alzheimer's disease
AHP	afterhyperpolarizatioin
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
α7nACh	α7 nicotinic Acetylcholine
AP	action potential
APOE	apolipoprotein E
BACE	beta-site APP cleaving enzyme
cDNA	complementary deoxyribonucleic acid
DG	dentate gyrus
DIC	differential interference contrast
DPLP	cipeptidyl peptidase-like proteins
EC	entorhinal cortex
EEG	electroencephalography
EPSP	excitatory postsynaptic potential
ERK	extracellular signal-regulated kinases
FDA	food and drug administration
fMRI	functional magnetic resonance imaging
FMRP	fragile X mental retardation protein
GABA	γ-aminobutyric acid
hAPP	human amyloid precursor protein
HCN	hyperpolarization-activated cyclic nucleotide-gated
HDAC	histone deacetylase
IA	A-type current
ICV	intracerebroventricular
IPSC	inhibitory presynaptic current
IV	current-voltage
KChips	K+ channel interacting proteins
KO	knock out
LEV	levetiracetam
LTD	long-term depression
LTP	long-term potentiation
MCI	mild cognitive impairment
mGluR	metabotropic glutamate receptors
MOT	motor cortex
mRNA	messenger ribonucleic acid
NFAT	nuclear factor of activated T-cells
NFT	neurofibrillary tangles
NMDA	N-Methyl-D-aspartate
NTG	nontransgenic
PET	positron emission tomography
PDGF	platelet-derived growth factor
PKA	protein kinase A
PKC	protein kinase C
PrP	cellular prion protein
PSEN	presenilin
SK	small conductance calcium-activated potassium channel
STDP	spike timing dependent plasticity
STEP	striatal-enriched protein tyrosine phosphatase
SV2A	synaptic vesicle protein 2A
TBI	traumatic brain injury
TLE	temporal lobe epilepsy
TTX	tetrodotoxin

INTRODUCTION

Alzheimer's Disease

Alzheimer's disease (AD) is the most common cause of dementia. Currently, 35 million people are afflicted worldwide and the prevalence is expected to reach 115 million by 2050 (Prince and Jackson, 2009). New diagnostic criteria recognize three stages of AD, with a shifting emphasis for earlier detection through biomarkers. In preclinical AD, the first stage, AD biomarkers are present but symptoms have not yet appeared (Sperling et al., 2011). In mild cognitive impairment (MCI), the second stage, patients have cognitive deficits but no functional impairments (Albert et al., 2011). In AD dementia, the third stage, decline in two or more cognitive domains has progressed to the point that functioning at work or in daily activities is impaired (McKhann et al., 2011). From time of diagnosis, AD symptoms typically worsen for 12 years before they are fatal.

Initial understanding of AD came from observations of brain pathology and genetics. AD brain pathology comprises protein aggregates, including extracellular amyloid plaques composed of predominantly amyloid-beta (Aβ) peptides and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau (Goedert, 1993). Clinical progression correlates better with the neurofibrillary tangle distribution pattern described by Braak staging. Stages I-II show mild or severe deposition in the transentorhinal layer, stages III-IV show deposition in the

transentorhinal region and entorhinal cortex and mild deposition in the hippocampal formation and stages V-VI show deposition spread to isocortical association areas (Braak and Braak, 1991). Notably, loss of synapses correlates best with cognitive decline (Terry et al., 1991, Honer, 2003).

Genetics

Examination of the genetic cause of AD has been paramount in forming the hypothesis that high levels of soluble Aβ at the synapse causes AD. Autosomal dominant AD accounts for less than 1% percent of AD cases (Campion et al., 1999). AD is caused by mutations in 3 genes: amyloid precursor protein (APP) and the presenilin genes (PSEN1 and PSEN2). All of the mutations identified alter the production of Aβ through APP processing. APP is a type I transmembrane protein that has a large amino-terminal extracellular domain. Aβ is made by first cleavage of the extracellular domain by β-secretase (beta-site APP cleaving enzyme, BACE). γ-secretase then cleaves the remaining carboxy-terminal fragment within the membrane. γ-secretase is composed of presenilin and other components and it can cleave APP at different sites, resulting in Aβ peptides of 40 or 42 amino acids. $A\beta_{42}$ is more toxic and is more prone to oligomerization than $A\beta_{40}$. Mutations in APP that cause AD are predominantly localized to Aβ cleavage sites. The K670N/M671L double mutation is at the β-secretase cleavage site (originally found in a Swedish family). It results in increased BACE cleavage and thus increased Aβ production (Citron et al., 1992, Suzuki et al., 1994). The γ-secretase cleavage site is the location of the London (V717I), Indiana (V717F), and other mutations that increase the ratio of the more toxic form, $A\beta_{42}$, relative to $A\beta_{40}$ (Charier-Harlin, 1991, Price and Sisodia, 1998). Increased APP copy number also causes AD (Heston, 1984, Cabrejo et al., 2006). A

coding mutation (A673T) was found in the APP gene that is near the β-cleavage site and reduces Aβ production by 40% (Jonsson et al., 2012). It actually protects against cognitive decline in AD patients (Jonsson et al., 2012). Mutations in the presenilin genes, the catalytic subunit of γ-secretase, also cause autosomal dominant Alzheimer's disease. Mutations in PSEN1 and PSEN2 are more common than APP mutations. AD-associated presenilin mutations increase the Aβ42/Aβ40 ratio (Sherrington et al., 1995, Sherrington et al., 1996, Duff, 1997, Oyama et al., 1998).

Most cases of AD are sporadic and late onset, but several genes modulate the risk of this more common form of the disease. The strongest risk factor gene is APOE, encoding apolipoprotein E (apoE). There are three alleles of APOE; the ε2 allele is protective, the ε3 allele is the most common, and the ε4 allele is associated with increased risk and earlier age of AD onset (Corder et al., 1993, Farrer et al., 1997). Each copy lowers the age of onset about 10 years (Corder et al., 1993). Evidence that APOE ε4 increases risk for AD is strong but how it increases risk is not completely understood. ApoE is mainly secreted by astrocytes (Xu et al., 2006) and assists in the transport and delivery of cholesterol and other lipids through cell surface apoE receptors (Mahley, 1988, Mahley and Rall, 2000). ApoE is involved in the binding and clearance of Aβ (Holtzman et al., 2000). Some studies indicate ApoE4 protein is less stable and is degraded more easily therefore less apoE is associated with higher Aβ levels (Shinohara et al., 2013). Other studies have shown a stronger interactions between Aβ and apoE3 than apoE4 (Petrlova et al., 2011) and is associated with an accelerated cellular uptake of Aβ (Li et al., 2012). Patients with the ε4 allele have elevated brain Aβ and increased plaque deposition (Schmechel et al., 1993, Tiraboschi et al., 2004). ApoE can be

produced in neurons under stress (Xu et al., 2006). In neurons, apoE4 undergoes a higher rate of proteolytic cleavage than apoE3, creating a fragment that escapes secretion and is neurotoxic (Harris et al., 2003, Xu et al., 2008). Therefore, APOE ε4 could have both Aβ-dependent and independent roles in AD.

Although knowledge about AD has grown exponentially in the past few decades this has not yet been translated into disease modifying treatments. Two drugs have been FDA-approved for treatment of AD: (1) acetylcholinesterase inhibitors to increase the level of acetylcholine and (2) NMDA receptor antagonists. But in the clinic these drugs provide modest symptomatic benefits (Cummings, 2004, Lopez et al., 2009). The field has focused on targeting Aβ processing and clearance. β- and γ-secretase inhibitors are being developed but difficulties arise with γ-secretase's other functions (Schor, 2011). For example, γ-secretase cleaves other substrates and is involved in endoproteolysis, cell adhesion, calcium homeostasis, transport, trafficking/ localization, and apoptosis (Thinakaran and Parent, 2004, Vetrivel et al., 2006). Moreover, both active and passive Aβ immunization have proceeded to clinical trials (Gilman et al., 2005, Salloway et al., 2014). So far, complications from side effects and perhaps late intervention have made it difficult to determine efficacy. Interest in tau-based therapies is growing. Since blocking tau aggregation may pose complications similar to those associated with targeting Aβ, it is only one line of inquiry. Targeting tau expression levels, phosphorylation and interactions have become a focus of effort (Brunden et al., 2009, Golde et al., 2011, Morris et al., 2011b). In addition to efforts for target identification, there have also been huge efforts toward earlier detection using biomarkers, allowing for earlier treatment

(Sperling et al., 2011). These ventures will hopefully change the unfortunate reality that no disease-modifying treatment exists.

Evidence of network hyperexcitability

There is a tight association between Aβ overexpression and seizure activity (Table 1). Late-onset sporadic and early-onset autosomal dominant cases of AD show an increased incidence of unprovoked seizures (Hauser et al., 1986, Romanelli et al., 1990, Amatniek et al., 2006). The increase in incidence is highest among early onset patients (age 50-60) with an 87-fold increase over age matched controls (Amatniek et al., 2006). 85% of Down's syndrome patients who develop AD also have seizures (Lai and Williams, 1989). A recent retrospective study found that patients with amnestic MCI who had epilepsy had cognitive decline 6.8 years earlier and patients with AD who had epilepsy had cognitive decline 5.5 years earlier (Vossel et al., 2013). Even patients with AD who had subclinical epileptiform activity also had early onset of cognitive decline (Vossel et al., 2013). Patients with AD and seizures also have more severe neuronal loss at autopsy than those without seizures (Forstl et al., 1992).

Gene	Mutation	Phenotype	Reference
APP	V717G	Seizures	(Rossor et al., 1993)
APP	T714A	Seizures	(Lindquist et al., 2008)
APP	Duplication	Seizures	(Cabrejo et al., 2006)
APP	Trisomy 21	Seizures	(Menendez, 2005)
Presenilin 1	M139V	Seizures	(Fox et al., 1997)
Presenilin 1	S169L	Seizures	(Takao et al., 2001)
Presenilin 1	L420R	Seizures	(Shrimpton et al., 2007)
Presenilin 1	E280A	Seizures	(Velez-Pardo et al., 2004)
Presenilin 1	Multiple	Seizures	(Larner, 2010)
Presenilin 2	M239V	Seizures	(Marcon et al., 2004)
Presenilin 2	N141L	Seizures	(Jayadev et al., 2010)

Table 1. Human AD gene mutations leading to elevated Aβ and epilepsy.

There is phenotypic overlap between AD and temporal lobe epilepsy (TLE). For example, increased adult neurogenesis is found in both TLE and early in AD (Jin et al., 2004, Sutula and Dudek, 2007). Additionally, there is a brain region overlap. MRI-based volumetric analysis show atrophy in the lateral temporal and frontal cortices in both AD and TLE patients (Thompson et al., 2003, Bernhardt et al., 2008) and PET imaging shows hypometabolism in inferior temporal regions in both AD and TLE patients (Carne et al., 2007, Edison et al., 2007). Originally, seizures in AD patients were thought to be a late-stage byproduct of neurodegeneration, instead of a contributor of cognitive deficits.

Given this association, the effectiveness of antiepileptic drugs is being examined in AD patients. Levetiracetam and lamotrigine have demonstrated higher antiepileptic efficacy than phenytoin, with levetiracetam being associated with improved cognitive performance and lamotrigine associated with better mood (Belcastro et al., 2007, Cumbo and Ligori, 2010, Vossel et al., 2013). Phenytoin, a sodium channel blocker, was poorly tolerated and worsened cognitive function in multiple studies (Rao et al., 2009, Cumbo and Ligori, 2010, Vossel et al., 2013). Those treated with valproic acid, an HDAC inhibitor, had moderate seizure control but more rapid brain atrophy (Fleisher et al., 2011). Phenobarbital also had negative cognitive side effects (Cumbo and Ligori, 2010). Importantly, the effect of levetiracetam to improve cognition in amnestic MCI was attributed to reduced hippocampal hyperactivity by fMRI (Bakker et al., 2012).

Network dysfunction appears to be due to early Aβ-induced hyperexcitability of neurons. While neuronal activity declines in the later stages of AD, a variety of evidence indicates that it is increased in the early stages of the disease. The hippocampus is seen to be hyperactive in functional imaging studies in MCI (Dickerson et al., 2005), the earliest

symptomatic stage of AD, and even in the asymptomatic preclinical stage of AD (Reiman et al., 2012). Cortical neurons in early AD patients are hyperexcitable by transcranial magnetic stimulation (Olazarán et al., 2010). Seizures and subclinical epileptiform activity are also more frequent in early AD, including mild cognitive impairment (Palop and Mucke, 2009, Vossel et al., 2013).

Mouse Models of AD

As previously mentioned, autosomal dominant AD accounts for relatively few cases of AD. But similarity between the phenotypes of sporadic and autosomal dominant AD, allow genetic mutations expressed in mouse models to be suitable and relevant. Many mouse models overexpress human APP with different promoters, isoforms and mutations mentioned such as PDAPP, J20, Tg2576, TASD41, TgCRND8 and APP23 (Games et al., 1995, Calhoun et al., 1998, Mucke et al., 2000, Chishti et al., 2001, Rockenstein et al., 2001, Westerman et al., 2002). Some mouse models overexpress both hAPP and PS1 mutations such as PSAPP, APP/PS1dE9 and 5XFAD (Holcomb et al., 1998, Jankowsky et al., 2004, Oakley et al., 2006). Also some mouse models overexpress APP, PS1 and Tau with mutations such as TAPP and 3xTg (Lewis et al., 2001, Oddo et al., 2003). Although these are not perfect models of AD because they combine mutations causing AD and frontotemporal dementia (FTD), they have been used to examine tau pathology. Overexpression of PS1 mutations alone doesn't result in a phenotype due to the differences in mouse and human Aβ (Jankowsky et al., 2007). Some models are based on a knockin of the human ApoE gene. ApoE4 knockin mice have higher Aβ and cognitive, synaptic plasticity and network impairments compared with apoE3 knockin mice (Fryer et al., 2005, Bales et al., 2009). These many mouse models recapitulate the

phenotypes of AD to difference extents (Hall and Roberson, 2012). For instance, hAPP transgenic mice show cognitive deficits and Aβ plaques. Human tau is required for neurofibrillary tangles and neurodegeneration in the mouse model.

Line	Transgene	Background	Seizure observation	Reference
Tg2576	Human APP_{SWE}	FVB/N	Generalized tonic-clonic seizures, occasionally terminated with sudden death	(Hsiao et al., 1995)
	Murine $A\beta_{42}$	FVB/N	Generalized tonic-clonic seizures, reduced life span	(LaFerla et al., 1995)
	Human APP/RK	FVB/N	Brief seizures of variable severity in young mice, extended generalized tonic-clonic seizures in old mice	(Moechars et al., 1996)
	Human APP/RK	C57BL	Very rare seizure incidence	(Moechars et al., 1996)
APP/Fl APP/Du	Human APP_{Fl} Human APP_{du}	FVB/N	Mild to severe tonic-clonic seizure in 15% of mice older than 6 month	(Kumar-Singh et al., 2000)
APP23	Human APP_{SWE}	C57BL/6J	Tonic-clonic seizures in 40% of mice	(Lalonde et al., 2005)
hAPPJ20	Human $APP_{SWE,Ind}$	C57BL/6J	Frequent generalized- sharp- synchronous-electrographic discharges; intermittent nonconvulsive; electroencephalographic seizures	(Palop et al., 2007b)
APPswe/PS1dE9	Human APP_{SWE} + PS1dE9	C57BL/6J	65% mice had at least one electrographic seizure in 3 weeks	(Minkeviciene et al., 2009)
	Human APP intracellular domain + Fe65	C57BL/6J	Increased electrographic spikes	(Vogt et al., 2011)

Table 2. Epileptiform activity in hAPP/Aβ mouse models. Adapted with permission from Dr. Zhiyong Li.

The plethora of mouse models that overexpress APP/Aβ and show network dysfunction is a strong indication that the phenotype is not a model artifact. Epileptiform activity or seizures appear on different background strains and with different mutations in human APP with or without PS1 (Table 2). This allows us to use mouse models to dissect the Aβ-induced network dysfunction at the level of circuit, cellular, synaptic, and molecular changes.

The mouse model used for the work described in this dissertation, hAPPJ20 mice, expresses the human APP minigene with Swedish and Indiana mutations under control of the PDGF promoter (Mucke et al., 2000). The PDGF is neuronal specific and is expressed in the hippocampus, cortex and brainstem (Sasahara et al., 1991). hAPPJ20 mice show spiking on EEG as early as 2.5 months and behavioral dysfunction at 3 months. They get their first Aβ plaques around 4 months, and at 6 months have mild plaque deposition (Mucke et al., 2000). They additionally show increased drug-induced seizure susceptibility (Palop et al., 2007a).

Hypothesis of AD-Induced Network Dysfunction

Synaptic dysfunction

The current hypothesis of Alzheimer's disease, termed the "amyloid hypothesis," says that high levels of soluble Aβ act at the synapse to cause synaptic failure and cognitive dysfunction (Selkoe, 2002, Tanzi, 2005). AD originally was thought to be caused by the Aβ plaques (Hardy and Higgins, 1992) but strong evidence has implicated soluble Aβ oligomers as the culprits (Hsia et al., 1999) (Kuo et al., 1996, Lue et al., 1999, McLean et al., 1999). This is also supported by findings that loss of synapses, not plaques, correlates more consistently with cognitive dysfunction in AD patients (Terry et al., 1991). The involvement of Aβ-induced synaptic dysfunction has become more accepted (Selkoe, 2002, Tanzi, 2005). More recently the Aβ hypothesis has incorporated the permissive role of tau; due to repeatable findings that Aβ-induced dysfunction requires tau (Palop and Mucke, 2010, Zempel and Mandelkow, 2014). Additionally the network dysfunction is becoming recognized as important (Palop and Mucke, 2010).

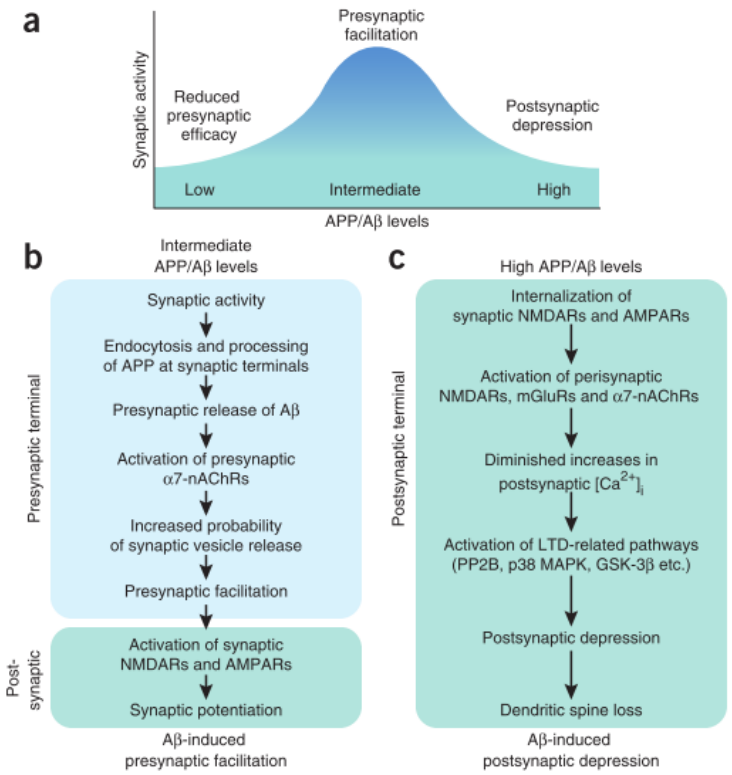

Figure 1. Presynaptic and postsynaptic regulation of synaptic transmission by Aβ. (a) Hypothetical relationship between Aβ level and synaptic activity. Intermediate levels of Aβ enhance synaptic activity presynaptically, whereas abnormally high or low levels of Aβ impair synaptic activity by inducing postsynaptic depression or reducing presynaptic efficacy, respectively. (b) Within a physiological range, small increases in Aβ primarily facilitate presynaptic functions, resulting in synaptic potentiation (c) At abnormally high levels, Aβ enhances LTD-related mechanisms, resulting in postsynaptic depression and loss of dendritic spines. Used with permission from (Palop and Mucke, 2010)

A lot of information can be gleaned about how the dysfunction is induced by Aβ by first understanding the normal function of Aβ at the synapse as a feedback loop for synaptic activity (Figure 2 and (Kamenetz et al., 2003)). Synaptic activity causes the release of Aβ, which causes presynaptic facilitation dependent on activation of α7-nicotinic receptors and calcium buildup which increases release probability (Figure 2b and (Kamenetz et al., 2003, Puzzo et al., 2008, Abramov et al., 2009)). But higher

activity, and thus higher levels of Aβ release, induces post-synaptic depression (Kamenetz et al., 2003, Hsieh et al., 2006, Shankar et al., 2007). Pathologically high levels of Aβ would thus drive suppression of synaptic activity post-synaptically (Figure 2c and (Kamenetz et al., 2003, Snyder et al., 2005, Shankar et al., 2007, Li et al., 2009)). Pathologically high levels of Aβ, potentially through impaired glutamate reuptake or desensitization of synaptic NMDA receptors, shift glutamate activation toward perisynaptic receptors (NMDAR and mGluR) and LTD mechanisms (Liu et al., 2004, Snyder et al., 2005, Hsieh et al., 2006). LTP is suppressed and LTD is enhanced (Kim et al., 2001, Walsh et al., 2002, Hsieh et al., 2006, Lesné et al., 2006, Li et al., 2009). There are many changes occurring within the dendrite (Figure 3 and (Cochran et al., 2014)).

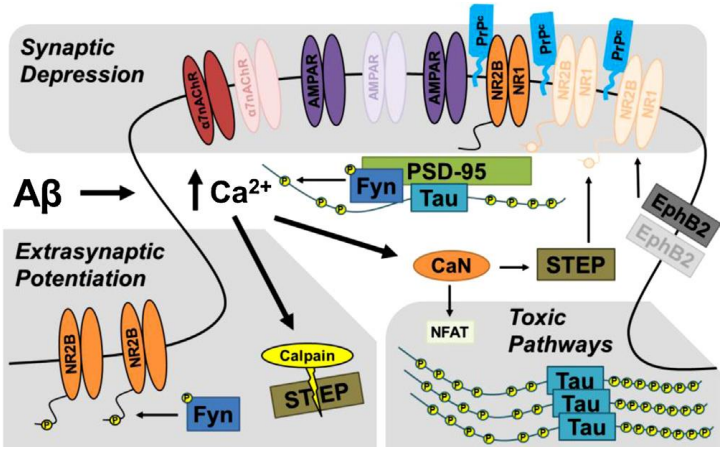

Figure 2. Key mechanisms of dendritic abnormality in AD. Aβ can cause a transient rise in calcium through α7nACh and NMDA receptors, leading to a transient synaptic potentiation, followed by synaptic depression mediated by STEP activation and EphB2 depletion (Snyder et al., 2005, Cisse et al., 2011a, Um et al., 2012). Loss of receptors at the synapse is indicated by light shading. Extrasynaptically, calpain can cleave STEP, allowing activated Fyn to keep NR2B-containing NMDA receptors at the surface, leading to extrasynaptic potentiation (Xu et al., 2009, Li et al., 2011a). These events can also lead to toxic CaN/NFAT signaling and phosphorylation of tau (Wu et al., 2010, Larson et al., 2012). Used with permission from (Cochran et al., 2014).

The mechanisms for how Aβ induces synaptic depression have received much attention. Aβ has been shown to bind a host of receptors at the synapse that together could induce synaptic depression. For example, Aβ induces degradation of EphB2, the NMDAR recruiting receptor (Cisse et al., 2011a). Aβ binds, clusters and enhances mGluR5 activation and binds and activates NMDARs (Renner et al., 2010). Downstream mechanisms involve increased calcium in spines and dendrites, activation of calcineurin, transcription factor NFAT and phosphorylation of tau (Wu et al., 2010, Larson et al., 2012). Calcineurin activates STEP which causes synaptic NMDAR internalization (Snyder et al., 2005, Um et al., 2012) which has been shown to be α7nAChR dependent (Snyder et al., 2005). Fyn enhances extrasynaptic NMDAR surface expression (Xu et al., 2009, Li et al., 2011a). Tau phosphorylation in the microtubule domain is associated with its mislocalization to dendrites (Zempel et al., 2010, Zempel and Mandelkow, 2012). Ultimately, these changes result in loss of synapses and dendritic spines (Mattson et al., 1992, Demuro et al., 2005, Wu et al., 2010, Zempel et al., 2010, Hudry et al., 2012).

Even small chronic increases in Aβ can lead to synaptic depression (Abramov et al., 2009). For the remaining circuit to continue functioning normally in spite of synaptic depression of many neurons, the patterns of neuronal activity in circuit changes (Palop et al., 2006). In the cortex and hippocampus of APP/PS1 mice, what likely occurs is many neurons are functionally impaired and have decreased activity and the remaining neurons become hyperactive to compensate (Figure 4 and (Busche et al., 2008, Busche et al., 2012)).

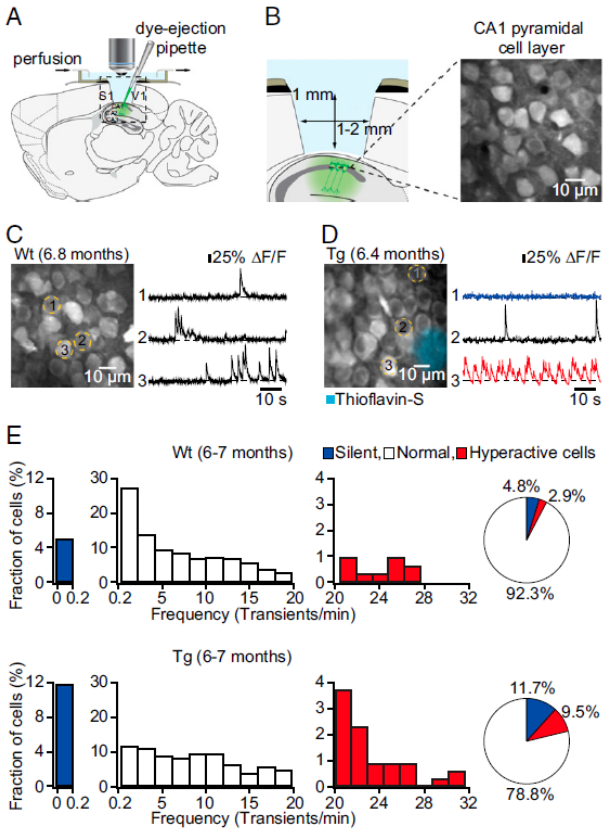

Figure 3. Altered neuronal activity in hippocampus of plaque-bearing transgenic mice. ***A***, Schematic of the experimental preparation for in vivo imaging. ***B,*** (Left) Detailed view of the boxed region in ***A***. (Right) In vivo image of a CA1 pyramidal cell layer. ***C,*** (Left) CA1 neurons imaged in vivo in a wild-type mouse. (Right) Spontaneous Ca2+ transients of the corresponding neurons marked (Left). ***D***, (Left) CA1 neurons imaged in vivo in a transgenic (Tg) mouse with thioflavin-S–positive plaques (light blue). (Right) Spontaneous Ca2+ transients of the corresponding neurons marked (Left). Traces are color-coded to mark neurons that were either silent (blue) or hyperactive (red) during the recording period. ***E***, Histograms showing the frequency distribution of Ca2+ transients in wild-type (Upper; n = 312 cells in five mice) and Tg (Lower; n = 349 cells in five mice) mice. Note increased fractions of silent and hyperactive neurons in Tg mice. Pie charts show the relative proportions of silent, normal, and hyperactive neurons. Used with permission from (Busche et al., 2012).

Aβ-induced neuronal hyperexcitability induces compensatory responses in the hippocampal circuit. In vivo imaging of neuronal activity patterns confirms early increases in hippocampal neuron firing attributable to soluble Aβ (Figure 4 and (Busche et al., 2012)). In the hippocampus, inhibitory remodeling occurs such as NPY positive

GABAergic interneuron sprouting in the molecular layer of the dentate gyrus and depletion of calcium- and activity-regulated proteins calbindin, arc, and Fos (Palop et al., 2003, Palop et al., 2007a). Consistent with GABAergic interneuron sprouting, dentate granule cells have increased frequency and increased large-amplitude mIPSCs (Palop et al., 2007a). This might further contribute to synaptic inhibition and synaptic plasticity deficits (Palop et al., 2007a). Although similar, it is important to note that the hippocampal remodeling does not fully replicate that seen in temporal lobe epilepsy, indicating that there must be changes induced by Aβ distinct from changes in TLE (Palop et al., 2007a). For example, hippocampal changes in mice with kainic acid-induced epilepsy induced excitatory sprouting of collateral mossy fibers in the inner molecular layer, whereas hAPPJ20 mice showed inhibitory sprouting (Palop et al., 2007a).

GABAergic dysfunction

In the hippocampus, Aβ induces synaptic depression as well as hyperactivity (Busche et al., 2012). Patch-clamp recordings from DG cells in hAPPJ20 mice show excitation/inhibition imbalance, with a shift toward more excitatory activity (Roberson et al., 2011). Part of the excitation/inhibition imbalance seems to be GABAergic interneuron dysfunction. hAPPJ20 mice have reduced action potential–driven inhibitory synaptic activity (sIPSCs) (Roberson et al., 2011). Nav1.1 protein is decreased in parvalbumin-positive GABAergic interneurons in the parietal cortex of hAPPJ20 mice and human AD parietal cortex (Verret et al., 2012). Nav1.1 is expressed in the somatodendritic compartment and regulates signal integration from the dendrites (Trimmer and Rhodes, 2004). The decrease in Nav1.1 contributes to decreased gamma

oscillations and increased network hypersynchrony. Restoring Nav1.1, using Nav1.1-BAC transgenic mice, partially restores cognitive function (Verret et al., 2012).

Tau Reduction

Tau is a microtubule-associated protein that accumulates in neurofibrillary tangles in AD brains. Recently, new roles for tau have been identified. Tau acts as a scaffolding protein at the dendrite (Morris et al., 2011b) and aberrantly moves into dendrites in the Aβ-induced pathological state (Ittner et al., 2010, Zempel et al., 2010, Frandemiche et al., 2014). It is required for Aβ-induced dysfunction in cultured neurons shown in studies examining Aβ-induced toxicity (Rapoport et al., 2002), Aβ-induced microtubule disassembly (King et al., 2006), Aβ-induced deficits in axon transport (Vossel et al., 2010), as well as Aβ-induced synaptic dysfunction with impairment of long term potentiation (Shipton et al., 2011). It has also been shown to be required for Aβ-induced network and synaptic dysfunction in multiple mouse AD models including hAPPJ20 (Roberson et al., 2007), APP23 (Ittner et al., 2010), apoE4 knock-in (Andrews-Zwilling et al., 2010), hAPPJ9/Fyn and TASD41/Fyn (Roberson et al., 2011). Tau reduction produces resistance to seizures and epileptiform activity in AD mouse models as well as in mouse and fly models of epilepsy (Devos et al., 2013, Holth et al., 2013, Gheyara et al., 2014). Tau reduction also prevents cognitive deficits associated with streptozotocin-induced type 1 diabetes-like disease (Abbondante et al., 2014). But tau reduction is not nonspecifically neuroprotective since it does not prevent increased mortality in mutant SOD1 mice or cell loss in a Parkinson's disease model (Morris et al., 2011a, Roberson et al., 2011). This indicates that tau contributes to AD-related neuronal hyperexcitability. However, exactly how tau regulates neuronal excitability remains unclear.

Kv4.2 Function

Features

Kv4.2 is a voltage-gated potassium channel that is part of the Shal-type family that also includes Kv4.1 and Kv4.3. These channels are composed of tetramers that form the pore. These three channels are activated at subthreshold potentials and generate a transient outward current, as they inactivate and recover from inactivation quickly (Birnbaum et al., 2004). They are expressed at high levels in the brain, heart and smooth muscle and are responsible for transient outward A-type K+ current in neurons and the Ca+2-independent A-type K+ current in cardiac myocytes (Birnbaum et al., 2004). In the brain, Kv4.2 is predominantly localized to the somatodendritic compartment of neurons and it tends to cluster at postsynaptic GABAergic synapses (Jinno et al., 2005). A-type current is hyperpolarizing and increases along apical dendrites (Kerti et al., 2012). In the apical dendrites Kv4.2 regulates back propagation of action potentials and in terminal dendrite Kv4.2 regulates the initiation of plateau potential or the all or nothing regenerative depolarization by NMDA and voltage-gated calcium conductance (Tang and Thompson, 2012). Knocking out the Kv4.2 gene, KCND2, eliminates A-type currents and enhances induction of long term potentiation (LTP) (Chen et al., 2006).

Regulation

There are multiple auxiliary and accessory subunits that affect both surface membrane levels and channel kinetics of Kv4.2 (recently reviewed in (Jerng and Pfaffinger, 2014). K+ channel-interacting proteins (KChips) are small proteins that bind Kv4.2 on the intracellular side and enhance trafficking, assembly, and stability of Kv4.2 channels and cause faster recovery from inactivation (Jerng and Pfaffinger, 2014).

Dipeptidyl peptidase-like proteins (DPLPs), DPP6 and DPPX bind to the extracellular portion of the Kv4 channels. They facilitate trafficking to increase surface membrane levels up to 18-fold, accelerate recovery from inactivation, and cause a hyperpolarizing shift in activation and inactivation threshold (Jerng and Pfaffinger, 2014). DPP6 is more often expressed with Kv4.2 in pyramidal neurons whereas DPPX more often with Kv4.3 in interneurons (Zagha et al., 2005). Other proposed accessory proteins for Kv4.2 in the brain include Kvβ1, Navβ1, filamin, kinesin, Kif17 and SAP97 (Birnbaum et al., 2004).

Kv4.2 protein and mRNA are both transported into the dendrites and Kv4.2 translation can be activity-dependent. Kif17 is the kinesin that transports Kv4.2 protein into the dendrites by binding to the extreme carboxyl-terminus of Kv4.2 (Chu et al., 2006). In hippocampal neurons, Kv4.2 mRNA was found in the dendrites and its transport into dendrites was microtubule-dependent. Part of the 3'-untranslated region of Kv4.2 mRNA was found to be necessary and sufficient for dendritic localization (Jo et al., 2010).

Kv4.2 is regulated by neuronal activity (Table 3). Kv4.2 phosphorylation by PKA at serine 552, and PKC at carboxyl-terminus serines 447 and 537 and an ERK triple phosphorylation decrease surface membrane levels (Schrader et al., 2009). In contrast, CAMKII phosphorylation at serine 438 and serine 459 increases Kv4.2 surface membrane levels (Varga et al., 2004). NMDA receptor activation is an important regulator of Kv4.2 expression at the membrane surface. The most consistent findings are that synaptic NMDAR activation induces internalization and degradation of Kv4.2. One group found that NMDAR activation induces increased dendritic translation of Kv4.2 (Lee et al., 2011). Interestingly, Kv4.2 current can actually have a bidirectional effect on

NR2B/NR2A composition. Enhanced Kv4.2 current decreases relative synaptic NR2B/NR2A subunits and results in no LTP, while blocking Kv4.2 expression by using a dominant negative for Kv4.2, or Kv4.2 KO mice, show an increased fraction of synaptic NR2B/NR2A and enhanced LTP (Jung et al., 2008).

Model	Induction	Kv4.2 changes	Reference
Dissociated hipp neurons	NMDA, unspecified	Internalization, clathrin-mediated endocytosis	(Kim et al., 2007)
Hipp slice culture	Glycine, LTP		
	Synaptic LTP		
Hipp culture	NMDA, not AMPA	Reduced total Kv4.2 levels	(Lei et al., 2008)
Hipp culture	NMDA, not AMPA	Hyperpolarized shift in the inactivation curve of A-type K(+) currents; activation curve unchanged	
Hipp culture	Synapse, NMDA	No change	(Lei et al., 2010)
	Glutamate + NR2B antagonist	Decrease total Kv4.2, clusters, inhibited by calpain inhibitor	
	Glutamate	Decrease total Kv4.2 levels, clusters, hyperpolarized shift of inactivation curve	
Hipp culture	Synapse, NMDA	Decrease at surface, ERK phosphorylation decrease total Kv4.2 levels	(Mulholland and Chandler, 2010)
	Extrasynaptic, NMDA	ERK dephosphorylation decrease total Kv4.2 levels	
Hipp culture	Synapse, NMDA	Increase mRNA at dendrite and cell body, transport to dendrite	(Jo and Kim, 2011)
Hipp slice	NMDA, unspecified	Kv4.2 degradation, FMRP suppression of Kv4.2 released	(Lee et al., 2011)

Table 3. Activity-dependent regulation of Kv4.2

Role of Kv4.2 in Memory

Kv4.2 is important for learning and memory. Kv4.2 is activated at subthreshold voltages and activation dampens back propagation of action potentials and dendritic excitability (Yuan et al., 2005, Andrásfalvy et al., 2008). Regulation of the timing of synaptic signals and back propagating dendritic APs is critical for synaptic plasticity called spike timing dependent plasticity (STDP) (Ramakers and Storm, 2002, Kim et al., 2005). Regulation of STDP by Kv4.2 affects induction of LTP at active synapses (Watanabe et al., 2002, Chen et al., 2006). LTP requires coincidence between incoming

synaptic binding of glutamate and postsynaptic depolarization, usually within a narrow window. In Kv4.2 knockout mice there is an expanded time window which makes induction of LTP less dependent on the timing of the pre-synaptic and post-synaptic activity (Zhao et al., 2011). Therefore, the induction threshold is lower for LTP and increased for LTD (Zhao et al., 2011).

Kv4.2 knockout mice show spatial deficits in the Morris water maze including longer latencies and distance to reach a hidden platform and no preference for target area (Lugo et al., 2012). This was attributed to an enhanced and stabilized EPSP-spike component of LTP, independent of basal transmission or short-term plasticity which was proposed to decrease signal to noise (Truchet et al., 2012). ICV injection of the Kv4.2 and Kv4.3 inhibitor AmmTX3 impaired reference memory on the radial arm maze (Truchet et al., 2012). Thus, lower levels of Kv4.2 in the hippocampus of hAPPJ20 mice could contribute to learning and memory deficits.

Kv4.2 in Diseases with Hyperexcitability

Mutation or loss of Kv4.2 leads to an epilepsy phenotype in humans. For example, a truncation mutation in the Kv4.2 gene causes temporal lobe epilepsy (Singh et al., 2006) and a missense mutation in Kv4.2 found in twins led to autism and seizures (Lee et al., 2014). Older studies using the voltage-gated K channel blocker, α-dendrotoxin injected into hippocampus of rats resulted in motor and cortical seizures (Bagetta et al., 1992) and similar results occurred with dendrotoxin I and 4-AP (Gandolfo et al., 1989). Kv4.2 knockout mice show increased susceptibility to bicculine and kainate stimulation (Andrásfalvy et al., 2008, Barnwell et al., 2009).

Traumatic brain injury (TBI) is considered a risk factor for AD (Van Den Heuvel et al., 2007). After TBI, APP/Aβ and phosphorylated tau levels increase (Tsitsopoulos and Marklund, 2013). TBI pathology also includes diffuse Aβ plaques and tau tangles (Blennow et al., 2012). Secretase inhibitors are shown to be protective in TBI mouse models (Loane et al., 2009). In the controlled cortical impact, TBI model, controlled cortical impact, Kv4.2 channels and IA current are decreased in the ipsilateral CA1 region (Lei et al., 2012). Antiseizure drugs, including LEV, are being used to treat TBI-induced epileptogenesis (Shetty, 2013).

Fragile X patients have seizures in addition to their cogintive impairment. Fragile X mental retardation protein (FMRP) normally represses translation of dendritic APP mRNA (Sokol et al., 2011). mGluR activation leads to the dislodging of FMRP from APP mRNA, increasing its translation. The Fragile X mouse model, fmr-1 KO, shows increased levels of APP and Aβ (Westmark and Malter, 2007). A cross between fmr-1 KO and an hAPP overexpressing mouse model showed dramatically increased mortality and lower seizure threshold, indicating a potential synergistic effect (Westmark et al., 2008). Changes in Kv4.2 have been examined in the fmr-1 KO mice by two different groups but with opposite results (Gross et al., 2011, Lee et al., 2011). The Lee paper convincingly showed that FMRP represses of Kv4.2 mRNA translation in the dendrites is regulated by NMDAR activation (Lee et al., 2011).

Kv4.2 protein levels have not been looked at in AD patients' brains. In a microarray on AD tissue from CA1 region of the hippocampus, KCND2 is upregulated compared with control tissue (Ginsberg et al., 2012).

Outstanding Questions

- Are the dendrites of hippocampal neurons in an Azheimer's disease mouse model (hAPPJ20) hyperexcitable?
- What causes the dendritic hyperexcitability? Is Kv4.2 decreased in the hippocampus of hAPPJ20 mice?
- What could cause the Kv4.2 depletion? Could epileptiform activity cause depletion of Kv4.2?
- Is Kv4.2 depletion detrimental or protective? Can Kv4.2 deficiency exacerbate behavioral abnormalities and epileptiform activity in Aβ-sensitized mice?

TAU-DEPENDENT KV4.2 DEPLETION AND DENDRITIC HYPEREXCITABILITY IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

by

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Abstract

Neuronal hyperexcitability occurs early in the pathogenesis of Alzheimer's disease (AD) and contributes to network dysfunction in AD patients. In other disorders with neuronal hyperexcitability, dysfunction in the dendrites often contributes, but dendritic excitability has not been studied in AD models. We used dendritic patch-clamp recordings to directly examine dendritic excitability in the CA1 region of the hippocampus. We found that dendrites, but not somata, of hippocampal neurons were hyperexcitable in mice overexpressing Aβ. This dendritic hyperexcitability was associated with depletion of Kv4.2, a dendritically-localized potassium channel important in regulation of dendritic excitability and synaptic plasticity. Epileptiform activity drives the reduction in Kv4.2, and blocking epileptiform activity by tau reduction prevented both Kv4.2 depletion and dendritic hyperexcitability. The dendritic hyperexcitability induced by Kv4.2 depletion exacerbates behavioral deficits induced by Aβ and further increases dendritic excitability, creating a detrimental feedback loop. Therefore, we conclude that Kv4.2 is a dendritic effector downstream of Aβ and that increased dendritic excitability may contribute to neuronal dysfunction in early stages AD.

Introduction

The incidence of Alzheimer's disease (AD) is increasing with the aging population and an astonishing 5.2 million Americans are affected by AD, the most common cause of dementia. Along with synaptic dysfunction, dysregulation of neuronal excitability plays an important role in the pathogenesis of AD. While neuronal activity declines in the later stages of AD, a variety of evidence indicates that it is increased in the early stages of the disease. The hippocampus is found to be hyperactive in functional

imaging studies of patients in the mild cognitive impairment stage of AD (Dickerson et al., 2005), the earliest symptomatic stage, and even in the asymptomatic preclinical stage of AD (Reiman et al., 2012). Cortical neurons in early AD patients are hyperexcitable by transcranial magnetic stimulation (Olazarán et al., 2010). Seizures and subclinical epileptiform activity are also more frequent in early AD stages, including mild cognitive impairment (Palop and Mucke, 2009, Vossel et al., 2013).

The cellular basis of AD-related neuronal hyperexcitability is unclear but many features are replicated in mouse models, enabling mechanistic studies. For example, electrophysiological recordings of spontaneous activity onto hippocampal neurons show excitation/inhibition imbalance, with a shift toward more excitatory activity (Roberson et al., 2011). In vivo imaging of neuronal activity patterns confirms early increases in hippocampal neuron firing attributable to soluble Aβ (Busche et al., 2012). And many mouse models of AD also show subclinical epileptiform activity and corresponding hallmarks of compensatory remodeling such as interneuron sprouting and depletion of calbindin (Palop et al., 2003, Palop et al., 2007).

Dendrites are the location of highly regulated interactions between synaptic signals and intrinsic conductance. Dendritic excitability has an important effects on synaptic plasticity and disorders of learning and memory (Nestor and Hoffman, 2012). For example, aberrant dendritic morphology and alterations in ion channels have been shown to contribute to dendritic hyperexcitability and thus improper integration of synaptic signals in mouse models of Fragile X syndrome, Rett syndrome, autism and epilepsy (Nestor and Hoffman, 2012). Therefore, we asked whether AD-related neuronal hyperexcitability could involve changes in dendritic excitability.

Results

We first determined if the dendrites in a mouse model of AD are hyperexcitable. Because of dendrites' electrotonic properties, accurately measuring their excitability requires directly recording from their membranes; recordings from the soma do not reflect dendritic potentials. We measured dendritic excitability in the hAPPJ20 mouse model of AD by patch-clamp recordings on apical dendrites of CA1 pyramidal neurons, approximately 150 μm from the soma. Action potentials (APs) induced in the axon travel retrogradely into the dendrites depending on the excitability of dendritic membranes. The amplitude of these back-propagating dendritic APs provides a standard measure of dendritic excitability (Magee et al., 1998).

Dendritic AP amplitude was increased by more than 50% in hAPPJ20 mice compared to wild type controls (Fig. 1), indicating dendritic hyperexcitability. To determine if this hyperexcitability was a general property of the cell, we measured AP firing properties (AP onset, threshold, amplitude, half-width, AHP amplitude and AP number) at the soma and found no differences (Table 1). Thus, somatic excitability of hippocampal neurons in hAPPJ20 mice is normal, while the dendrites are hyperexcitable, suggesting that AD-related neuronal hyperexcitability originates in dendrites.

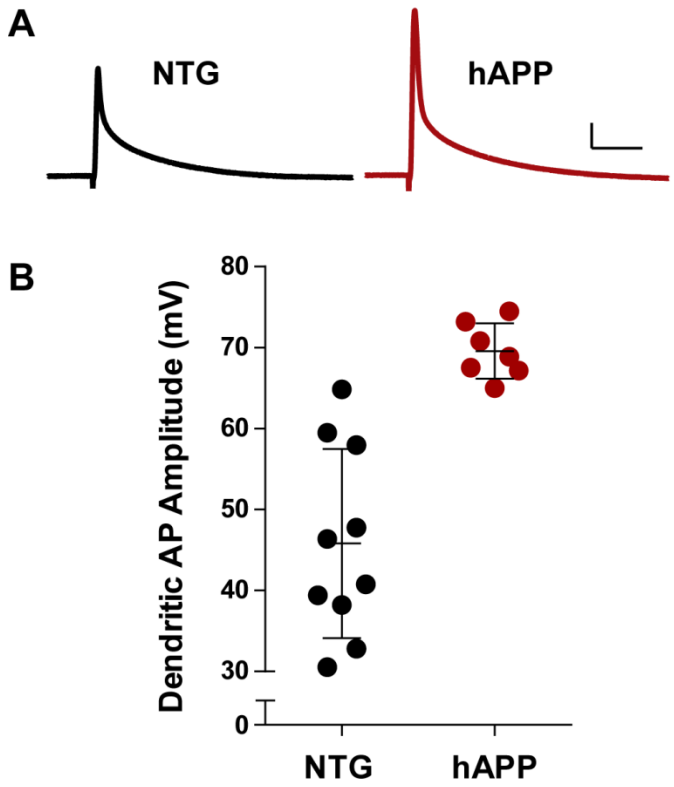


Figure 1. Increased dendritic excitability in hAPP mice. ***A***, Representative back-propagating dendritic action potentials (APs) recorded in apical dendrites of CA1 pyramidal neurons in nontransgenic (NTG) and hAPPJ20 mice. Scale bars: 10 mV, 12.5 ms. ***B***, Dendritic AP amplitude was increased by 52 ± 3% in hAPPJ20 mice, indicating increased dendritic excitability (t-test: $P < 0.0001$; n = 7–10 per genotype; age 6–9 month).

	NTG (n = 31)	**hAPPJ20 (n= 23)**
AP onset (ms)	18.57 ± 1.45	18.52 ± 1.46
AP threshold (mV)	-46.10 ± 0.65	-46.13 ± 0.46
AP amplitude (mV)	96.78 ± 1.00	96.37 ± 1.04
AP half-width (ms)	0.76 ± 0.12	0.73 ± 0.13
AHP amplitude (mV)	6.85 ± 0.47	7.48 ± 0.45
AP number	34.84 ± 1.38	32.99 ± 1.15

Table 1. Somatic firing properties were calculated from the first action potential (AP) elicited upon a 1 S, +200 pA current injection in whole-cell current-clamp recordings from the soma of non-transgenic (NTG) and hAPPJ20 mice. No significant differences were found between genotypes. Neurons were held at –65 mV and series resistance was monitored between each trial. 3 trials were recorded per cell and the results were averaged by cell. Threshold was defined as the point when the slope reached 10 mV/ms. AP onset was the time between initiating the current injection and threshold. Maximum after-hyperpolarization potential (AHP) and peak AP amplitude were measured relative to threshold, and AP half-width was measured at 50% maximum amplitude. AP number is the total number of APs elicited by the current injection.

We next probed the molecular mechanisms underlying dendritic hyperexcitability in hAPPJ20 mice. Dendritic APs are regulated by several voltage-gated ion channels that are selectively expressed in dendrites (Jinno et al., 2005). In hippocampal neurons, the primary dendritic ion channels regulating dendritic APs are Kv4.2 potassium channels and voltage-gated Na^+ channels (Frick and Johnston, 2005). Alterations in voltage-gated Na^+ channel levels were unlikely to mediate the effect, as the maximal rate of dendritic AP rise, an indication of maximal Na^+ conductance, was normal (data not shown) and a prior study had found no changes in voltage-gated Na+ channels in the hippocampus of hAPPJ20 mice (Verret et al., 2012).

On the other hand, Kv4.2 levels were reduced in area CA1 of hAPPJ20 mice, an effect consistent with increased dendritic excitability (Fig. 2A). A similar pattern of Kv4.2 reduction was observed in the dentate gyrus (Fig. 2B). This was not a generalized effect on dendritic ion channels, as levels of HCN1 and HCN2 channels, which are also selectively expressed in dendrites, were unchanged (Fig. 2A,B). Levels of Kv4.2 mRNA were decreased in both CA1 and DG (Fig. 2C), indicating that Kv4.2 depletion occurs due to downregulation of gene expression. We next asked whether these changes were

widespread in the brain. Kv4.2 levels were normal in several cortical regions (Fig. 2D), suggesting hippocampal specificity. Finally, we determined if similar changes were observed in other mouse models of AD, and found a similar decrease in Kv4.2 in hAPPswe/PS1dE9 mice (Fig. 2E).

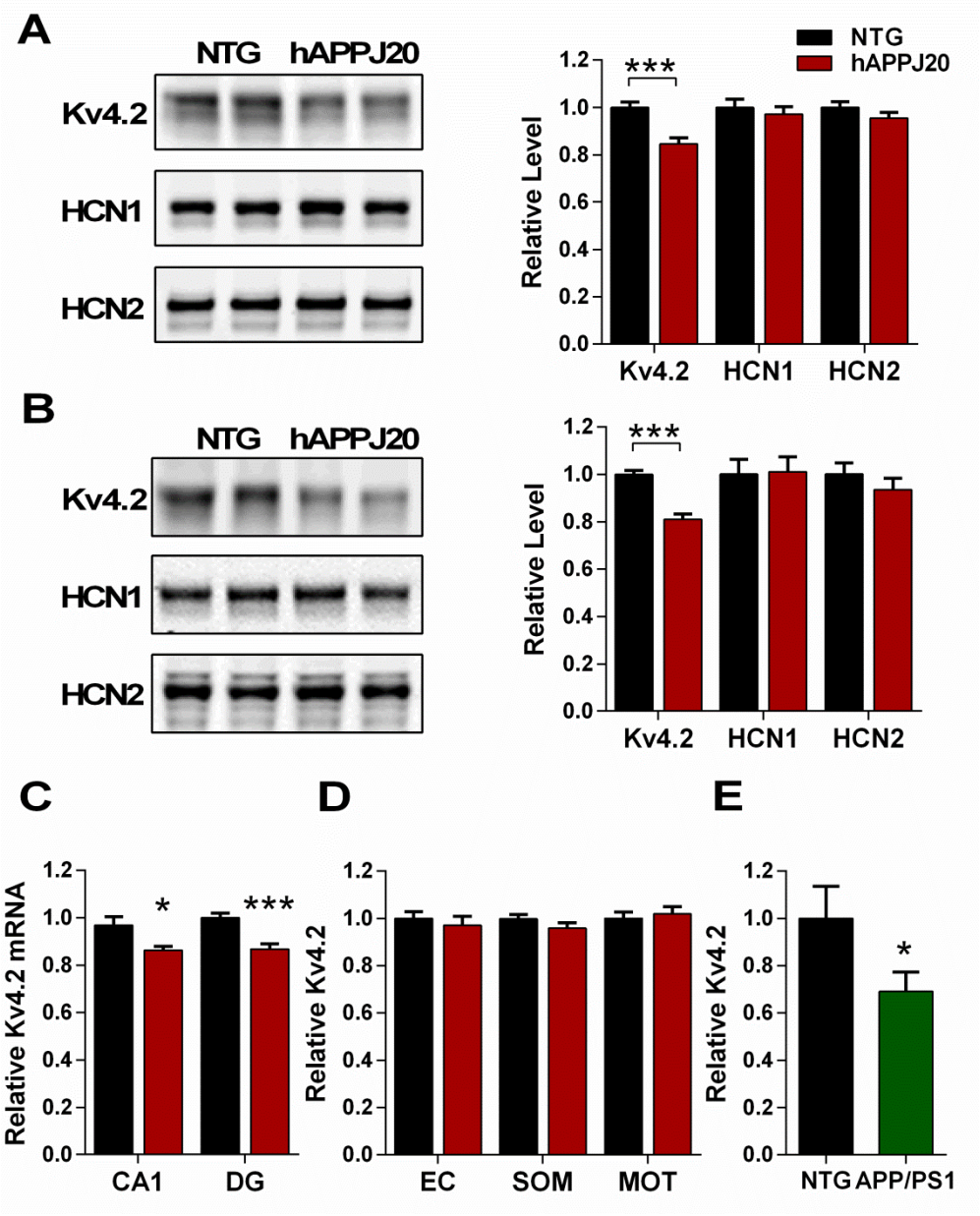
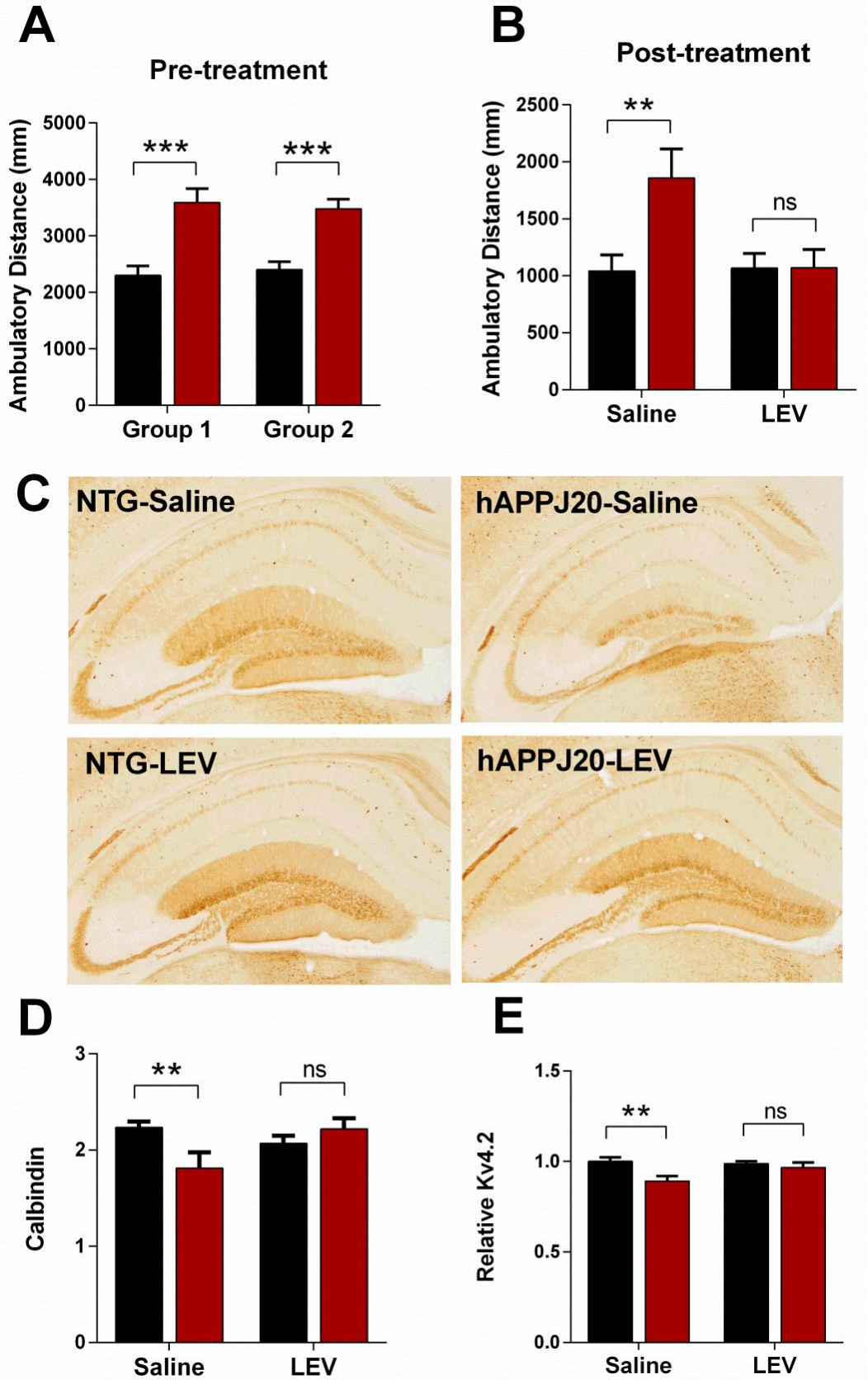


Figure 2. Kv4.2 is depleted in hippocampal regions in hAPPJ20 mice. A, Immunoblots for dendritic ion channels in area CA1. Kv4.2 was decreased in hAPPJ20 mice (ANOVA: hAPP x Channel interaction, $P < 0.05$, *** $P < 0.0001$ on post hoc test; n = 21–38 per genotype; age 4–6 months). B, Immunoblots for dendritic ion channels in dentate gyrus. Kv4.2 was decreased in hAPPJ20 mice (ANOVA: hAPP x Channel interaction, $P < 0.05$, *** $P < 0.0001$ on post hoc test; n = 13–71 per genotype; age 4–6 months). C, Kv4.2 mRNA levels were decreased in area CA1 and DG of hAPPJ20 mice (* $P < 0.05$ by one-tailed t-test; *** $P < 0.0001$ by one-tailed t-test; n = 26–32 per genotype for each region). D, Kv4.2 levels were unchanged in cortex in hAPPJ20 mice. EC, entorhinal cortex; SOM, somatosensory cortex; MOT, motor cortex (n = 25–37 per genotype for each region). E, Kv4.2 is depleted in the hippocampus of hAPPswe/PS1dE9 mice. Representative immunoblots and quantification of Kv4.2 in hAPPswe/PS1dE9 mice (t-test, n = 5-7 per genotype; age 6 months).

To determine why Kv4.2 is depleted in hAPPJ20 mice, we considered factors known to influence Kv4.2 expression, including epileptiform activity and seizures (Tsaur et al., 1992, Pei et al., 1997, Bernard et al., 2004, Lugo et al., 2008, Aronica et al., 2009) which occur in hAPPJ20 mice (Palop et al., 2007). To determine if epileptiform activity contributes to Kv4.2 depletion, we used levetiracetam (LEV), an antiepileptic drug that has been shown to block epileptiform activity and behavioral abnormalities in hAPPJ20 mice (Sanchez et al., 2012). First, we pretested mice before LEV treatment to ensure that both saline and LEV-treated groups were comparable at baseline (Fig. 3A). Mice were implanted with osmotic pumps and treated with saline or LEV for 28 days. We confirmed the prior findings of Sanchez et al. (2012) that LEV corrects behavioral abnormalities in hAPPJ20 mice (Fig. 3B) and blocks calbindin depletion (Fig. 3C,D), which serves as an indicator of epileptiform activity in hAPPJ20 mice (Palop et al., 2011). When we measured Kv4.2 levels, we found that LEV treatment blocked the Kv4.2 depletion in hAPPJ20 mice (Fig. 3E). We conclude that epileptiform activity contributes to Kv4.2 depletion in hAPPJ20 mice.

Tau reduction is also known to have anti-epileptic properties (Roberson et al., 2007, Ittner et al., 2010, Roberson et al., 2011, Devos et al., 2013), so we examined whether the changes in Kv4.2 and dendritic excitability were tau-dependent by crossing hAPPJ20 mice with *Tau*$^{-/-}$ mice. Using dendritic patch-clamp recordings on CA1 pyramidal neurons, we confirmed the increased dendritic excitability in hAPPJ20/*Tau*$^{+/+}$ mice (Fig. 4B), which have normal tau levels and are genetically identical to the hAPPJ20 mice in Fig. 1. However, in hAPPJ20/*Tau*$^{-/-}$ mice, dendritic APs were normal (Fig. 4A,B), indicating that tau is required for the increase in dendritic excitability. Similarly, loss of Kv4.2 was observed in hAPPJ20/*Tau*$^{+/+}$ but not in hAPPJ20/*Tau*$^{-/-}$ mice, both in area CA1 (Fig. 4C) and in dentate (Fig. 4D). Thus, tau is required for Aβ-induced dendritic hyperexcitability and loss of Kv4.2.


A
Pre-treatment
Ambulatory Distance (mm)
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Saline
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1.5
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Figure 3. Leveteracitam (LEV) blocks Kv4.2 depletion in hAPPJ20 mice. ***A***, hAPPJ20 mice showed increased ambulatory distance in open field when pretested before LEV treatment (t-test P<0.0001; n=21-23; 4-4.5 months). ***B***, After 18 days of LEV treatment, hAPPJ20 mice show normal ambulatory distance (ANOVA: hAPPx Treatment interaction, P<0.05, **P<0.01 in post-hoc test; n=10-11; 5-5.5 months). ***C***, Representative images of calbindin after 28 days of LEV treatment. ***D***, Quantification of calbindin after 28 days of LEV treatment. hAPPJ20 mice treated with LEV show normal levels of calbindin (ANOVA: hAPPx Treatment interaction, P<0.05, **P<0.01 in post hoc test; n=10-11; 5-5.5 months). ***E***, Quantification of Kv4.2 after LEV treatment for 28 days. LEV blocks the Kv4.2 depletion in the hAPPJ20 mice. Representative blots and quantification of Kv4.2 in the DG of hAPPJ20 mice. (ANOVA: hAPP effect, P<0.05, **P<0.01 in post-hoc test; n=10-11; ages 5-5.5 months).

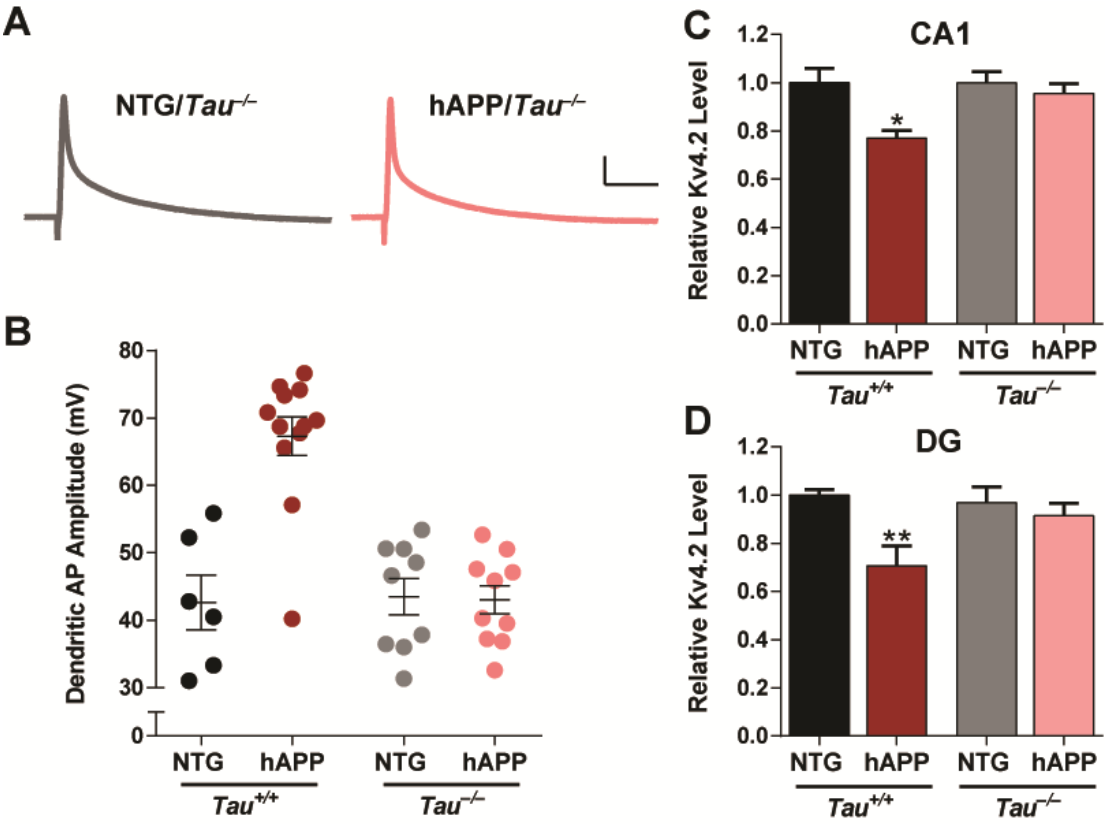

Figure 4. Tau is required for Aβ-induced increase in dendritic excitability and loss of Kv4.2 in hAPPJ20 mice. ***A***, Representative back-propagating dendritic APs recorded in apical dendrites of CA1 pyramidal neurons from *Tau*$^{-/-}$ mice. Scale bars: 10 mV, 12.5 ms. ***B***, Dendritic AP amplitude is increased in hAPPJ20/*Tau*$^{+/+}$ mice, but not in hAPPJ20/*Tau*$^{-/-}$ mice (ANOVA: hAPP x Tau interaction, P = 0.001; on *post hoc* tests, hAPPJ20/*Tau*$^{+/+}$ differs from all other groups, P < 0.0001; n = 6–12

per genotype; age 6–9 months). ***C***, Tau reduction blocks the loss of Kv4.2 in area CA1 (ANOVA: $P = 0.02$; * on post hoc tests, hAPP/*Tau*$^{+/+}$ differs from other groups, $P = 0.01$; $n = 6–14$ per genotype; age 5.5–7.5 months). ***D***, Tau reduction blocks the loss of Kv4.2 in the dentate gyrus (ANOVA: $P = 0.01$; ** on post hoc tests, hAPP/*Tau*$^{+/+}$ differs from other groups, $P = 0.005$; $n = 9–13$ per genotype; age 5.5–7.5 months).

If epileptiform activity is driving changes in Kv4.2 and dendritic excitability, these changes could be either part of a compensatory response or part of positive feedback loop. To determine if loss of Kv4.2 is protective or detrimental in hAPP mice, we crossed Kv4.2-deficient mice with hAPPJ9 mice. hAPPJ9 mice express lower levels of the same minigene of human APP with mutations as the hAPPJ20 mice, and they have no overt phenotype but they are sensitized to Aβ-induced dysfunction (Mucke et al., 2000, Roberson et al., 2011). We predicted that if Kv4.2 depletion were detrimental, hAPPJ9/Kv4.2$^{-/-}$ mice would have greater neuronal dysfunction than hAPPJ9/Kv4.2$^{+/+}$ mice with normal Kv4.2 levels. Consistent with this hypothesis, hAPPJ9/Kv4.2$^{-/-}$ mice had increased exploratory activity and spend more time in the center of the open field, changes also seen in hAPP20 mice (Fig. 5A,B). We found that in the elevated plus maze, hAPPJ9/Kv4.2$^{-/-}$ mice had more total entrances and spent a higher percent of their time in the open arms compared with the hAPPJ9 mice (Fig. 5C,D). Therefore, Kv4.2 deficiency exacerbates behavioral abnormalities in the hAPPJ9 mice.

We also wanted to know whether Kv4.2 deficiency could exacerbate epileptiform activity in hAPPJ9 mice, so we recorded electroencephalograms (EEGs). We found that hAPPJ9/Kv4.2$^{-/-}$ mice had a significant increase in epileptiform spiking compared to NTG mice (Fig 6A,B). Thus, Kv4.2 depletion in the hippocampus of hAPPJ20 mice could be contributing to the behavioral abnormalities and epileptiform activity.

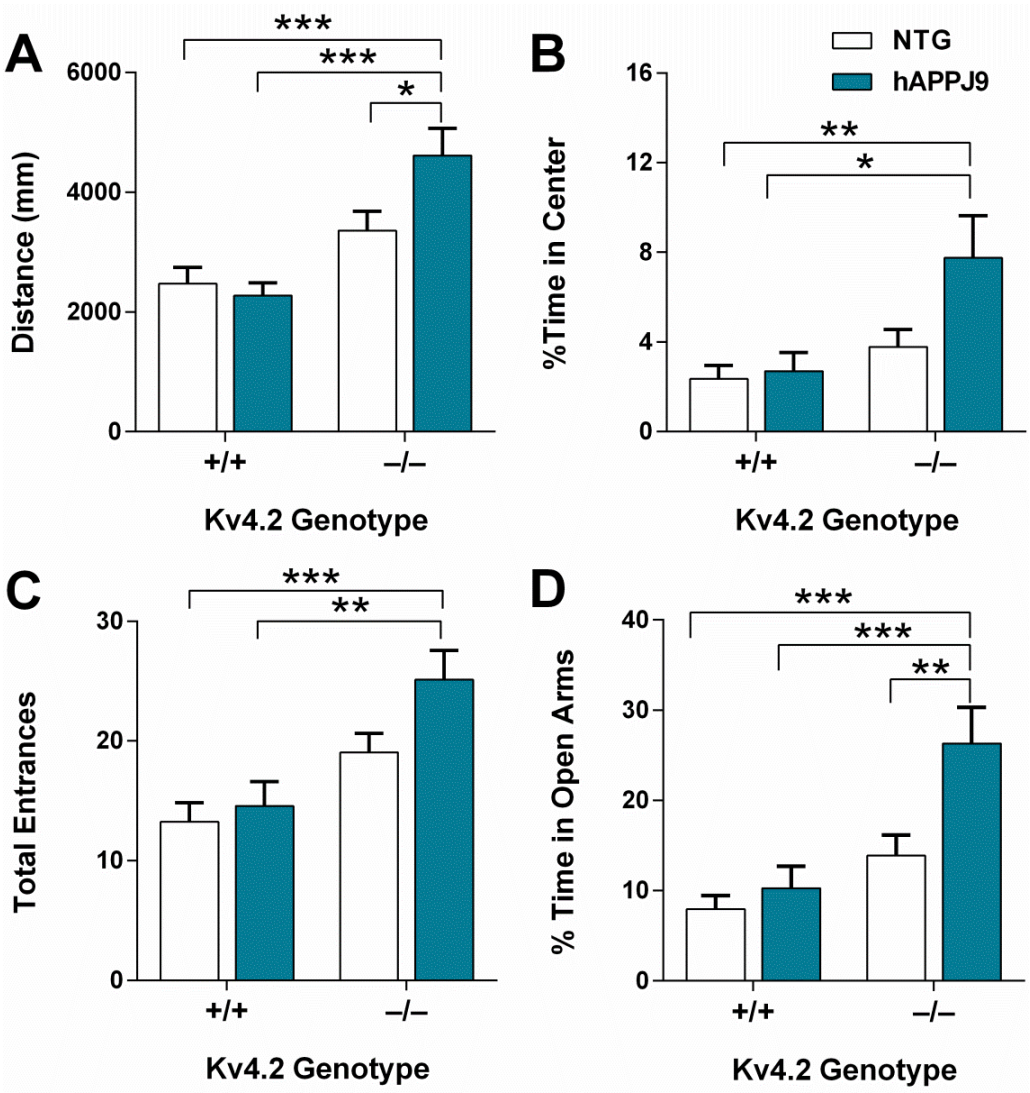

Figure 5. Kv4.2 deficiency exacerbates behavioral abnormalities in the open field and elevated plus maze tests in the low hAPP-expressing (hAPPJ9) mice. ***A***, In the open field test, Kv4.2$^{-/-}$/hAPPJ9 mice show increased ambulatory distance (mm) compared with the hAPPJ9 group. (ANOVA: hAPPxKv4.2 interaction, P<0.05; *P<0.05 and ***P<0.0001 on post hoc test; n=20-22; 5.5-7.5 months) ***B***, In open field test, hAPPJ9/Kv4.2$^{-/-}$ show increased % time in the center compared with the hAPPJ9 mice (ANOVA: Kv4.2 effect, P<0.01; *P<0.05 and **P<0.01 on post hoc test; n=20-22, 5.5-7.5 months). ***C***, In the elevated plus maze test, hAPPJ9/Kv4.2$^{-/-}$ mice showed increased total entrances compared with the hAPPJ9 mice. (ANOVA: Kv4.2 effect, P<0.0001; **P<0.01 and ***P<0.001 on post hoc test, n=20-22, 5.5-7.5 months) ***D***, In elevated plus maze test, hAPPJ9/Kv4.2$^{-/-}$ mice showed increased % time in the open arms compared with the hAPPJ9 mice. (ANOVA: hAPP effect P<0.0001; Kv4.2 effect P<0.001; **P<0.01 and ***P<0.001 on post hoc test, n=20-22, 5.5-7.5 months).

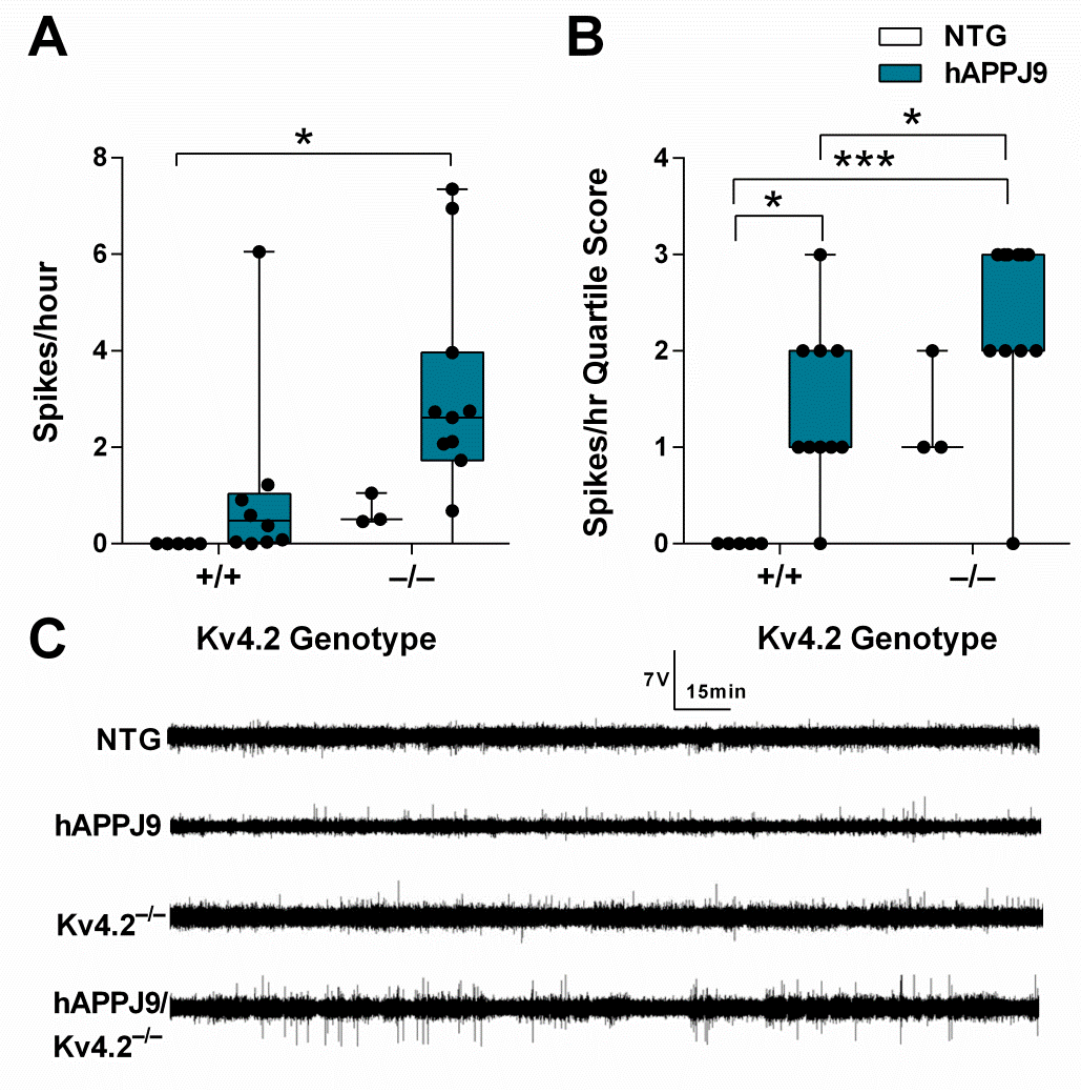

Figure 6. Kv4.2 deficiency exacerbates epileptiform activity in low hAPP-expressing (hAPPJ9) mice. ***A***, Electroencephalography (EEG) performed on hAPPJ9 mice crossed with K4.2$^{-/-}$ mice shows hAPPJ9/Kv4.2$^{-/-}$ mice as the only group with a significant increase in spikes/hour from NTG (ANOVA: hAPP effect, P<0.05; *P<0.05 on post hoc test; n=3-11, 10-12 months). ***B***, Quartile transformation of EEG results show hAPPJ9/Kv4.2$^{-/-}$ mice have a significant increase in spikes/hr compared to hAPPJ9 mice (ANOVA: hAPP effect, P<0.001, Kv4.2 effect P<0.001; *P<0.05 post hoc test, ***P<0.0001, n=3-11, 10-12 months). ***C***. Representative traces from the four genotypes of mice: NTG, hAPPJ9, Kv4.2$^{-/-}$ and hAPPJ9/Kv4.2$^{-/-}$.

Discussion

In this study, we asked whether AD-related neuronal hyperexcitability involves changes in dendritic excitability. We showed that Aβ-induced hyperexcitability originates in the dendrites in hippocampal neurons and that dendritic hyperexcitability is associated with reduction in the level of Kv4.2 potassium channels. Epileptiform activity drives the reduction in Kv4.2, and blocking epileptiform activity by tau reduction prevented both Kv4.2 depletion and dendritic hyperexcitability. The dendritic hyperexcitability induced by Kv4.2 depletion exacerbates behavioral deficits induced by Aβ and further increases dendritic excitability, creating a detrimental feedback loop.

Kv4.2 is predominantly localized to distal dendrites (Jinno et al., 2005) and has a hyperpolarizing current, so its activation dampens back propagation of action potentials and dendritic excitability (Yuan et al., 2005, Andrásfalvy et al., 2008). Regulation of the timing of synaptic signals and back propagating dendritic APs is critical for a form of synaptic plasticity called spike timing dependent plasticity (STDP) (Ramakers and Storm, 2002, Kim et al., 2005). Regulation of STDP by Kv4.2 affects induction of LTP at active synapses (Watanabe et al., 2002, Chen et al., 2006). Kv4.2 knockout mice, which have increased dendritic APs, have impaired learning and memory (Lugo et al., 2012). Thus, lower levels of Kv4.2 in the hippocampus of hAPPJ20 mice might contribute to learning and memory deficits.

It is increasingly apparent that dendrites are a key site of AD pathophysiology. Aβ reduces dendritic complexity, induces spine loss, and causes aberrant dendritic signaling through its direct binding to dendritic receptors and downstream effectors (reviewed in Cochran et al., 2014). Our data show that Kv4.2 is a dendritic effector

downstream of Aβ and that changes in excitability are likely an important aspect of dendritic changes in AD. Previous imaging studies showed increased levels of calcium in dendrites of hAPP mice, likely a reflection of this increased dendritic excitability (Kuchibhotla et al., 2008). Tau is required for Aβ-induced dysfunction and tau reduction blocks the increased seizure susceptibility seen in hAPP mice (Roberson et al., 2007, Ittner et al., 2010, Roberson et al., 2011). Recent data suggest that tau is present in dendrites and that its dendritic localization is regulated by both Aβ and neuronal activity (Ittner et al., 2010, Zempel et al., 2013, Frandemiche et al., 2014). Our finding that hAPPJ20 mice show tau-dependent hyperexcitability in the dendrites supports the hypothesis that tau acts in the dendrites to increase excitability, contributing to AD pathophysiology. The ability to patch and directly record from dendrites was critical in this study as dendritic excitability cannot be evaluated using conventional whole-cell patch-clamp or extracellular field recordings because of the electrotonic properties of dendrites.

Dendritic abnormalities and hyperexcitability are increasingly being appreciated in CNS diseases, particularly those with cognitive impairment (Nestor and Hoffman, 2012). In Fragile X syndrome, both misregulation of Kv4.2 (Gross et al., 2011, Lee et al., 2011) and improper dendritic arborization (Vetter et al., 2001) have been proposed to contribute to dendritic hyperexcitability and thus improper integration of synaptic signals (Brager and Johnston, 2014). Mouse models of autism and Rett syndrome also show aberrant dendritic morphology (Nestor and Hoffman, 2012). Finally, there is a strong association between traumatic brain injury and risk for Alzheimer's disease and in the controlled cortical impact traumatic brain injury model shows lower Kv4.2 levels and

current in the hippocampus (Lei et al., 2012), highlighting a point of possible mechanistic overlap.

Our data suggest that increased dendritic excitability may be an early event in AD pathophysiology. Additional studies should further explore the mechanisms underlying this increase, which could potentially be targeted therapeutically early in the course of AD.

Materials and Methods

Mice. The hAPPJ20 line overexpresses the hAPP minigene with Swedish (K670M/N671L) and Indiana (V717F) mutations under control of the PDGF promoter (Mucke et al., 2000). These mice were also crossed with the $Tau^{-/-}$ mice (Dawson et al., 2001). Both of these lines were backcrossed more than 12 generations onto the C57BL/6J background before use. The hAPPJ9 line expresses low levels of the same minigene as the hAPPJ20 line and is also on the C57BL/6J background. $Kv4.2^{-/-}$ mice were crossed with hAPPJ9 mice (Mucke et al., 2000). The hAPPJ9/$Kv4.2^{-/-}$ cross was on C57BL/6J/129SvEv mixed background. Male and female mice were used for all experiments. The APPswe/PS1dE9 double transgenic (Jankowsky et al., 2001, Jankowsky et al., 2004) were originally obtained from the Jackson Laboratory (Bar Harbor, ME) with strain name B6C3-Tg ((APPswe, PSEN1dE9) 85Dbo/J; stock number 004462). These mice express a chimeric mouse/human APP transgene that contains the Swedish mutations (K595N/M596L) and a mutant human PS1 transgene carrying the deleted exon 9 variant under control of mouse prion promoter elements. These mice have been backcrossed for more than twelve generations to C57BL/6 background. For experiments with the hAPPswe/PS1dE9 line only male mice were used. Mice were kept

on a 12-hr light/ 12-hr dark cycle and had *ad libitum* access to food and water. The studies were approved by the Institutional Animal Care and Use Committees of the University of Alabama at Birmingham and the National Institute of Child Health and Human Development, and were conducted in compliance with the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals."

Electrophysiology. Mice aged 6–9 months were deeply anesthetized with isoflurane, transcardially perfused with oxygenated cutting solution, and decapitated. Perfusion and cutting stock solution contained (in mM): 2.5 KCl, 1.25 NaH_2PO_4, 28 $NaHCO_3$, 7 dextrose, 0.5 $CaCl_2$, and 7 $MgCl_2$, to which 235 sucrose, 1 ascorbic acid, and 3 sodium pyruvate were added immediately before use (pH 7.3). Transverse hippocampal slices (250 μm thick) were prepared using a vibratome (Leica VT1200S) and incubated in ACSF for 30 minutes at 37°C before being transferred to room temperature. ACSF contained (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 25 dextrose, 2 $CaCl_2$, and 1 $MgCl_2$ (pH 7.3). Ascorbic acid (1 mM) and sodium pyruvate (3 mM) were added to the incubating solution immediately before use.

For somatic and dendritic patch-clamp recordings, slices were transferred to the recording chamber with warmed (31–33°C), oxygenated ACSF. DNQX (10 μM), D-AP5 (50μM), and bicuculline (20 μM) were added to the ACSF to block ligand-gated channels for all recordings. CA1 pyramidal neuron cell bodies and primary apical dendrites were visualized using a Zeiss Axioskop 2 FS *plus* microscope fit with differential interference contrast (DIC) optics. Patch pipettes were pulled from borosilicate filaments (3–5 MΩ for soma, 10–12 MΩ for dendrite) and filled with internal solution containing (in mM): 20 KCl, 125 K-Gluconate, 10 HEPES, 4 NaCl, 0.5 EGTA, 4 Mg-ATP, 0.3 Tris-GTP, and 10

phosphocreatine (pH 7.2). All neurons had resting membrane potentials between –60 and –75 mV. Series resistances ranged from 6–15 MΩ and 15–35 MΩ for somatic and dendritic recordings, respectively, and were monitored throughout the experiments. All recordings were made using a Multiclamp 700B amplifier and Clampex 10.2 software. Signals were digitized at 10 kHz with a Digidata 1440A and filtered at 10 kHz for recordings of back-propagating action potentials and 50 kHz for recordings of firing properties. All recordings were analyzed using IGOR Pro (WaveMetrics).

Single back-propagating action potentials measured in the soma and primary apical dendrite were stimulated every 5 s for 10 sweeps by a 0.2 ms constant current pulse through a stimulus isolator (WPI A385) using bipolar glass stimulating electrodes placed in the alveus. Minimal stimulation to reliably elicit back-propagating action potentials was used. Series resistance was monitored using a 100 ms, 20 mV hyperpolarizing step 1 s before stimulation. The maximum back-propagating action potential amplitude was calculated from the resting potential for each sweep and averaged together for each cell.

Somatic properties were recorded using a 1 s current injection starting with a –200 pA sweep increasing to +200 pA in 20 pA steps with 8 s between sweeps. Neurons were held at –65 mV and series resistance was monitored between each trial. 3 trials were recorded per cell and the results were averaged by cell. Sag was measured as the ratio between the steady state and peak voltage change during the –200 pA current injection. Input resistance was calculated from the slope of the IV curve between –20 and +20 pA. Firing properties (number of spikes, threshold, AHP, etc.) were calculated from the first action potential during the +200 pA current injection. Threshold was defined as the point

when the slope reached 10 mV/ms. Time to onset was the time between initiating the current injection and threshold. Maximum AHP and amplitude of action potentials were measured relative to threshold, and spike half-width was measured at 50% maximum amplitude.

Immunoblots. Saline perfused mouse brains were frozen. Hemibrains were chopped into 450 µm slices (McIlwain tissue chopper) and brain regions were microdissected. Tissue was homogenized in a lysis buffer (50 mM Tris Base, 150 mM NaCl, 0.5% sodium deoxycholate, 5 mM EDTA, 1 mM DTT, 0.1% Triton-X100), protease inhibitors (Halt) and phosphatase inhibitors (Sigma). Samples, were placed in sample buffer and DTT and were heated for 10 min at 70°C then separated in reducing NuPAGE gels (Life Technologies) and transferred to PVDF Immobilon-FL membrane (Millipore). Membranes were incubated with primary antibody (Kv4.2, 1:2,000 from Alomone Labs; Kv2.1, 1:500 from Antibodies Inc.; Kv1.4, 1:500 from Antibodies Inc.; Kv4.3, 1:500 from Antibodies Inc.; α-tubulin, 1:2,000 from Sigma; HCN1, 1:3000 DMC51.1; HCN2, 1:2000 DMC52.2 gifts from Dr. Dane Chetkovich). Membranes were incubated with fluorescent secondary antibody (LI-COR) at 1:20,000 for 1 hour at room temperature, scanned by Odyssey Imager (LI-COR) and analyzed using Odyssey Imager software. For hAPPswe/PS1dE9 experiments, lysates were a gift from Dr. Qin Wang. Mice were perfused with PBS and the hippocampus extracted. The hippocampus was homogenized in carbonate buffer (100 mM Na_2CO_3, 50 mM NaCl, pH 11.5. 2X Lysis buffer (75 mM NaCl, 75 mM Tris-HCl, pH 6.8; 15% glycerol, 3% SDS) was added and samples were triturated 10 times with a 25G needle. Lysates were then centrifuged at 14,000rpm for 30min at 4℃. Supernatants were collected and separated on a SDA-

PAGE. After transfer, membrane was probed with primary antibody for Kv4.2 (1:2000 from Sigma P0233) as outlined above.

Immunohistochemistry. Saline perfused brains were fixed for 24 hours in 4% paraformalydehyde, and then sectioned at 30µm on a sliding microtome (Leica SM200R). One set of serial sections was first treated with 3% H_2O_2 for 10 min then blocked with 1% milk and 10% normal goat serum for 1 hour at room temperature. Then the sections were incubated with anti-calbindin antibody (1:30,000, from Swant) overnight at 4ºC. This was followed by a 1hr at room temperature incubation with biotinylated goat anti-rabbit antibody (1:500, Vector), then incubated for another hour in avidin-biotin complex (Vectastain Elite ABC Kit, Vector). Peroxidase activity was detected with 3,3'-diaminobenzidine and 0.036% H_2O_2 in 100mM Tris-HCl for 6 min at room temperature. Sections were mounted on slides and cover-slipped using Vectashield HardSet mounting medium with DAPI (Vector). Sections were imaged using a Nikon H600L microscope. Calbindin was quantified using ImageJ to measure staining intensity of the dentate gyrus and CA1. The ratio of dentate/CA1 was graphed.

qRT-PCR. RNA was isolated using Trizol Reagent (Invitrogen). cDNA was made from RNA with the SuperScriptIII 1st Strand cDNA Kit (Invitrogen). cDNAs were quantified using BioRad iQ SYBER green and primers sets, Kv4.2, 5'-TCTGTGCACTTA CAATGAGCTGATT-3' and 5'-TGCATCCCCATGAGAAACACT-3', GAPDH, 5'-GGGAAGCCCATCACCATCTT-3', 5'-GCCTTCTCCATGGTGGTGAA-3'. Amplification was on the Roche LightCycler 480 and Kv4.2 mRNA was normalized to GAPDH mRNA.

Drug treatment. Levetiracetam (Sequoia Research Products, Pangbourne, United Kingdom) was dissolved in sterile saline solution. For chronic treatment, Model 2004 osmotic minipumps (Alzet, Durect, Cupertino, CA) were filled with saline or levetiracetam solution and were implanted subcutaneously in the interscapular region of the mice. The Model 2004 minipump delivers fluid at a rate of 0.25 µl/hr for 28 days. Calculations were done to achieve a concentration of 75 mg/kg/day. Minipumps were primed for 24 hr at 37°C. Then mice were anesthetized with 3% isoflurane and the pumps were surgically implanted. After surgery mice were given fluids and pain medicine and placed in a heated caged for recovery. Mice continued to be group housed after surgery.

Behavioral assessment. All behavioral testing was conducted during the light cycle, at least 1 hour after the lights came on. Mice were transferred to the testing room with dim lights and white-noise for acclimation at least 1 hour before experiments. Testing apparatuses were cleaned with 75% ethanol between experiments and disinfected with 2% chlorhexidine after experiments were finished each day. Investigators were blind to genotype. For elevated plus maze assessment, mice are place in the center hub and allowed free access to explore the arms for 10 min (Med Associates, Inc.). The apparatus was 1 meter high with 2-inch-wide arms. Exploration of each arm was determined by the manufacturer's software. For open field assessment, mice were placed into a 40 cm by 40 cm open field apparatus and allowed to explore for 10 min (Med Associates, Inc.). Ambulatory distance and time was determined by the manufacturer's software.

EEG acquisition: For electroencephalography (EEG) experiments, mice were anesthetized with 2.5% isofluorane. Six small holes were drilled bilaterally through the cranium, ~2-2.5 mm posterior and lateral to Bregma, ~4 mm posterior to Bregma and 5

mm lateral to the midline, and ~6mm posterior to Bregma and 3-4 mm lateral to midline, using a dental drill with a 1 mm bit. Three 1.6 mm stainless steel screws (Small Parts, Inc.) were screwed halfway into alternate holes. An EEG electrode (Plastic One, Inc.) with 2 lead wires and a ground, cut to rest on the dura, were fitted into the remaining holes. The lead wires were placed bilaterally on the cortical surface of the parietal hemispheres in the cortical region over the underlying hippocampi. Once the wires were positioned, the electrode unit was stabilized with dental acrylic, and the scalp closed with skin glue (3M Vetbond). This two electrode system did not allow us to identify the anatomical origin of epileptic activity. One week after electrodes were placed, animals were housed individually in specially-constructed EEG monitoring cages. EEG data was acquired at a sampling rate of 500 using Biopac Systems amplifiers (Biopac EEG100C) and AcqKnowlege 4.2 EEG Acquisition and Reader Software (BIOPAC Systems, Inc.). All data were manually analyzed in digital format by an experienced observer blinded to genotype. In addition, cages were equipped with IR Digital Color CCD cameras (Digimerge Technologies) to visually record animals concurrent with EEG acquisition. Video was acquired using security system hardware and software (L20WD800 Series, Lorex Technology, Inc.) to compare EEG abnormalities with behavior.

EEG recordings were assessed for epileptiform activity including isolated spikes, repetitive spiking, and seizures. Spikes were defined as having a duration of < 200 milliseconds with 5x baseline amplitude and repetitive spiking activity was defined as ≥3 spikes lasting ≤ 5 s. Seizures were defined as high-frequency and high-amplitude repetitive spiking, or low frequency, high-amplitude spike-wave patterns ≥ 10 s. The

corresponding video of the animal was analyzed for associated behavior during these abnormal events.

Statistical Analysis. Data were analyzed using the statistical methods indicated in the figure legends (GraphPad Prism 6). Error bars indicate standard errors of the mean.

Author Contributions:

A.M.H., D.A.H., L.S.O.W., and E.D.R. designed experiments, A.M.H., B.T.T., S.C.B., S.J.M., Q.W., and E.D.R. performed experiments, A.M.H., B.T.T., D.A.H., S.C.B., S.J.M., and E.D.R. analyzed data, and A.M.H., D.A.H., and E.D.R. wrote the paper with input from the other authors.

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SUMMARY AND DISCUSSION

Summary of Findings

Here, we demonstrated dendritic hyperexcitability in hippocampal excitatory neurons of hAPPJ20 mice. We further showed that the level of Kv4.2 is decreased in the hippocampus of both hAPPJ20 and hAPP/PS1dE9 mice. These changes in Kv4.2 are caused by epileptiform activity and exaccerbate Aβ-induced epileptiform activiy. Tau was required for the Aβ-induced dendritic hyperexcitability and Kv4.2 depletion.

Sources of Neuronal Excitability in hAPPJ20 Mice

The Aβ-induced neuronal hyperexcitability in the hippocampus of hAPPJ20 mice has been observed previously but its mechanism is unknown. We have identified one source of neuronal hyperexcitability, as dendritic hyperexcitability in these neurons. Dissecting the molecular mechanisms involved in Aβ-induced neuronal excitability allows us to contribute a new piece of the AD puzzle. Dendritic hyperexcitability can result from misregulation of ion channels. Epilepsy models suggest Kv4.2 and HCN channels as likely contributors to dendritic hyperexcitability (Tang and Thompson, 2012). We have identified depletion of Kv4.2 associated with the neuronal hyperexcitability. Dendritic hyperexcitability is not necessarily the only source of neuronal hyperexcitability. GABAergic tone is another critical regulator of neuronal excitability and the GABAergic signal from parvalbumin inhibitory neurons is decreased in the parietal cortex of hAPPJ20 mice. This GABAergic signal loss is due to misregulation of

the somatodendritic ion channel, Nav1.1, which is involved in signal summation in GABAergic neurons.

The depletion of Kv4.2 parallels the Nav1.1 story nicely but occurs in excitatory neurons. Kv4.2 is a critical regulator of excitability in the dendrites of excitatory neurons and is involved in signal summation. Kv4.2 depletion in excitatory neurons could cause dendritic and neuronal hyperexcitability. The finding that Kv4.2 is depleted specifically in the hippocampus provides a potential mechanistic link between the seizure activity and, given the importance of Kv4.2 in learning and memory, the cognitive deficits. Thus, we have found Aβ-induced changes in the hippocampus that could promote hyperexcitability and contribute to the cognitive deficits.

Mechanisms for Aβ-induced Kv4.2 Depletion

We found that Kv4.2 mRNA and protein were depleted in DG and CA1 regions of hAPPJ20 mice. There are many mechanisms by which Aβ could cause Kv4.2 depletion. For example, Aβ could directly cause Kv4.2 protein and mRNA depletion coinciding with the molecular changes that lead to synaptic depression. It could be part of the circuit's response to synaptic depression, a result of the epilpetogenesis, or part of the circuit's response to epileptiform activity.

It has been shown in many cell culture models that acute application of pathological levels of Aβ blocks I_A (Table 4). One group has investigated this mechanism using whole-cell recordings from cholinergic basal forebrain neurons. They found that Aβ reduced outward current and the decrease was prevented by anti-PrPC (cellular prion protein) and in $PrPC^{-/-}$ cells (Alier et al., 2011). They went on to determine that PrPC modulates A-type current via DPP6 by increasing peak amplitude, shifting the voltage-

dependent steady state inactivation curve to the right, slowing inactivation, and decreasing recovery time from steady state inactivation. This would increase A-type current (Mercer et al., 2013). Therefore, Aβ binding PrPC might block the effect of PrPC on A-type current. Aβ has been shown to bind directly to PrPC (Lauren et al., 2009). But there are conflicting results about the necessity for PrPC in Aβ-induced dysfuntion. In some models, PrPC is required for Aβ-induced dysfunction (Gimbel et al., 2010, Barry et al., 2011, Freir et al., 2011) while in others it is not (Balducci et al., 2010, Calella et al., 2010, Kessels et al., 2010, Cisse et al., 2011b). In the hAPPJ20 line, PrPC is not necessary for the Aβ-induced effects (Cisse et al., 2011b). Interestingly, AD patients have increased PrPC in the inferior temporal gyrus (Larson et al., 2012).

There have only been two groups that have looked at the effect of Aβ in a longer time course. One group used 10-fold lower concentrations of Aβ than the acute experiments mentioned and found potentiation at 2 and 24 hours, so it is unclear if this is a pathological or more likely a physiological effect of Aβ (Plant et al., 2006). No cell culture models have conclusively determined the long-term effect of pathological levels of Aβ on Kv4.2 protein or mRNA levels. In an in vivo study, rats were given ICV injections of aggregated $A\beta_{25-35}$ and tested eight days later. $A\beta_{25-35}$ contains the neurotoxic region of Aβ (Monji et al., 2002). The rats showed Morris water maze deficits and an increase in Kv4.2 mRNA and protein in the cortex (Pan et al., 2004). It might be of interest that an increase was seen at this time point; eight days of incubation might represent an earlier stage of the disease progression. Monji et al. did not determine whether the rats had epileptiform activity. Alternatively, ICV injection of Aβ may induce different network changes than global overexpression. The literature is convincing in

showing that Aβ acutely blocks I_A current. An extended block of I_A current which might occur with extended Aβ treatment could contribute to initiation of hyperexcitability. Blocking I_A in multiple models induces epilepsy (Gandolfo et al., 1989). Although this is possible it wouldn't explain the Kv4.2 protein and mRNA depletion. Another thing to consider is that blocking of I_A might result in a change of NMDAR composition, increasing the synaptic NR2B/NR2A ratio, potentially enhancing the early Aβ potentiation at the synapse (Jung *et al.* 2008).

Model	Length	Aβ species	Effect on Kv4.2/ I_A current	Reference
hipp neurons	acute	$A\beta_{1-39}$ and $A\beta_{1-29}$	Reduces I_A	(Good et al., 1996)
dissociated hipp neurons	acute	$A\beta_{25-35}$	Blocks I_A	9746905
basal forebrain neurons	acute	$A\beta_{1-42}$ oligomers	Inhibits outward currents-I_K & I_A	(Alier et al., 2011)
Cortical neurons	acute	Protofibrils, 2hr incubation of $A\beta_{1-42}$ at 25°C, 1-2uM	Inhibits 4-AP sensitive current-I_A &I_D	(Ye et al., 2003)
		Low molecular weight $A\beta_{1-42}$	No effect	
CA1 dendrite of hipp slices	acute	$A\beta_{1-42,}$ 1uM	Inhibits I_A, Increase amplitude of APs (34%) in dendrites	(Chen, 2005)
		$A\beta_{25-35,}$ 1uM	Increases amplitude of APs 15%	
		$A\beta_{42-1,}$ 1uM	Increases amplitude of APs 5%	
Cerebellar & cortical neurons	24hr	Unaggregated $A\beta_{1-40}$, 1uM	Increases in IA	(Ramsden et al., 2001)
		Aggregated $A\beta_{1-40,}$ 1uM	No effect	
Cerebellar and cortical neurons	2hr & 24 hr	$A\beta_{1-40,}$ 100nM	Enhances I_A; Increases Kv4.2 protein in a cycloheximide dependent manner	(Plant et al., 2006)
		$A\beta_{25-35,}$ 100nM	No effect	
Rats with single ICV injection	8 days	$A\beta_{25-35}$ incubated 4 days at 37°C	Water maze deficits, increases Kv4.2 mRNA and protein in cortex	(Pan et al., 2004)

Table 4. The effect of acute and chronic Aβ treatment on K 4.2 protein and mRNA.

It is difficult to predict how the effect of Aβ on the synapse would affect Kv4.2. As discussed in the Introduction, Aβ causes a shift toward activation of extrasynaptic NMDA and mGluR and LTD mechanisms. There is no clear consensus from studies looking at extrasynaptic activation of NMDARs as to what happens to Kv4.2 (Table 3 from the Introduction). Both studies that looked specifically at extrasynaptic NMDAR activation saw a decrease in total Kv4.2, but their models showed different effects on synaptic NMDAR activation, and one saw ERK dephosphorylation, which should increase Kv4.2 surface membrane levels (Lei et al., 2008, Lei et al., 2010). Even if extrasynaptic NMDAR activation could explain the decrease in Kv4.2 protein, it does not address the mRNA decrease.

As an alternative to a direct effect of Aβ, Kv4.2 mRNA and protein may be downregulated by the epileptiform activity, or be part of the compensatory mechanisms to epileptiform activity. There could be changes in transcription factors induced by epileptiform activity that regulate Kv4.2 gene expression. Data from multiple rodent seizure models indicate that seizure activity causes a temporary downregulation of Kv4.2 mRNA as well as ERK phosphorylation of Kv4.2 protein (Table 5). Although this mechanism fits with our data, Aβ-induced network hypersynchrony/epileptiform activity in AD is not entirely equivalent to epilepsy. For example, hippocampal changes in mice with kainic acid-induced epilepsy induced excitatory sprouting of collateral mossy fibers in the inner molecular layer, whereas hAPPJ20 mice showed inhibitory sprouting (Palop et al., 2007a). Also, proposed epilepsy mechanisms involve excessive synaptic NMDAR activation, enhanced LTP and more synapses, while Aβ appears to shift to LTD signaling

mechanisms, LTD and fewer synapses. Despite these caveats, this is a potential mechanism of Kv4.2 protein and mRNA depletion worth further investigation.

Teasing out the mechanism of Kv4.2 depletion is also complicated by seizure activity increasing the release of APP/Aβ (Kamenetz et al., 2003). This probably creates a feedforward loop. Thus, if it is either the Aβ or the epileptiform activity that causes the Kv4.2 mRNA depletion, Kv4.2 mRNA depletion could exacerbate the epileptiform activity and Aβ release.

One mechanistic overlap is the increase in calcineurin that occurs in AD and epileptogenesis (Vazquez-Lopez et al., 2006, Shin et al., 2012, Casanova et al., 2013). Although it hasn't been looked at in brain, Kv4.2 gene transcription is regulated by calcineurin in cardiomyocytes (Perrier et al., 2004, Gong et al., 2006).

Seizure induction	Effect on Kv4.2 protein or mRNA	Reference
Rats plus PTZ	Transient decrease in Kv4.2 mRNA at 3, 6 and normal at 12 hrs. Diazapam blocked PTZ-induced seizures and decrease in Kv4.2 mRNA	(Tsaur et al., 1992)
Rats shock-acute ECS	Transient decrease in Kv4.2 mRNA in DG	(Pei et al., 1997)
Rats plus multiple shocks-chronic ECS	24hrs: Increase in Kv4.2 mRNA in the DG	
Rats plus kainic acid–induced seizures	3 hrs: decrease in Kv4.2 mRNA in DG granule cells 6 hrs: decrease in DG cells and hilar cells	(Francis et al., 1997)
	24 hrs: Kv4.2 mRNA is increased in DG, decrease in CA3/CA4	
Rat plus pilocarpine-induced temporal lobe epilepsy	1 week: Kv4.2 channels in molecular layer of DG went from evenly distributed to concentrated in the outer two thirds. In CA1 region Kv4.2 decrease in striatum radiatum	(Monaghan et al., 2008)
Rats plus pilocarpine	Increased APs in the CA1 dendrites	(Bernard et al., 2004)
Rats plus kainic acid to induce SE	1 and 3 hrs: In hippocampus, decrease Kv4.2 channels at the membrane; increase P- Kv4.2 at the ERK site but decrease at the CamKIII site	(Lugo et al., 2008)
Patient hippocampal sclerosis tissue	Reduction of Kv4.2 levels in neuropil in CA1 and CA3 with intense levels in soma of remaining neurons and granule cells. P-Kv4.2 by ERK has similar pattern in CA1 but increase in DG	(Aronica et al., 2009)
PTZ and bicuculline	Increase ERK phosphorylation of Kv4.2 in hippocampal neurons	(Mulhollan d and Chandler, 2010)
Rats with post-stroke	Reduction of Kv4.2 expression in hippocampus and cortex	(Lei et al.,

seizures		2014)

Table 5. Effect of seizure activity on Kv4.2 protein and mRNA.

Kv4.2 Depletion and Treatment

It is substantial that we observed Kv4.2 depletion in two mouse models of AD, hAPPJ20 and hAPPswe/PS1dE9 (Figure 2). This finding provides stronger evidence that it is not an artifact of a particular mouse line. The ultimate validation of Kv4.2 depletion as an essential part of AD pathology would be to see the change in human AD hippocampal brain tissue.

Although determining which step in AD pathology leads to Kv4.2 depletion is critical, it can be argued that knowing whether Kv4.2 depletion is compensatory or pathological may be even more important, particularly in regards to treatment. A good example of this was the finding that Nav1.1 was decreased in inhibitory interneurons of hAPPJ20 mice (Verret et al., 2012). Before this finding, a sodium channel blocker, phenytoin, was used to treat AD patients but was actually made the patients worse (Carter et al., 2007). This was probably because phenytoin was further blocking the inhibitory input and exacerbating the epileptiform activity. In our case, Kv4.2 depletion made the epileptiform activity worse in hAPPJ9 mice, suggesting that Kv4.2 depletion is part of the pathology. The ultimate test would be to restore Kv4.2 in the hippocampus of hAPPJ20 mice and see if they improve.

Another factor for treatment is timing. AD involves multiple stages, and different drugs might be more appropriate for different stages. For example, choline-acetyltransferase activity is high in MCI and decreases in later stages of disease, which makes timing of acetycholineserase inhibitor treatment relevant for efficacy (DeKosky et

al., 2002). Since it is likely that AD treatment might involve multiple drugs, it will be important to determine the best stage for treatment with each drug.

Tau Reduction

One of the main goals of our research is to understand how tau reduction has its protective effects. Tau is unmistakably required for Aβ-induced neuronal hyperexcitability but the mechanism in unclear. In the disease state tau is altered in multiple ways. It is aggregated, is post-transnationally modified and is mislocalized to dendrites. Similar to Aβ, there is reason to suspect soluble tau as the culprit for dysfunction. Soluble tau mislocalization can be induced by Aβ on primary neurons (Zempel et al., 2010) and tau mislocalization occurs early in the disease pathogenesis, before tau aggregates form (Braak et al., 1994, Braak and Del Tredici, 2011). Tau phosphorylation correlates well with its dendritic mislocalization (Li et al., 2011b), whereas unmodified tau has a high affinity for microtubules (Congdon et al., 2008). There are currently two gain-of-function hypotheses for what tau might be doing in the dendrites. Tau may transport the Src-family kinase Fyn to the synapse, essentially acting as an aberrant scaffold enabling Fyn to enhance NR2B phosphorylation and exacerbate initial excitotoxicity (Ittner et al., 2010). Alternatively, dendritic tau may destabilize microtubules (Zempel et al., 2013). The proposed mechanism involves accumulation of tubulin tyrosine ligase-like 6 which catalyzes polyglutamylation of microtubules, which recruit spastin to cleave microtubules (Zempel et al., 2013). This also helps explain the previous findings that both tau reduction and microtubule stabilizers are protective in AD models (Ballatore et al., 2012).

Looking at similarities in treatment mechanisms might help disclose function. For example the antiepileptic drug levetiracetam (LEV) has been shown to block the epileptiform activity, behavioral abnormalities, as well as the synaptic depression in an AD mouse model (Sanchez et al., 2012). LEV's many functions have not been fully elucidated but its main binding partner is synaptic vesicle protein 2A (SV2A) and it inhibits presynaptic release of glutamate. During high frequency of activity, LEV is endocytosed and binds synaptic vesicle protein 2A (SV2A) intracellularly to reduce neurotransmitter release, likely through calcium channel inhibition (Lynch et al., 2004, Meehan et al., 2011). SV2A interacts with synaptotagmin, and is required for priming of vesicles to be fully calcium-responsive (Chang and Sudhof, 2009). Other proposed functions involve inhibition of calcium channels. LEV was shown to inhibit P/Q-type (Lee et al., 2009), L-type (Yan et al., 2013), N-type (Lukyanetz et al., 2002), and intraneuronal (Nagarkatti et al., 2008) calcium channels in hippocampal cultures.

It is likely that both LEV and based on the current knowledge about tau in the dendrites, the lack of tau at the synapse prevent the initial overexcitation of the synapse. This then might prevent the synaptic depression and network hypersynchrony in hAPPJ20 mice. Evidence to support this hypothesis includes, LEV and tau reduction prevent both epileptiform activity and the synaptic deficits in hAPPJ20 mice. Whereas restoring Nav1.1, which is probably downstream of the initial synaptic depression, only partially blocked the cognitive deficits and epileptiform activity in hAPPJ20 mice. It would be insightful to know if the synaptic activity in the hippocampus was normalized by enhancement of the GAMA activity. It might clarify if the synaptic deficits and/or the epileptiform activity are required for cognitive deficits. One hypothesis is that the

epileptiform activity alone causes the cognitive deficits and another is that the synaptic deficits, which are made worse by the epileptiform activity, cause cognitive deficits.

Future Directions

Although we have obtained data showing that Kv4.2 is depleted and that there is an increase in bpAPs in CA1 pyramidal neurons in hAPPJ20 mice, their connection is only correlative. We presented data showing that I_A was decreased in DG cells, but these data did not show convincing that only Kv4.2 I_A is involved. We also saw a decrease in input resistance and sag ratio in CA1 pyramidal cells (data not shown). HCN channels can affect input resistance (Tang and Thompson, 2012). In the Kv4.2 KO mice, input resistance is decreased, which might be explained by Kv4.2 reduction or a compensatory changes that occurs during development (Carrasquillo et al., 2012). But we wouldn't predict a decrease in input resistance and sag ratio with Kv4.2 depletion. I propose to look at HCN current (I_H) to determine if the gating might be changed although the channel levels are unchanged in this model. This could be done using outside-out patch clamp. There is also evidence in literature in seizures models and patients that HCN channels are altered in epilepsy (Jung et al., 2010, Surges et al., 2012, Phillips et al., 2014). So although Aβ-induced epileptiform activity and epilepsy are not equivalent, there are indicators to continue to examine I_H.

In the hAPPJ20 mice we saw a relatively small change in Kv4.2 mRNA, and protein levels, and only a 5% decrease by immunohistochemistry but we saw a large increase in bpAPs. It is possible that these small decreases in Kv4.2 might account for the larger increase in bpAPs or there could be a bigger change in the surface expression of Kv4.2 that was not seen in the western blot on whole tissue lysate. To answer this

question we could do a surface biotinylation experiment to quantify changes specifically in Kv4.2 surface expression in the hAPPJ20 mice. Theoretically, if there is abundant Kv4.2 in vesicles, surface biotinylaiton would enhance our signal.

Another piece of information that is lacking from our study is relevance to the human condition. I would like to determine if there is Kv4.2 depletion in human hippocampal tissue samples to verify relevance.

The experiment that would demonstrate the impact of Kv4.2 depletion on dendritic excitability in the hAPPJ20 model and possibility of treatment would be to restore the Kv4.2 level to normal in the hAPPJ20 mice. We could inject a virus expressing Kv4.2 or use a Kv4.2-BAC transgenic. Then we could perform EEG recordings and behavioral analysis to determine if the epileptiform activity and cognitive function are normalized. We could also determine if synaptic activity in the hippocampus is normalized. If the EEG recordings and behavioral analysis are normalized this would implicate Kv4.2 as a key contributor to neuronal hyperexcitability and network dysfunction. If the EEG recordings and behavioral analysis is normalized but the synaptic activity in the hippocampus remains depressed, this would indicated that network dysfunction is necessary for cognitive dysfunction. Whereas if the synaptic activity in the hippocampus is also normalized it would indicate that the network dysfunction exacerbates the synaptic dysfunction and if either are sufficient for cognitive deficit cannot be determined. Alternatively, we could treat the hAPPJ9 x Kv4.2$^{-/-}$ mice with saline or LEV. We found Kv4.2 deficiency exacerbates the behavioral abnormalities and epileptiform activity in hAPPJ9 mice. In this experiment, we could block the epileptiform activity and behavioral abnormalities that are associated with Aβ without blocking the

dendritic hyperexcitability. This allows us to determine whether dendritic hyperexcitability alone could still drive epileptiform activity and behavioral abnormalities. If LEV completely blocks the epileptiform activity and behavioral abnormalities in hAPPJ9x Kv4.2$^{-/-}$ mice this would indicate that dendritic hyperexcitability neither drives nor contributes to the epileptiform activity and behavioral abnormalities. If LEV does not block the epileptiform activity and behavioral abnormalities seen in hAPPJ9x Kv4.2$^{-/-}$ mice this would indicate that the dendritic hyperexcitability can drive and contribute to the epileptiform activity and behavioral abnormalities.

As previously mentioned, epilepsy models and AD models have increased calcineurin levels in the hippocampus (Vazquez-Lopez et al., 2006, Shin et al., 2012, Casanova et al., 2013). Also calcineurin (using overexpression and inhibitors) has been shown to regulate Kv4.2 gene transcription in cardiomyocytes (Perrier et al., 2004, Gong et al., 2006). We first would want to confirm if calcineurin regulates Kv4.2 transcription in hippocampal neurons. Then we could determine if blocking calcineurin, by using calcineurin inhibitors, prevents the downregulation of Kv4.2 transcription under epileptic conditions and/or with Aβ treatment.

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APPENDIX:

IACUC APPROVAL FORM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Animal Care and Use Committee (IACUC)

NOTICE OF APPROVAL

DATE: September 26, 2014

TO: ERIK ROBERSON, M.D.
SHEL-1110
(205) 996-9486

FROM:

Robert A. Kesterson, Ph.D., Chair
Institutional Animal Care and Use Committee (IACUC)

SUBJECT: Title: Kv 4.2 Potassium Channels in Alzheimer's Disease Related Neuronal Dysfunction
Sponsor: Internal
Animal Project_Number: 140909515

As of September 26, 2014 the animal use proposed in the above referenced application is approved. The University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) approves the use of the following species and number of animals:

Species	Use Category	Number In Category
Mice	A	16
Mice	B	120

Animal use must be renewed by September 25, 2015. Approval from the IACUC must be obtained before implementing any changes or modifications in the approved animal use.

Please keep this record for your files, and forward the attached letter to the appropriate granting agency.

Refer to Animal Protocol Number (APN) 140909515 when ordering animals or in any correspondence with the IACUC or Animal Resources Program (ARP) offices regarding this study. If you have concerns or questions regarding this notice, please call the IACUC office at (205) 934-7692.

Institutional Animal Care and Use Committee (IACUC)
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933 19th Street South
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1530 3rd Ave S
Birmingham, AL 35294-0019

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Animal Care and Use Committee (IACUC)

MEMORANDUM

DATE: September 26, 2014

TO: ERIK ROBERSON, M.D.
SHEL-1110
(205) 996-9486

FROM: Robert A. Kesterson, Ph.D., Chair
Institutional Animal Care and Use Committee (IACUC)

SUBJECT: **NOTICE OF APPROVAL - Please forward this notice to the appropriate granting agency.**

The following application was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) on September 26, 2014.

Title: Kv 4.2 Potassium Channels in Alzheimer's Disease Related Neuronal Dysfunction
Sponsor: Internal

This institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), is registered as a Research Facility with the USDA, and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

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