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**Publication Date**

2014

Peer reviewed|Thesis/dissertation

**Roles of Apolipoprotein E and GABAergic Interneurons in the  
Pathogenesis of Alzheimer's Disease**

by

**Leslie M Tong**

**DISSERTATION**

**Submitted in partial satisfaction of the requirements for the degree of**

**DOCTOR OF PHILOSOPHY**

in

**Biomedical Sciences**

in the

**GRADUATE DIVISION**

of the

**UNIVERSITY OF CALIFORNIA, SAN FRANCISCO**



This work is dedicated

to both my grandfathers, who suffered from Alzheimer's Disease; you taught me so much  
to all other caregivers of AD patients; remember laughter is therapy too  
and to Baku, my unconditionally loving pet; may you rest in peace

## ACKNOWLEDGEMENTS

I would like to give special thanks to my dissertation committee members, Arturo Alvarez-Buylla, Bruce Miller, and Yadong Huang. Without their unwavering support, thoughtful advice, and phenomenal clinical and scientific expertise, the following research presented simply would not have been possible. I owe particular gratitude to my mentor, Yadong Huang who always manages to find just the right balance between inspirational encouragement and challenging pressure. His adaptive style has given me enormous freedom from a scientific and professional standpoint which has allowed me to grow tremendously on both fronts and feel fully prepared for my next career steps. My qualifying exam committee members, Arturo Alvarez-Buylla, Li Gan, Robert Mahley, and John Rubenstein, have also been instrumental in guiding the direction and rigor of the experiments conducted for my projects. I have continued to seek and generously receive their advice for technical procedures and career development.

An integral part of all my scientific endeavors have also been the informal but thoughtful conversations with many members of the Gladstone Institute of Neurological Disease (GIND) and members of collaborating labs. I've had the pleasure of enjoying the daily social and scientific company of lab members Helen Fong, Johanna Knoferle, Laura Leung, Jessie Carr, Phil Nova, Anna Gillespie, Reeny Balestra, Victoria Yoon, Chengzhong Wang, David Walker, and Biljana Djukic. Those beyond our lab of special note are Meaghan Morris, Caroline Tang, Rob Lindquist, Christine Arnold, Mercedes Paredes, Derek Southwell, Daniel Vogt, Shawn Sorrells, Tim Stowe, Michael Ward, Robert Hesse, and Ricardo Romero. I'm also grateful for a number of college friends,

many from Lloyd House, who've provided moral support and served as a sounding board for technical considerations.

I'm thankful to Christine for introducing me to the use of SS forceps and Mercedes for all the tips on making glass needles. Both were incredibly gracious and patient while I learned to dissect the MGE. Biljana also spent countless hours developing and troubleshooting methods for recording from cells of old, fragile animals. Gui-Qiu Yu taught me about and provided materials for running the ELISA experiments. Ravikumar Ponnusamy and Iris Lo assisted in reserving and maintaining the behavioral facility. Max Wang, a diligent summer intern now a graduate student himself at Yale, was quite helpful in conducting and analyzing various immunohistochemistry experiments; Diane Nathaniel, an enthusiastic smile-generating research assistant from City College San Francisco, helped in physiology experiment analysis; and Olivia Zhang, a quick-minded high school intern also now at Yale, helped with both. I would be remiss to not express my immeasurable gratitude to Teodoro Meneses, our tireless animal technician, who always helped out in all aspects of animal husbandry.

I've also had the good fortune to have wonderful previous scientific mentors: David Hansen, Natalya Nikitina, Marianne Bronner, Dennis Clegg, and Bruce Hay who helped prepare me scientifically and mentally for graduate school.

Thomas Duda and Shirley Freeman of Gladstone admin and all of the admins of GIND, specifically Linda Turney, are also deserving of many thanks for all things logistics and innumerable smiles, particularly in tough times. Shannon Noonan and Paul Li, the outreach coordinators, helped enable me to recognize my own passion for

improving our education system, which is something I hope to be able influence later in my career.

The following work presented was supported by the J. David Gladstone Institutes, the California Institute for Regenerative Medicine, the National Institutes of Health, and gifts from the S.D. Bechtel, Jr. Foundation, the Roddenberry Foundation, and the Hellman Foundation.

## CONTRIBUTIONS

Components of the following text and figures in this dissertation are reprinted material as it appears in Tong et al., published on July 16, 2014 in *The Journal of Neuroscience* 34:9506–9515.

Biljana Djukic conducted the electrophysiological recordings, analysis, and trained and coordinated the assistance from Diane Nathaniel and Olivia Zhang on data analysis. She also advised on which types of physiology experiments might be the most informative and once done, how to most effectively organize and present the data generated.

Christine Arnold assisted with dissections, closing the surgery, and post-operative recovery throughout the entire first cohort of transplant recipients. She also provided advice on stereotaxic techniques and helped develop transplantation coordinates.

Victoria (Seo Yeon) Yoon bred and maintained the apoE knock-in (KI) mouse colonies, assisted with preparation for behavioral experiments (blinding and single-housing), and performed transcardial perfusions for tissue collection. Anna Gillespie maintained the apoE-KI/hAPP<sub>FAD</sub> mice and assisted in the ELISA experiments.

Max Wang assisted with immunostaining, confocal image processing, quantification of A $\beta$  plaque load both histologically and biochemically.

Olivia Zhang assisted in electrophysiological data organization and analysis. She also conducted post-hoc fluorescent immunostaining, imaging, and analysis of thick electrophysiology slices.

Johanna Knoferle assisted with spinning disk confocal microscopy of A $\beta$  fluorescent immunohistochemistry.



Gui-Qiu Yu from Lennart Mucke's lab provided expertise, antibodies, and reagents for the A $\beta$  ELISAs. She also provided positive control tissue samples. Sakura Minami and Michael Ward from Li Gan's lab shared protocols and expertise for microglia antibodies Iba1 and CD68.

John Rubenstein and Arturo Alvarez-Buylla participated in the discussions that drove the experimental design, troubleshooting, and evaluating the thoroughness of the study. Arturo's lab also provided the initial  $\beta$ -Actin GFP males used for breeding MGE cells and the system for making glass capillary injection needles.

Yadong Huang was involved in all of the prior studies demonstrating the deficit of interneurons in the hilus of aged apoE4-KI female mice and provided the inspiration that these interneurons might be the crux of apoE4-caused decline of learning and memory. He was extremely supportive of the proposal to transplant interneurons from an embryonic source and to collaborate with labs with expertise in the technique. He helped me in shaping the experimental design and the structure of presenting the study and helped prepare the manuscript and figures. Finally, and importantly, he also wrote the grants that were awarded to fund this research.

## **ABSTRACT**

### **Roles of Apolipoprotein E and GABAergic Interneurons in the Pathogenesis of Alzheimer's Disease**

**Leslie Tong**

Excitatory and inhibitory balance of neuronal network activity is essential for normal brain function and may be of particular importance to the formation of memories. Apolipoprotein (apo) E4 and amyloid-beta ( $A\beta$ ) peptides, two major players in Alzheimer's disease (AD), cause inhibitory interneuron impairments and death and aberrant neuronal activity in the hippocampal dentate gyrus in AD-like mouse models and in humans, leading to learning and memory deficits. To determine if replacing the lost or impaired interneurons rescues neuronal signaling and behavioral deficits, we transplanted embryonic medial ganglionic eminence (MGE)-derived interneuron progenitors into the dentate gyrus hilus of aged apoE4 knock-in mice without or with  $A\beta$  accumulation. In both conditions, the transplanted cells developed into mature interneurons, functionally integrated into the hippocampal circuitry, and restored normal learning and memory. Thus, largely restricted hilar transplantation of inhibitory interneurons restores normal cognitive function measured by Morris water maze, open field, and elevated plus maze in two widely used AD-related mouse models, highlighting the importance of interneuron impairments in AD pathogenesis and the potential of cell-replacement therapy for AD. More broadly, it demonstrates that excitatory and inhibitory balance is crucial for learning and memory and suggests an avenue for investigating the processes of learning and memory and their alterations in healthy aging and diseases.

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# CHAPTER 1

## Introduction

### Alzheimer's Disease

Alzheimer's disease (AD) is characterized clinically by progressive behavioral decline and loss of memory, language, regulation of emotion, and other cognitive functions. After the first symptoms become noticeable, patients live an average of eight years, during which time, this devastating illness causes patients to increasingly need extensive help in activities of daily living often directly from or paid for by their closest most beloved family members (Alzheimer's Association, 2014). With no cure or even disease-modifying treatments, patients ultimately die in a completely helpless state. In 2010 alone, AD was estimated to have cost the US \$172 billion and the world \$604 billion (Thies and Bleiler, 2011; Wimo et al., 2011), staggering prices that don't account for emotional toll of powerlessly watching one's spouse, parent, best friend wither to become the empty shell of a human being. The looming tidal wave of AD is that the number of worldwide cases, currently estimated at 36 million, including 1 in 3 seniors over age 85, is expected to triple by 2050 (Wimo et al., 2013; Alzheimer's Association, 2014).

The complex and multifactorial nature of AD poses unique challenges for pathogenic studies and therapeutic developments (Huang and Mucke, 2012). The underlying loss of neurons and neuronal processes is likely due to intricate interactions among multiple genetic, epigenetic, and environmental factors. Efforts to target AD-related pathways have shown promise in animal studies, only to fail during human trials (Golde et al., 2011; Huang and Mucke, 2012; Krstic and Knuesel, 2013). The long list of seemingly

encouraging preclinical animal studies that have failed to yield treatments for the human disease highlight the limitations of our rodent models, which primarily focus on the amyloid hypothesis (discussed below) using overexpression and/or mutations in the amyloid pathway. These models are unidimensional so that studies can target one or two isolated genetic pathways, but might explain why therapies exclusively targeting the amyloid pathway have thus far been insufficient (Jucker, 2010). Important insights into the mechanisms of disease pathology have certainly been gained through animal studies, but carefully evaluate and acknowledge their limitations of models used when we interpret the results (Jucker, 2010). It is therefore likely worth examining additional models that have physiological levels of non-mutant proteins involved in the AD.

There is a pressing need to identify novel mechanisms and investigate theories that unify the relationships between proposed mechanisms in order to develop new therapeutic strategies for AD. There is emerging consensus that treatment of moderate-to-advanced AD patients with currently developed drugs comes too late, probably due to widespread severe neuronal loss in the brain and the limited number of brain systems those therapies target, which may additionally represent only some of the branches downstream of the precipitating pathology (Huang and Mucke, 2012).

Prominent early hypotheses for AD pathogenesis centered around the cholinergic system because biochemically, patients with the disease have decreased levels of acetylcholine (ACh) (Minati et al., 2009). The basal forebrain cholinergic cells are lost early in the disease, and ACh, a dynamic neurotransmitter, is important for sleep-wake cycle, consciousness, learning, and memory (Schliebs and Arendt, 2011; Vega and Newhouse, 2014). Currently, most of the drugs actually approved to treat AD target the

cholinergic system, many in the form of cholinesterase inhibitors. At best, these treatments marginally delay behavioral decline for a short period of time in one third of AD patients, but essentially they only substitute disease symptoms with a host of unpleasant side effects including gastrointestinal, cardiorespiratory, genitourinary, and sleep disturbances (Thompson et al., 2004).

It is well-known that the brains of AD patients accumulate two misfolded proteins (Huang and Mucke, 2012). The first of these proteins is amyloid-beta ( $A\beta$ ), which is the pathological cleavage product of amyloid precursor protein (APP), which forms plaques and smaller oligomers. Mutations in APP or in those involved with its processing, such as presenilin (PS)-1 or PS-2, are well-documented to be linked to inherited familial AD (FAD), an early-onset autosomal-dominant form of the disease that begins before age 65 but only accounts for <2% of all AD cases (Campion et al., 1999; Bertram et al., 2010; Huang and Mucke, 2012). Many of the failed drugs in clinical trials directly or indirectly target this pathway with small molecules or antibody therapies to decrease  $A\beta$  production and accumulation or promote  $A\beta$  clearance (Golde et al., 2011). The second of the misfolded AD proteins is Tau, the stabilizing microtubule associated protein, which collects as tangles and paired filaments, typically after post-translational modifications such as phosphorylation or acetylation (Huang and Mucke, 2012). Aggregated Tau is the pathological feature that most closely correlates with cognitive decline in AD, however mutations in this protein are associated with frontotemporal dementia rather than AD (Giannakopoulos et al., 2003; Morris et al., 2011; Huang and Mucke, 2012). Emerging evidence suggest that the disease spreads through functionally connected neuronal networks, especially in the case of tau, which starts in the entorhinal-hippocampal

system, a critical learning and memory center in the brain (Braak and Braak, 1991; Palop and Mucke, 2010; Pooler et al., 2013).

### **Apolipoprotein E4**

The remaining >98% of AD cases, where patients don't have mutations in the A $\beta$  processing pathways, are considered sporadic and most often affect patients over age 65 (Huang and Mucke, 2012). For this vast majority of the population, strongest predictor of developing AD aside from age itself, is the genetic risk factor apolipoprotein E isoform 4 (apoE4) (Huang and Mucke, 2012). All well-conducted genome-wide association studies for sporadic AD in various population from around the world have identified apoE4 to be associated with the disease at extremely high confidence levels, which has led apoE4 to be described as “major gene with semi-dominant inheritance” (Bertram et al., 2010; Genin et al., 2011).

Each individual carries two copies of the *APOE* gene which exists in three alleles:  $\epsilon$ 2,  $\epsilon$ 3, and  $\epsilon$ 4 (Mahley, 1988). They encode three corresponding isoforms: apoE2, apoE3, and apoE4, which in most populations exist at frequencies of roughly 7%, 78%, and 14%, respectively (Mahley, 1988). Importantly, apoE4 carriers (heterozygotes and homozygotes) make up 60–75% of AD cases even though those individuals represent only about 25% of the population. AD patients with apoE4 have a younger average age of disease onset relative to non-carrier patients (Farrer et al., 1997; Genin et al., 2011). Relative to apoE3 homozygotes, apoE4 carriers, especially women, have a dramatically increased odds ratio of ~4.5 in heterozygous women, and ~10.5 and ~12 in homozygous apoE4 men and women respectively (Farrer et al., 1997). ApoE3 homozygotes, ~60% of the population, are considered to have a normal lifetime risk for developing AD (~11%),



and carriers of apoE2, the rarest isoform, are somewhat protected (Corder et al., 1994; Farrer et al., 1997; Genin et al., 2011; Alzheimer's Association, 2014). ApoE isoforms differ from each other at only one or two positions, 112 and 158, in the amino acid sequence. At these two positions respectively, apoE2 has Cys, Cys; apoE3 has Cys, Arg; and apoE4 has Arg, Arg (Mahley, 1988). The Arg-112 in apoE4 allows for a conformational change and the formation of a salt bridge between residues Arg-61 and Glu-255 in a process referred to as "domain interaction" (Mahley et al., 2006; Zhong and Weisgraber, 2009).

As its name suggests, apoE functions as a lipid transport protein by forming a protein-lipid complex, such as in high-density lipoprotein (HDL), and is endocytosed via interaction with membrane-bound receptors on target cells (Mahley, 1988). Expression of apoE is highest in the brain, second only to its expression in the liver (Mahley et al., 2006; Huang, 2010). In the brain, it takes on additional functions that include neurite remodeling and redistribution of lipids to cells for proliferation, membrane repair, and axon myelination (Mahley et al., 2006; Huang, 2010; Huang and Mucke, 2012). In the brain, apoE is primarily synthesized and secreted by astrocytes, but is expressed by neurons under conditions of stress, such as kainic acid-induced excitotoxicity and aging, as indicated by the expression of eGFP in a heterozygous mouse where eGFP is knocked-in at the mouse *apoe* locus (Xu et al., 2006). The same model also suggests that apoE is expressed in type I radial astrocytes and proliferating neuroblasts in the subgranular zone of the dentate gyrus (described below), which give rise to adult-born excitatory granule cells throughout life (Kriegstein and Alvarez-Buylla, 2009; Li et al., 2009).

The molecular mechanisms by which apoE4 leads to cognitive decline are incompletely understood, but studies indicate that apoE4 has a variety of detrimental roles in the brain (Mahley et al., 2006; Kim et al., 2009; Huang, 2010; Huang and Mucke, 2012). Some of apoE4's toxic effects involve the A $\beta$  pathway (Mahley et al., 2006; Kim et al., 2009; Huang, 2010). For example, apoE4 is less efficient at clearing A $\beta$  (Kim et al., 2009), and higher levels of apoE (especially apoE4) are associated with increased amyloid deposition (Bien-Ly et al., 2012). Although all AD patients have amyloid plaques (by definition) many healthy patients also show plaques, a demonstration that plaque loads do not correlate well with cognitive decline (Giannakopoulos et al., 2003).

ApoE4 alone causes independent impairments in the following: intraneuronal trafficking of apoE4, neurite outgrowth, mitochondrial integrity and function, and astrocyte function (Chang et al., 2005; Mahley et al., 2006; Zhong and Weisgraber, 2009; Huang, 2010; Brodbeck et al., 2011; Chen et al., 2011). Furthermore, because of domain interaction, apoE4, relative to apoE3, is more prone to proteolytic cleavage when expressed in neurons (Mahley et al., 2006; Huang, 2010; Huang and Mucke, 2012). This leads to the production of neurotoxic fragments that, in turn, are responsible for the aforementioned defects (Harris et al., 2003; Brecht et al., 2004; Chang et al., 2005). Most recently, a study has not only shown that apoE4 produced in neurons, rather than astrocytes, is more detrimental to learning and memory in mice, but that apoE4 produced specifically in inhibitory interneurons is the primary culprit (Knöferle et al., 2014).

### **Interneurons**

Local circuit inhibitory interneurons exist throughout the central nervous system as a sparse yet diverse neuronal population which functions to coordinate and regulate brain

activity via the inhibitory neurotransmitter GABA (Kepecs and Fishell, 2014). When acting through GABA-A receptor chloride channels in mature cells, GABA causes chloride influx and hyperpolarization, which makes the action potential more difficult to reach, hence an “inhibitory” signal (Eldefrawi and Eldefrawi, 1987). It’s easy to imagine that any network, neuronal or otherwise, would require inhibition in order to function on a reasonable time-scale without becoming intrinsically unstable (Kepecs and Fishell, 2014). These inhibitory cells constitute only 10-25% of all neurons in any given brain region, but dynamically modify whole networks on the order of milliseconds (Tamamaki et al., 2003; Markram et al., 2004; Chamberland and Topolnik, 2012; Le Magueresse and Monyer, 2013; Marín, 2013; Tyson and Anderson, 2014). Thus, computational power and variety of signaling dynamics are enriched by having a wide variety of interneurons in different brain layers with different sub-cellular targets and different types of timing or firing patterns (Kepecs and Fishell, 2014). They can precisely control individual cells as well as coordinate as pacemakers to establish entire rhythms at various frequency signaling bands (Le Magueresse and Monyer, 2013; Kepecs and Fishell, 2014). An individual interneuron can control as many as 10,000 other cells, both excitatory principal cells and other inhibitory neurons (Amaral et al., 2007; Myers and Scharfman, 2009; Savanthrapadian et al., 2014).

As might be imagined, interneurons are critical in pre- and postnatal development for plasticity, setting up a functional cortex, and maintaining a balance of brain network activity. Thus, their disruption or dysfunction likely contributes to a wide range of neurological and psychiatric diseases (Marín, 2012; Le Magueresse and Monyer, 2013). There are an increasing number of studies in humans and multiple animal models that AD

may be among these disorders that involve inhibitory signaling dysfunction, and increased focus has been brought to the potential vulnerability and impairment of interneurons during AD pathogenesis.

A growing body of evidence points toward a region where interneurons are particularly vulnerable. This region is the dentate gyrus of the hippocampus, a critical structure for the encoding of new episodic memories and spatial learning and navigation (Wang and Morris, 2010; Squire and Wixted, 2011; Fellini and Morellini, 2013). The dentate gyrus can be thought of as a signaling gatekeeper between the entorhinal cortex and the hippocampus in the processing of learning and memory tasks, and the GABAergic system is crucial for this task (Amaral et al., 2007; Andrews-Zwilling et al., 2012). The space within the dentate gyrus is referred to as the polymorph layer, or hilus, and contains most of the interneurons that are responsible for controlling the sparse encoding of information in the primary cells of the dentate's granule cell layer and the maturation of new adult-born neurons in the subgranular zone. The neural stem cells that give rise to these adult-born neurons also endogenously express apoE (Li et al., 2009; Wang et al., 2014). Normal learning and memory is shaped by a balance of excitatory and inhibitory neuronal network activity. During normal healthy aging in humans and wildtype rodents, there is a decrease in the number of hilar interneurons and a slight waning of cognition, likely the result of network activity imbalance due to the natural progressive inhibitory interneuron loss or dysfunction (Stanley et al., 2012; Spiegel et al., 2013; Koh et al., 2014).

### **Mouse Models of AD**

Several mouse models of AD, including one carrying the genetic risk-factor apoE4, exhibit an accelerated and dramatic decline of interneuron number in the dentate hilus (Andrews-Zwilling et al., 2010; Leung et al., 2012; Knoferle et al., 2014; Tong et al., 2014). Aged apoE4 knock-in (KI) mouse models, where an isoform of human apoE gene homozygously replaces the endogenous mouse *apoe* gene in a C57Bl6J strain background, have enabled further study of physiological impairments caused by apoE4 (Hamanaka et al., 2000). These mice display age- and sex-dependent water maze learning and memory deficits which are most severe in individuals with the greatest loss of hilar interneurons (Andrews-Zwilling et al., 2012; Knoferle et al., 2014). Using these models, studies from our lab have shown that with increasing age, homozygous apoE4-KI female mice are most significantly impaired in learning and memory (Raber et al., 1998) relative to homozygous apoE3-KI mice (of either gender) (Andrews-Zwilling et al., 2010; Leung et al., 2012). In most aspects of comparison, apoE3-KI mice of either gender are indistinguishable from wildtype mice (Andrews-Zwilling et al., 2010; Leung et al., 2012). This is consistent with human data, which shows that among carriers of apoE4, females are at greater risk for developing AD (Farrer et al., 1997). For this reason, behavioral studies involving apoE-KI mice usually use females. Although the detrimental effects of apoE4 can be observed in middle-aged mice (~12 months old), apoE4-KI mice are more significantly and robustly impaired by old age (~ 16 months old), in learning and memory tasks such as the Morris Water Maze (MWM) (Andrews-Zwilling et al., 2010), which will be further detailed in Materials and Methods.

Examination of the pathological effects of apoE4 alone is important, but it is also essential to consider that A $\beta$  accumulation is a hallmark of AD in human patients.

ApoE4-KI mice have endogenous mouse APP and thus never develop amyloid plaques. Furthermore, several studies in mice and humans have shown that apoE4 is typically associated with greater A $\beta$  deposition, or perhaps reduced clearance, (Huang and Mucke, 2012). Confidence in any results obtained by studying apoE4-KI mice can be further increased by repeating experiments using apoE-KI mice that have been crossed with a model for A $\beta$  accumulation, such as the J20 line harboring Swedish (K670N,M671L) and Indiana (V717F) mutations in the APP gene, henceforth referred to as hAPP<sub>FAD</sub> mice (Mucke et al., 2000; Palop et al., 2007; Verret et al., 2012). Alone, hAPP<sub>FAD</sub> mice have robust learning memory impairments at a relatively young age, the severity and onset of which are even more dramatic and earlier in apoE4-KI/hAPP<sub>FAD</sub> animals (Bien-Ly et al., 2012). A $\beta$  overproduction and accumulation alone, has also been shown to functionally impair interneurons in rodent models (including hAPP<sub>FAD</sub> mice) and humans which lead to abnormal dentate gyrus activity (Palop et al., 2007; Verret et al., 2012).

### **Embryonic Origins of Cortical GABAergic Local-Circuit Neurons**

Because these aging and AD models reflect a selective loss of hilar interneurons, key regulators of network activity balance and located within a critical memory processing center of the brain, logical reasoning dictates that improving network balance may be effective for preventing or treating cognitive decline in various disease states to promote healthy aging. The question is what could be used to regulate such a complicated and delicately balanced process?

The answer lies in interneurons themselves. By their very nature, they receive feedback to fine tune and regulate a signaling system. Because they inhibit each other as well as principal excitatory cells, they are unlikely to cause over-inhibition in a system.

During embryonic development, they originate in a concentrated location in the embryonic brain from which they migrate autonomously into pre-existing developing brain tissue (Pleasure et al., 2000; Marín and Rubenstein, 2001; Wonders and Anderson, 2006; Kriegstein and Alvarez-Buylla, 2009; Bartolini et al., 2013).

Many cortical GABAergic interneurons are produced in the embryonic medial ganglionic eminence (MGE) (Anderson et al., 1999; Wichterle et al., 2001; Tricoire et al., 2011). The MGE is a transient embryonic germinal zone in the ventral telencephalon in which progenitors of cortical interneurons originate and migrate to become distributed throughout the cortex and hippocampus (Marín and Rubenstein, 2001, 2003). Remarkably, these cells migrate tangentially through the cortical excitatory neurons, which migrate radially from the ventricle (Marín and Rubenstein, 2001, 2003). Multiple studies have shown mouse MGE-derived immature interneurons, when micro-dissected and grafted into the neonatal and young adult CNS, have the unique ability to migrate, mature, and functionally integrate to modulate local circuitry (Wichterle et al., 1999; Alvarez-Dolado et al., 2006; Southwell et al., 2010; Alvarez-Dolado and Broccoli, 2011; Tanaka et al., 2011; Bráz et al., 2012; Hunt et al., 2013). Some of these transplanted cells survive and remain functional for the life of the host animal (Alvarez-Dolado et al., 2006; Calcagnotto et al., 2010). Importantly, grafted inhibitory interneurons ameliorate the excitatory-inhibitory imbalance in animal models of neurological disorders, including epilepsy, stroke, anxiety, Parkinson's disease, and pain (Baraban et al., 2009; Daadi et al., 2009; Martínez-Cerdeño et al., 2010; Zipancic et al., 2010; Bráz et al., 2012; Hunt et al., 2013; Perez and Lodge, 2013; Valente et al., 2013); reviewed in (Tyson and Anderson, 2014).

## **Interneuron Subtypes**

There is great diversity among interneurons. Broadly speaking, they have a spectrum of phenotypes based on morphology, physiology, and expression of neurochemicals (Fishell and Rudy, 2011). In the hilus of the hippocampus, there are several prominent subtypes of interneurons. Interneurons expressing somatostatin (SOM) are typically distributed throughout the hilus and have axons typically synapsing onto the apical dendrites of target cells (Fishell and Rudy, 2011). Physiologically, they are characterized by low-threshold regular spiking patterns. Interneurons expressing parvalbumin (PV) are typically called basket cells. Anatomically, many are located close to the interface with the granule cell layer and have axons that synapse onto cell bodies (Marín, 2012). Physiologically, they are associated with fast-spiking bursts of axon potentials. Both subtypes can inhibit other interneurons, which sharpens the temporal precision of action potential generation to create frequency oscillations in the network (Savanthrapadian et al., 2014).

PV+ and SOM+ interneurons primarily originate within the MGE (Xu et al., 2004). Embryonic interneurons are also subject to relatively late, context-dependent maturation, meaning the anatomical location of their destination appears to have an effect on the subtype they become (Fishell and Rudy, 2011; Hunt et al., 2013). This characteristic likely contributes to successful interneuron integration in studies where the MGE has been dissected and transplanted.

## **Hypothesis to Be Tested**

Aberrant dentate activity in AD patients and mouse models with dentate-specific interneuron loss are indications that balanced interneuron function is critical for normal



cognition in healthy aging. Furthermore, embryonic interneurons have been transplanted and shown to restore neuronal signaling balance in other mouse models with inhibitory interneuron deficits. Thus, I hypothesize that transplanting mouse MGE-derived inhibitory interneuron progenitors could restore the behavioral deficits by functionally replacing the lost inhibitory interneurons in apoE4-KI mice. Here, we demonstrate for the first time that these cells transplanted into the hippocampal hilus of adult recipient mice with advanced age (14 months) are (1) viable and functional, (2) sufficient to restore normal learning and memory in aged apoE4-KI mice, and (3) able to do so in the presence of both apoE4 and hAPP<sub>FAD</sub> in the apoE4-KI/hAPP<sub>FAD</sub> mice.

## CHAPTER 2

### Materials and Methods

#### Animals

All protocols and procedures followed the guidelines of the Laboratory Animal Resource Center at the University of California, San Francisco (UCSF). Experimental and control animals had identical housing conditions from birth through sacrifice (12-hour light/dark cycle, housed 5/cage, PicoLab Rodent Diet 20). All mouse lines were maintained on a C57Bl/6J background strain. ApoE3-KI and apoE4-KI homozygous mouse lines (Taconic) (Hamanaka et al., 2000) were born and aged under normal conditions at the Gladstone Institutes/UCSF animal facility. ApoE4-KI/hAPP<sub>FAD</sub> mice were generated by crossing apoE4-floxed-KI (apoE4-fKI) mice (Bien-Ly et al., 2012) to hAPP<sub>FAD</sub> mice (J20 line) (Mucke et al., 2000; Palop et al., 2007; Verret et al., 2012) harboring Swedish (K670N,M671L) and Indiana (V717F) mutations. Experiment timeline diagramed (Fig. 1A).

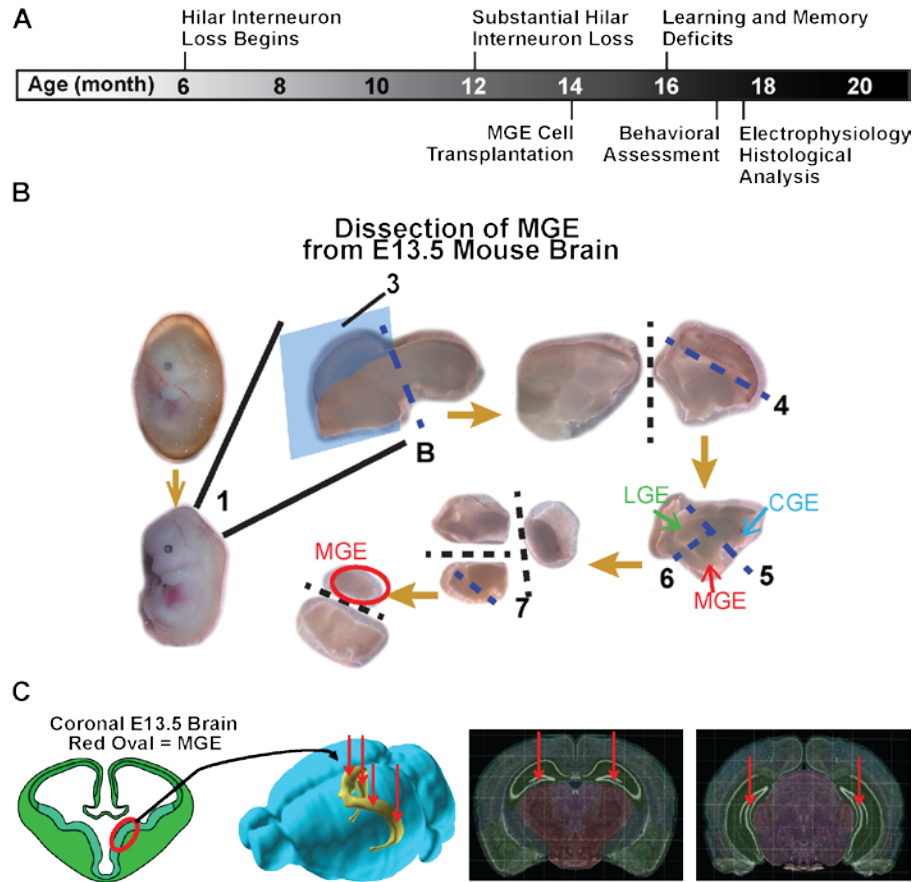
#### Tissue Dissection

Donor MGE cells, also on C57Bl/6J background strain, were generated by breeding male transgenic  $\beta$ -actin promoter-driven eGFP mice (strain 6567, Jackson Laboratory) with wild-type females for 24 hours. Embryonic day 0.5 was defined as the time when the sperm plug was detected. Embryonic MGE cells were dissected at E13.5 in Leibovitz L-15 medium (Cell Culture Facility, UCSF) containing 100  $\mu$ g/ml DNaseI (Roche), dissociated by pipetting into a single-cell suspension, and collected by centrifugation (3000g x 3 min) (Wichterle et al., 1999; Alvarez-Dolado et al., 2006; Martínez-Cerdeño

et al., 2010; Southwell et al., 2010; Alvarez-Dolado and Broccoli, 2011; Hunt et al., 2013).

The micro-dissection of MGE from GFP+ E13.5 mouse embryos is diagrammed in figure 1. (1) The embryonic brain was first removed. (2) The telencephalon was isolated by removing the hindbrain. (3) A sagittal cut separated the two hemispheres. (4) The ventral telencephalon was exposed by tearing away the dorsal cortex. The MGE was isolated by removal of the (5) caudal and (6) lateral ganglionic eminences. (7) Finally, the dorsal MGE was collected after rotating the remaining tissue onto its side and removing the mantle zone and preoptic area (Fig. 1B).

The tools used for the dissection were a pair of 13.5 cm Dumont SS Fine Forceps (Fine Science Tools 11200-33), a Sharpoint Microsurgical Straight Stab Knife (Fisher Scientific NC9854830), and utility 9 cm Fine ToughCut Tissue scissors (Fine Science Tools 14058-09).



**Figure 1. Experimental timeline, dissection protocol, and hilar targeting of MGE cells.**

(A) Experimental timeline for hilar MGE cell transplantation and evaluation. (B) Dissection protocol of MGE from GFP+ E13.5 mouse embryos. [1] Removal of embryonic brain, [2] isolation of the telencephalon by removing hindbrain, [3] sagittal cut to separate two hemispheres, [4] isolation of ventral telencephalon by removing dorsal cortex, [5] removal of caudal (CGE) and [6] lateral (LGE) ganglionic eminences, [7] removal of the mantle zone and preoptic area for dorsal MGE collection. (C) The dissected MGE cells from GFP+ E13.5 mouse embryos were bilaterally transplanted into the rostral and caudal hilus of 14-month-old apoE4-KI mice. Diagram adapted from (Allen Institute for Brain Science, 2014).

## Cell Transplantation

Glass micropipette needles (Drummond Scientific Company 3-000-203-G/X 0.02 in ID, 0.044 in OD, 3.5 in length) were vertically pulled and the finest part of the filament was broken on a taught Kimwipe to an inner diameter of 50  $\mu\text{m}$  and an outer diameter of <200  $\mu\text{m}$  at a distance of 2 mm from the tip. They were then beveled at a 30° on a circular diamond hone with N<sub>2</sub> back pressure to keep the tip clear during the grinding process for about 60 seconds until the tip had an inner diameter of ~60  $\mu\text{m}$ .

Female apoE4-KI and apoE3-KI mice at 14 month of age and apoE4-KI/hAPP<sub>FAD</sub> mice at 10 months of age were anesthetized with 80  $\mu\text{l}$  of ketamine (10 mg/ml) and xylazine (5 mg/ml) in saline (Hospira), intraperitoneally (i.p.) and maintained on 0.8–1.0% isoflurane (Henry Schein) in 1.5 L/min O<sub>2</sub> (Laboratory Animal Resource Center, UCSF).

Concentrated GFP+ MGE cell suspensions (~600 cells/nl) were loaded into the glass needles which were capillary-action backfilled with mineral oil (Sigma) on a Nanoject II (Drummond Scientific Company). The coordinates for the bilateral rostral and caudal stereotaxic sites used for hilar transplantation were X =  $\pm 1.65$ , Y = 2.00, Z = 1.7 and X =  $\pm 2.90$ , Y = 3.20, Z = 2.2, where Z was measured from the surface of the brain (Fig. 1 C) (Paxinos and Franklin, 2001; Allen Institute for Brain Science, 2014). All procedures were done using Kopf Small Animal Frame 940 using Model 943 Ear Bar Adaptors (David Kopf Instruments). The sites were marked with non-toxic Sharpie and scratched with a metal needle then hand-drilled with a 0.5-mm microburr (Foredom, Fine Science Tools) up to but not penetrating the dura which was hand-punctured with a metal needle to prevent unnecessary breakage of the glass needle. At each transplantation site, ~34 nl

(~20,000 cells) were injected over the course of ~45 seconds and allowed to diffuse for 3 minutes. For recovery, mice were given 1.5 L/min O<sub>2</sub> for 10 minutes and sutured with 6-0 monofilament non-absorbent nylon sutures (Ethicon). While still under anesthesia, they were administered analgesics ketophen (100 µl at 1 mg/ml) in saline (subcutaneous between shoulders) and buprenorphine (100 µl at 7.5 µg/ml) in saline, i.p., then monitored on a heating pad. Control transplant mice received an equivalent volume of heat-shocked dead MGE cells, which were generated by 4 alternating cycles of 3 minutes at 55°C and 3 minutes in dry ice before centrifugation collection (Alvarez-Dolado et al., 2006; Baraban et al., 2009; Southwell et al., 2010).

### **Behavioral Tests**

Behavioral tests were done for MGE cell-transplanted and control-transplanted mice at 70–80 days after transplantation (DAT). All mice were singly housed with a randomly assigned number so the experimenter is blinded to treatment group and genotype during behavioral tests.

Learning in the Morris water maze (MWM) is measured by forcing the mouse to swim through opaque water to find a submerged platform, where it is rewarded by removal or "escape" from the maze (Morris, 1984). The mouse is evaluated for 20 trials over the course of 5 days, referred to as "hidden days", and learning is measured by the improvement in escape time as a function of trial or hidden day number (Andrews-Zwilling et al., 2010). The pool is 122 cm diameter with room temperature water (21–23°C with a 10 cm<sup>2</sup> platform submerged 1.5 cm below the surface of water made opaque with 1/3-3/4 bottle white Powder Temptra Paint (Discount School Supply #CPTWH) during hidden trials (Andrews-Zwilling et al., 2010; Leung et al., 2012). Mice habituated

to the room for 48 hours and pre-trained to confirm their ability to swim and to familiarize the animals with the concept of a submerged platform. In the experiment itself, mice were trained to locate the hidden platform over 4 trials per day for 5 hidden days (HD1–5), where HD0 is the first trial on the first day, with a maximum of 60 seconds per trial. The cohort was divided into “squads” of 11-16 animals, and a given squad swam two trials in a “session” before the next squad swam. A squad received about 3 hours between sessions (swimming two sessions per day and two trials per session).

Each memory trial was conducted for 60 seconds in the absence of the platform at 24, 72, and 120 hours after the final learning session. Memory is assessed after hidden days by removing the platform and recording the amount of time the mouse spends in the (former) platform quadrant or the number of times the mouse crosses the exact location of the platform position, called "probe trials" (Andrews-Zwilling et al., 2010). Preference is calculated by the percentage of time spent in the target quadrant that contained the platform during the learning trials compared to the average of time spent in the non-target quadrants. Following the probe trials, the visible trials involved marking the platform location with a black-white striped mast (15 cm high) and placing it in each of the three “unused” quadrants for the three visible trials. The platform location and room arrangement remained constant throughout the assay with the exception of moving the platform during the visible trials. Speed was calculated by distance traveled divided by trial duration. Performance was objectively monitored using EthoVision video-tracking software (Noldus Information Technology).

Open field test assesses habituation and general activity behavior by allowing the mice to explore a new, but empty, environment (Andrews-Zwilling et al., 2012). After at

least 2 hours of room habituation, mice were placed in an odor-standardized chamber cleaned with 30% EtOH for 15 minutes. Activity behavior was monitored and analyzed by software from San Diego Instruments. The elevated plus maze evaluates anxiety and exploratory behavior by allowing mice to explore an open, illuminated area (open arm) or hide in a dark, enclosed space (closed arm) (Bien-Ly et al., 2011). Here, mice were placed in an odor-standardized maze cleaned with 30% EtOH for 10 minutes after at least 2 hours of room habituation. Behavior was analyzed by infrared photo-cells interfacing with Motor Monitor software (Kinder Scientific).

### **Hippocampal Slice Preparation and Electrophysiology**

Acute coronal brain slices (350  $\mu\text{m}$ ) were prepared from MGE cell-transplanted and control-transplanted mice 80–90 DAT at 12 or 17 months of age. The modified protective slicing and recovery method of Zhao et al. (Zhao et al., 2011) was used to improve health of the slices from aged animals (Zhao et al., 2011). Mice were deeply anesthetized with isoflurane and trans-cardially perfused with 30 mL of chilled oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) slicing artificial cerebrospinal fluid (ACSF; 92 mM N-methyl-D-glucamine (NMDG), 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 30 mM NaHCO<sub>3</sub>, 20 mM HEPES, 25 mM glucose, 2 mM thiourea, 5 mM Na-ascorbate, 3 mM Na-pyruvate, 12 mM N-acetyl-L-cysteine, 0.5 mM CaCl<sub>2</sub>, 10 mM MgSO<sub>4</sub>). Brains were quickly removed, sliced with the HM650V vibration microtome (Thermo Scientific) and incubated in slicing ACSF for 10 min at 35°C, followed by a recovery for 1 hour at room temperature in recovery ACSF (92 mM NaCl, 2.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 30 mM NaHCO<sub>3</sub>, 20 mM HEPES, 25 mM glucose, 2 mM thiourea, 5 mM Na-ascorbate, 3 mM Na-pyruvate, 12 mM N-acetyl-L-cysteine, 2 mM CaCl<sub>2</sub>, 2 mM MgSO<sub>4</sub>). After the recovery period, slices were



transferred to a holding chamber containing room temperature oxygenated recording ACSF (126 mM NaCl, 3 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 26 mM NaHCO<sub>3</sub>, 20 mM glucose, 2 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>). For recording, slices were placed in the recording chamber of a BX51WI microscope (Olympus) equipped with infra-red DIC optics (900 nm) and epifluorescence, and perfused with warmed (30°C) oxygenated recording ACSF at a rate of 3 mL/min. Whole-cell patch-clamp recordings were obtained from visually identified granule cells and hilar interneurons using borosilicate glass pipettes (4–5 M $\Omega$ ), Multiclamp 700B amplifier (Molecular Devices), and WinLTP acquisition software (University of Bristol). Recordings were discarded if series resistances (R<sub>s</sub>) > 25 M $\Omega$ , or if R<sub>s</sub> varied by more than 25% during the course of the experiment. All data analyses were performed off-line with Clampfit 10.2 software (Molecular Devices).

Spontaneous postsynaptic excitatory and inhibitory currents were recorded in the same cells by voltage-clamping the membrane potential at the reversal potential of the GABAergic current (-50 mV) and glutamergic current (10 mV), respectively. To stabilize recordings at depolarizing membrane potential, patch pipettes were filled with an internal solution containing: 120 mM CsMeSO<sub>3</sub>, 0.5 mM EGTA, 10 mM BAPTA, 10 mM HEPES, 2 mM Mg-ATP, 0.3 mM Na-GTP, 5 mM QX-314. Events were sampled for 200 sec and detected with a cutoff of  $\pm 5$  pA.

Intrinsic excitability of interneurons was assessed by measuring the firing rate in response to a series of depolarizing current injections (1 sec; 0.05 to 1 nA). Patch pipettes were filled with an internal solution containing: 100 mM K-gluconate, 20 mM KCl, 10 mM HEPES, 4 mM Mg-ATP, 0.3 mM Na-GTP, 10 mM phosphocreatine, 0.2 % biocytin.

After the experiment, slices were fixed and stained for biocytin, SOM, and PV to confirm the identity of the recorded cells.

### **Immunohistochemistry**

Animals were transcardially perfused with 0.9% (w/v) saline then with 4% (w/v) paraformaldehyde (PFA) and whole brains were collected and drop-fixed in 4% PFA for 24 hours at 4°C. After rinsing in PBS, tissue was cryoprotected in 30% (w/v) sucrose and sectioned coronally (30 µm) with a frozen sliding microtome (Leica) for floating section immunohistochemistry. Post-hoc electrophysiology slices (vibratome-cut 350 µm thick) were also stained as floating sections. Floating sections were immunostained overnight with the following primary antibodies: rabbit anti-GFP (1:2000; Abcam), goat anti-GFP (1:3000; Abcam), chicken anti-GFP (1:2000; Abcam), biotinylated mouse anti-NeuN (1:300; Abcam), rabbit anti-GABA (1:2500; Sigma), rat anti-SOM (1:100; Millipore), goat anti-SOM (1:250; Santa Cruz), rabbit anti-Neuropeptide-Y (NPY) (1:6000; Sigma), rabbit anti-PV (1:1000; Millipore), goat anti-ChAT (1:300; Millipore), rabbit anti-GFAP (1:2000; Dako), rabbit anti-Olig2 (1:300; Millipore), rabbit anti-Iba1 (1:3000; Wako), rat anti-mouse CD68 (1:300; Serotec), and biotinylated mouse anti-human Aβ 3D6B (1:700; Elan). Secondary antibodies were: Alexa Fluor 488 donkey anti-goat, 488 donkey anti-rabbit, 488 goat anti-chicken, 594 donkey anti-rabbit, 594 donkey anti-goat, 594 donkey anti-rat, 647 donkey anti-goat (1:2000; Life Technologies); biotinylated goat anti-rabbit (1:200; Vector Labs), biotinylated donkey anti-mouse (1:250; Jackson ImmunoResearch), and biotinylated rabbit anti-rat (1:250; Vector Labs). Alexa Fluor 594 streptavidin was used to detect biotin and biocytin (1:500; Life Technologies). Diaminobenzidine (DAB) immunohistochemistry development was done using Vectastain ABC amplification kit

(Vector Labs) and DAB (Sigma) as a chromagen substrate for 1–5 minutes. There were no tumors observed under gross and histological examinations.

**Table 1. Antibodies Used for Immunohistochemistry.**

<u>Primary Antibodies</u>			
rabbit	anti-GFP	1:2000	Abcam
goat	anti-GFP	1:3000	Abcam
chicken	anti-GFP	1:2000	Abcam
biotinylated mouse	anti-NeuN*	1:300	Abcam
rabbit	anti-GABA*	1:2500	Sigma
rat	anti-SOM	1:100	Millipore
goat	anti-SOM	1:250	Santa Cruz
rabbit	anti-NPY	1:6000	Sigma
rabbit	anti-PV	1:1000	Millipore
goat	anti-ChAT	1:300	Millipore
rabbit	anti-GFAP	1:2000	Dako
rabbit	anti-Olig2	1:300	Millipore
rabbit	anti-Iba1	1:3000	Wako
rat	anti-mouse CD68	1:300	Serotec
biotinylated mouse	anti-human A $\beta$ 3D6B	1:700	Elan
<u>Secondary Reagents</u>			
Alexa Fluor 488 donkey	anti-goat	1:2000	Life Technologies
Alexa Fluor 488 donkey	anti-rabbit	1:2000	Life Technologies
Alexa Fluor 488 goat	anti-chicken	1:2000	Life Technologies
Alexa Fluor 594 donkey	anti-rabbit	1:2000	Life Technologies
Alexa Fluor 594 donkey	anti-goat	1:2000	Life Technologies
Alexa Fluor 594 donkey	anti-rat	1:2000	Life Technologies
Alexa Fluor 647 donkey	anti-goat	1:2000	Life Technologies
Alexa Fluor 594	streptavidin	1:500	Life Technologies
biotinylated goat	anti-rabbit	1:200	Vector Labs
biotinylated donkey	anti-mouse	1:250	Jackson Immunoresearch
biotinylated rabbit	anti-rat	1:250	Vector Labs

\*No TritonX-100 used with these antibodies

## **Image Collection and Cell Quantification**

Histological images were collected using Biorevo BZ-9000 Keyence digital microscope, Leica spinning disk confocal microscope, or Biorad scanning confocal microscope. Quantification of interneuron subtypes was obtained manually by observation done on an upright epifluorescent DM500B Leica microscope and on the Keyence digital microscope. Quantification of microglia (markers of Iba1 and CD68) was obtained using semi-automated Image-based Tool for Counting Nuclei plugin (ITCN, University of California Santa Barbara Center for Bio-Image Informatics) in ` (National Institutes of Health) for images covering 0.5mm<sup>2</sup> of the dentate gyrus. Quantification of GAD67+ and GFP+ cells was conducted in the hilus on every 10th coronal section (30 µm) across the entire hippocampus using ITCN/ImageJ and verified manually visually for captured images.

## **Aβ Analysis**

Aβ was immunostained as reported (Bien-Ly et al., 2011, 2012). Hippocampal plaque loads were quantified using ImageJ Measure tool on manually outlined hippocampal images of Aβ immunostaining. Hippocampal Aβ concentrations were measured by sandwich ELISA with capture antibodies mouse anti-Aβ1-x 266 (10 µg/ml) and mouse anti-Aβ42 21F12 (5 µg/ml). The detection antibody was mouse anti-human Aβ 3D6B (0.5 µg/ml) (Bien-Ly et al., 2011, 2012). Development and detection was done with HRP-Avidin and TMB-ELISA substrate (Thermo Scientific) and read on Spectramax 190 Plate Reader (Molecular Devices).

## **Statistical Analysis**

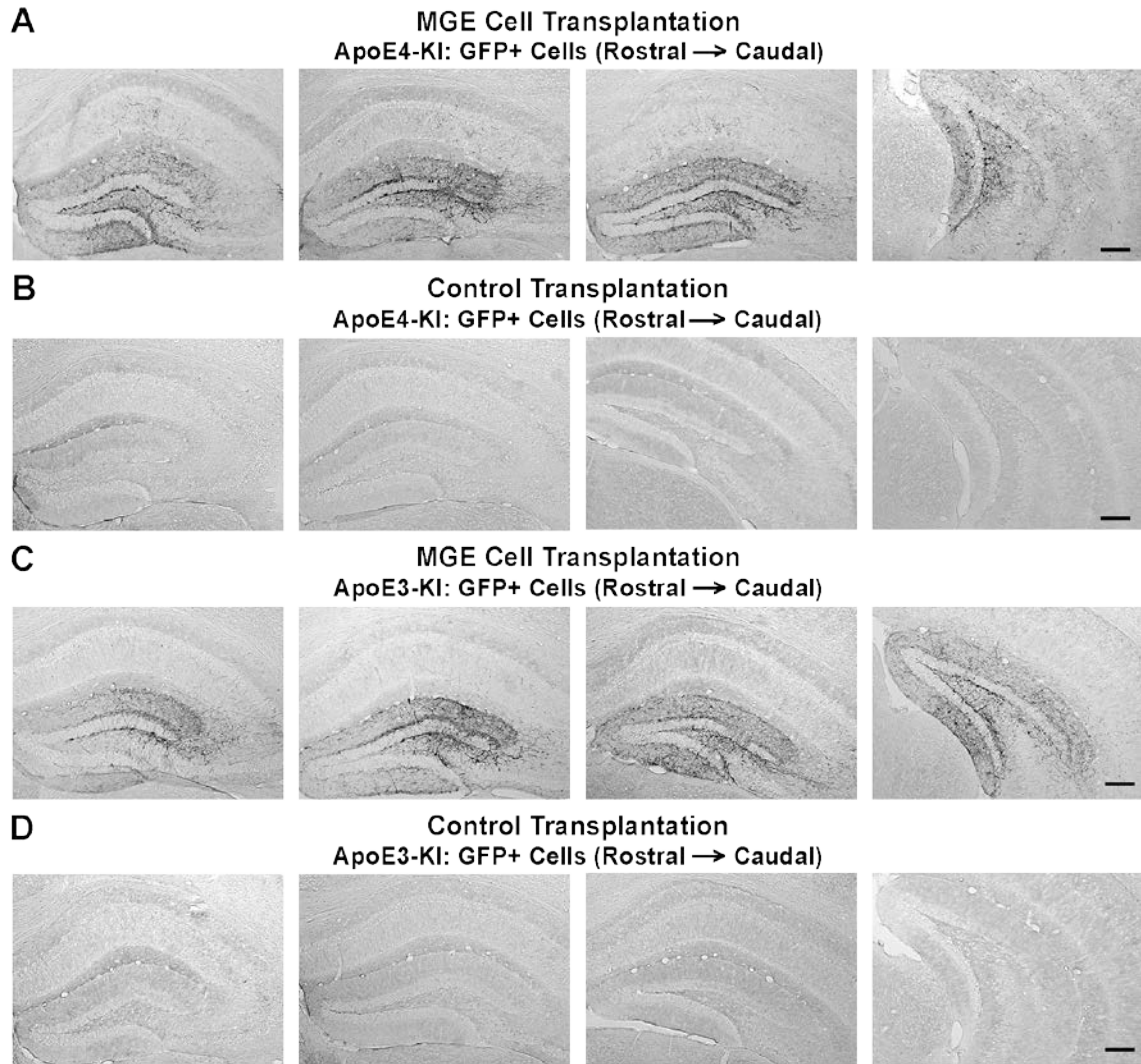
All statistical analyses for electrophysiology were done using IGOR Pro software (WaveMetrics) and all other analyses were done using Prism 6 software (GraphPad). Differences between means were assessed by t-test, one-way ANOVA, or repeated measures ANOVA, followed by Bonferroni or Tukey-Kramer post hoc tests, as noted in text and figure legends. In all cases, p value of less than 0.05 was considered statistically significant. All error bars shown are  $\pm$  standard error from the mean (SEM).

## CHAPTER 3

### **Inhibitory Interneuron Progenitor Transplantation Restores Normal Learning and Memory in ApoE4 Knock-In Mice without or with A $\beta$ Accumulation**

#### **Hilar Transplantation and Survival of MGE-derived GABAergic Interneuron Progenitors**

Freshly dissected MGE cells were transplanted bilaterally into the hilus of 14-month-old female apoE4-KI mice with substantial hilar interneuron loss at rostral and caudal sites (Fig. 1A–C) (Andrews-Zwilling et al., 2010; Leung et al., 2012). Donor cells were collected from the MGE of E13.5 mouse embryos that express green fluorescent protein (GFP) in all cells (Fig. 1B,C) (Alvarez-Dolado et al., 2006; Baraban et al., 2009; Calcagnotto et al., 2010; Southwell et al., 2010; Zipancic et al., 2010; Tanaka et al., 2011; Hunt et al., 2013). Controls included MGE cell-transplanted apoE3-KI mice and control-transplanted apoE3-KI and apoE4-KI mice (Alvarez-Dolado et al., 2006; Baraban et al., 2009; Southwell et al., 2010). The transplanted GFP<sup>+</sup> MGE cells survived at least 90 DAT and migrated throughout the hilus, with neurites extending into the molecular layer of the dentate gyrus in both apoE4-KI (Fig. 2A) and apoE3-KI (Fig. 2C) mice. No GFP<sup>+</sup> cells were found in hippocampi of control-transplanted apoE4-KI and apoE3-KI mice (Fig. 2B,D).

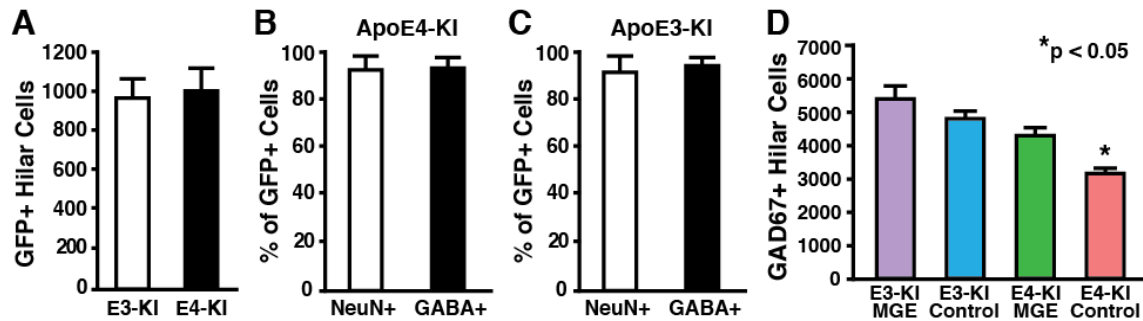


**Figure 2. Immunostaining of GFP+ cells in hippocampal sections (1.2 mm apart) from mice at 80–90 DAT.**

(A) Living MGE cell-transplanted apoE4-KI mice. (B) Control-transplanted apoE4-KI mice. (C) Living MGE cell-transplanted apoE3-KI mice. (D) Control-transplanted apoE3-KI mice. Scale bars: 250  $\mu$ m.



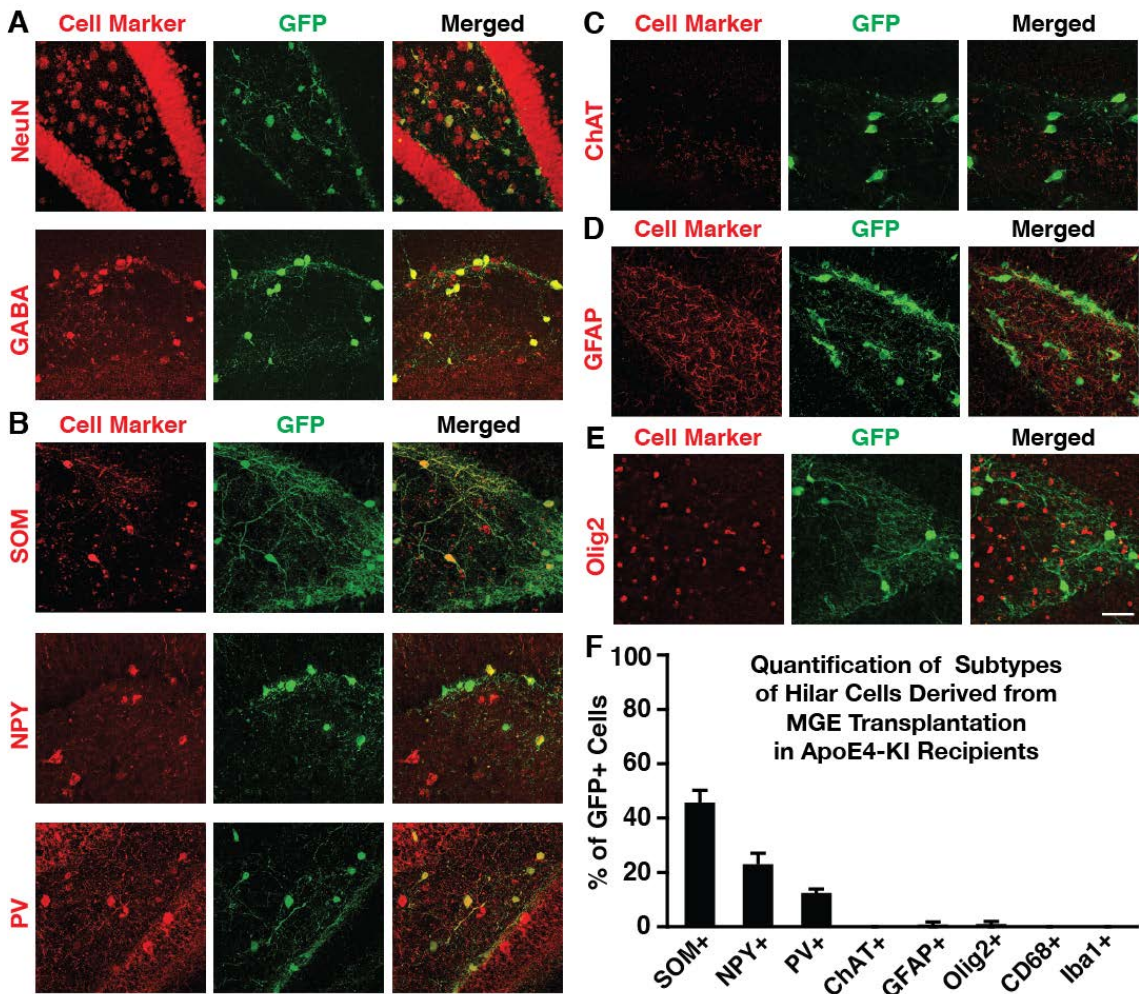
Transplanted MGE cells survived and matured into inhibitory interneurons, as marked by NeuN and GABA, at similar levels in apoE4-KI ( $94.8 \pm 2.3\%$  and  $96.1 \pm 2.1\%$ , respectively) (Fig. 3A,B and Fig. 4A) and apoE3-KI ( $93.6 \pm 4.7\%$  and  $93.8 \pm 3.4\%$ ) (Fig. 3A,C and Fig. 5A) recipients. Importantly, transplantation significantly increased the total number of GAD67+ hilar interneurons in apoE4-KI mice, reaching a level similar to control apoE3-KI mice (Fig. 3D). Transplanted MGE cells developed predominantly into somatostatin (SOM)+ interneurons (E4-KI:  $45.7 \pm 4.4\%$ ; E3-KI:  $45.0 \pm 5.3\%$ ), followed by neuroptide-Y (NPY)+ (E4-KI:  $23.0 \pm 4.1\%$ ; E3-KI:  $23.8 \pm 3.5\%$ ) and parvalbumin (PV)+ interneurons (E4-KI:  $12.5 \pm 2.4\%$ ; E3-KI:  $12.1 \pm 2.6\%$ ), in apoE4-KI (Fig. 4B,F) and apoE3-KI (Fig. 5B,F) mice.



**Figure 3. Quantification of GFP+ and total hilar GABAergic interneurons in MGE cell-transplanted apoE4-KI and apoE3-KI mice at 80–90 DAT.**

(A) Quantification of total GFP+ cells in the hilus (n=9–12 sections per brain, 11–13 mice per group). (B–C) Quantification of GFP+ cells that were also positive for the mature neuronal marker NeuN and inhibitory neurotransmitter GABA in apoE4-KI (B) and apoE3-KI (C) mice (n=5–8 sections per brain, 3–5 mice per group). (D) MGE cell transplantation significantly increased the total number of GAD67+ cells in the hilus of apoE4-KI mice (n=9–12 sections per brain, 11–13 mice per group). Values are mean  $\pm$  SEM. \*p<0.05 versus other groups (*one way ANOVA*).

### Subtypes of Hilar Cells Derived from MGE Transplantation in ApoE4-KI Recipients



**Figure 4.** Immunofluorescent analyses of neuronal subtypes derived from transplanted MGE cells in the hippocampal hilus of apoE4-KI mice at 80–90 DAT.

(A) Transplanted MGE cells were stained positive for NeuN and GABA. (B)

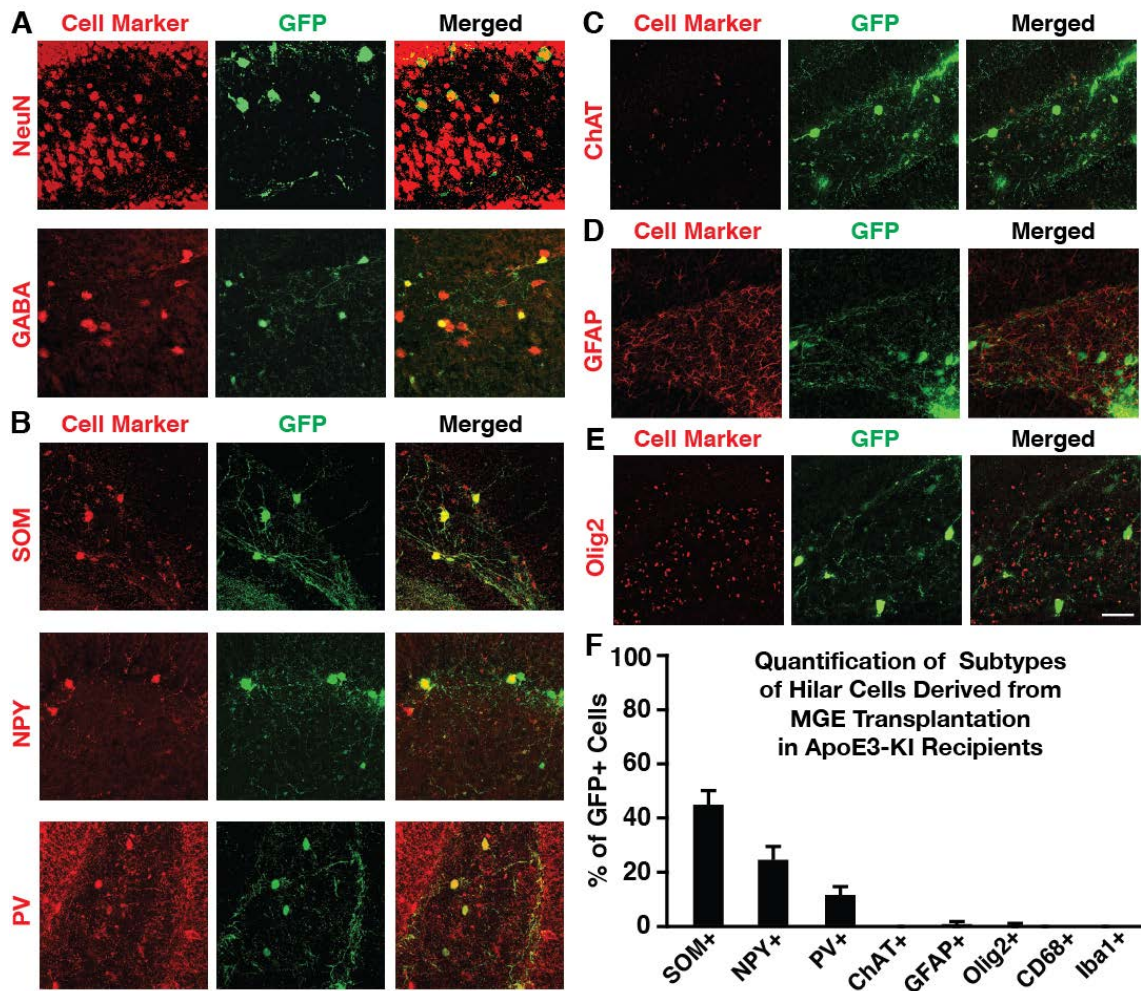
Immunofluorescent co-staining of inhibitory interneuron subtypes positive for SOM, NPY, and PV. (C–E), Immunostaining of cells that were positive for ChAT (C), GFAP (D), or

Olig2 (E). (F) Quantification of percentage of GFP+ cells that were also positive for

different cell markers (n=5–8 sections per brain, 3–5 mice per group for each cell marker).

Values are mean  $\pm$  SEM. Scale bar 50  $\mu$ m.

## Subtypes of Hilar Cells Derived from MGE Transplantation in ApoE3-KI Recipients



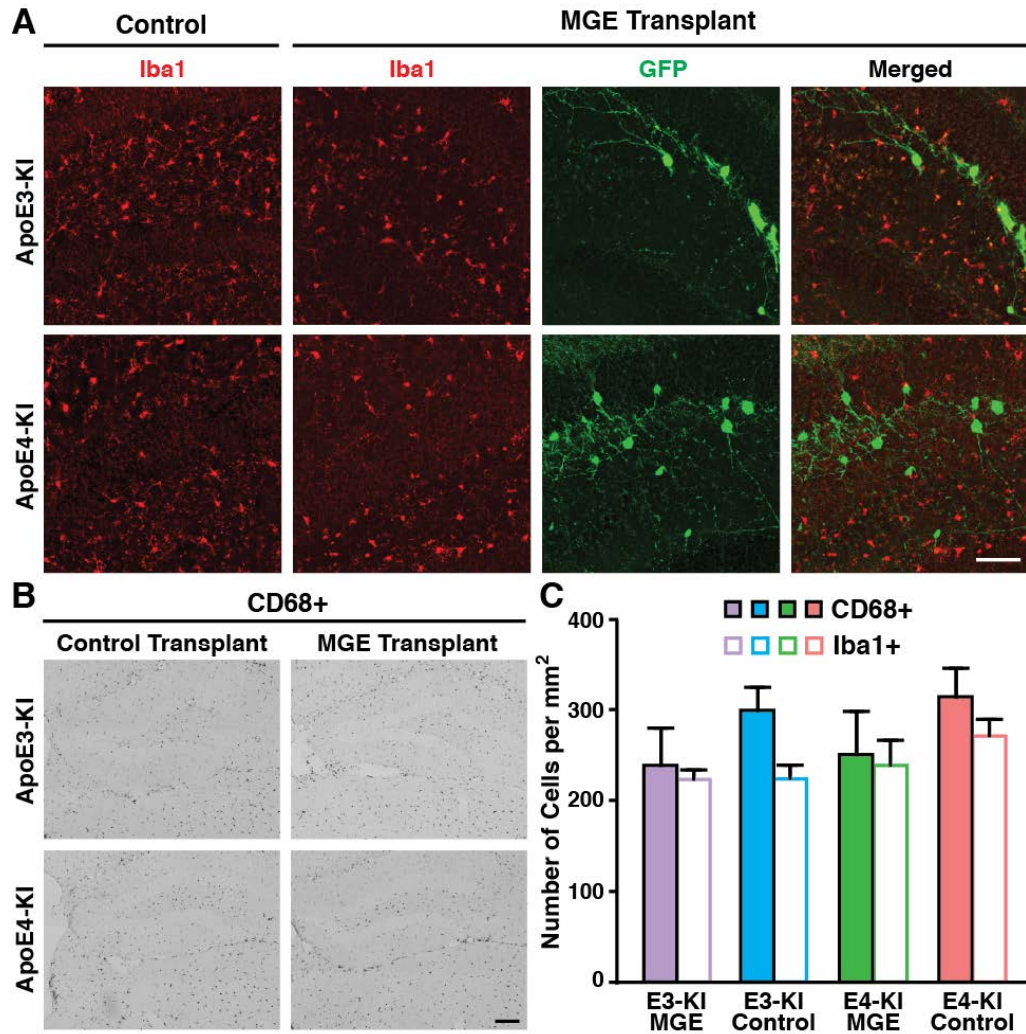
**Figure 5. Immunofluorescent analyses of neuronal subtypes derived from**

**transplanted MGE cells in the hippocampal hilus of apoE3-KI mice at 80–90 DAT.**

(A) Transplanted MGE cells were stained positive for NeuN and GABA. (B) Immunofluorescent co-staining of inhibitory interneuron subtypes positive for SOM, NPY, and PV. (C–E), Immunostaining of cells that were positive for ChAT (C), GFAP (D), or Olig2 (E). (F) Quantification of percentage of GFP+ cells that were also positive for different cell markers (n=5–8 sections per brain, 3–5 mice per group for each cell marker). Values are mean  $\pm$  SEM. Scale bar 50  $\mu$ m.

There were no significant differences of the GFP+ MGE-derived interneuron subtypes between apoE4-KI and apoE3-KI mice (Fig. 4F and Fig. 5F). Negligible numbers of ChAT+, GFAP+, Olig2+, CD68+, or Iba1+ cells were derived from the transplanted GFP+ MGE cells at 90 DAT (Fig. 4C–F and Fig. 5C–F). No neuroinflammatory response to MGE cell transplantation was detected, as indicated by similar microglia densities in MGE- and control-transplanted mouse hippocampal sections immunostained at 90 DAT for Iba1 and CD68 (Fig. 6).





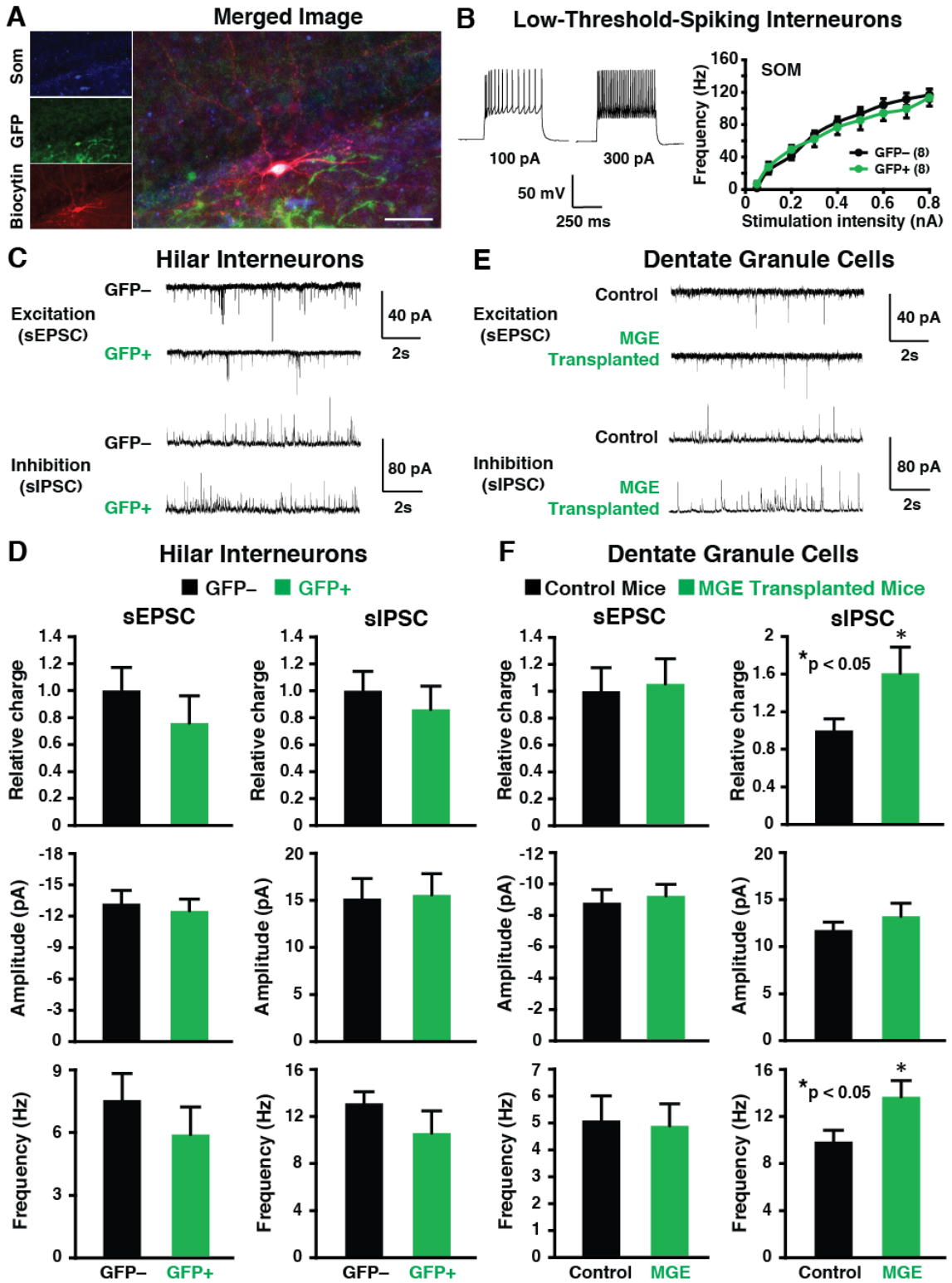
**Figure 6. Examination of microglia as a marker for inflammation in transplant and control mice.**

(A) Immunofluorescent staining of microglial marker allograft inflammatory factor 1 (Iba1) showed no microglia clustering around the grafted cells. (B) Immunostaining of activated microglia marker CD68 showed no significant differences between MGE cell-transplanted and control mice. (C) Quantification of CD68+ and Iba1+ cells per mm<sup>2</sup> (n=5–8 sections per brain, 3–5 mice per group). Values are mean ± SEM. Scale bars are 50 μm in A and 250 μm in B.

## **Functional Maturation and Integration of Transplanted MGE-derived GABAergic Interneurons in the Hippocampus**

Electrophysiological recordings in acute hippocampal slices from 17-month-old mice at 80–90 DAT confirmed functional maturation and integration of transplanted MGE cells. Patch-clamp recordings from most transplanted GFP<sup>+</sup> cells (Fig. 7A and Fig. 8A) revealed intrinsic firing properties typical of SOM<sup>+</sup> low-threshold spiking interneurons that did not differ significantly from endogenous (GFP<sup>-</sup>) interneurons in apoE4-KI (Fig. 7B) and apoE3-KI (Fig. 8B) mice. Post-hoc staining of these cells filled with biocytin during recording revealed that they were indeed SOM-positive interneurons (Fig. 7A). The transplanted GFP<sup>+</sup> MGE cells displayed spontaneous excitatory and inhibitory post-synaptic currents of comparable amplitude and frequency as endogenous hilar interneurons (Fig. 7C,D and Fig. 8C,D), suggesting their complete integration in the host hippocampal network in both apoE4-KI (Fig. 7C,D) and apoE3-KI (Fig. 8C,D) recipients. Most importantly, the dentate granule neurons in MGE cell-transplanted brains showed increased frequency of spontaneous inhibitory currents and increased inhibitory charge transfer relative to control-transplanted brains, indicating that the grafted interneurons functionally modified the hippocampal circuitry (Fig. 7E,F and Fig. 8E,F).

# Electrophysiological Analyses of Hippocampal Slices from ApoE4-KI Mice



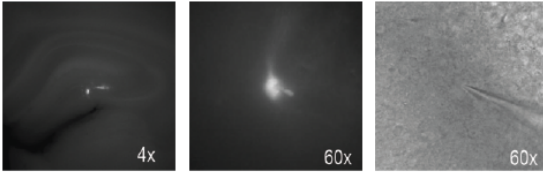


**Figure 7. Electrophysiological analyses of transplanted and endogenous cells in acute hippocampal slices from 17-month-old apoE4-KI mice at 80–90 DAT.**

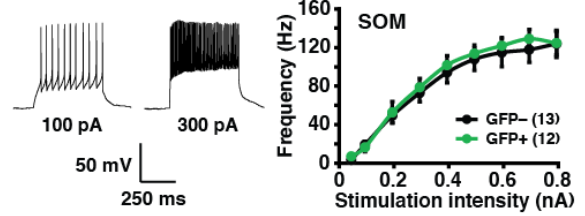
(A) Post-hoc staining of a low-threshold-spiking GFP+ interneuron filled with biocytin during recording was positive for SOM. Scale bar, 50  $\mu$ m. (B) Representative voltage traces from a GFP+ hilar cell are shown. The intrinsic excitability study (frequency of firing versus stimulation intensity) of GFP+ hilar cells revealed firing properties indistinguishable from endogenous (GFP-) hilar cells. (C-D) Transplanted GFP+ cells displayed spontaneous excitatory (sEPSC) and inhibitory (sIPSC) post-synaptic currents (C) at frequencies and amplitudes (D) comparable to endogenous (GFP-) hilar interneurons. (E-F) Current traces (E) from dentate granule neurons in control and MGE cell-transplanted apoE4-KI mice show no quantifiable changes (F) in sEPSC, but a significant increase in the frequency of sIPSC, leading to an overall increase in inhibitory charge transfer. Values are mean  $\pm$  SEM. \* $p < 0.05$  *t*-test. n=8–15 cells per group.

# Electrophysiological Analyses of Hippocampal Slices from ApoE3-KI Mice

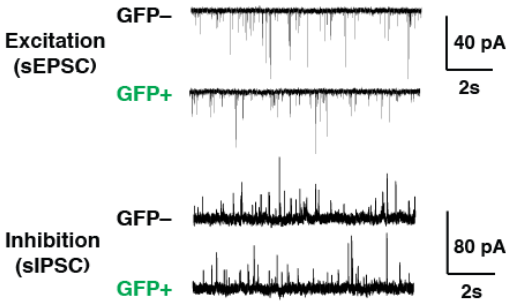
## A Recording from GFP+ Hilar Cells



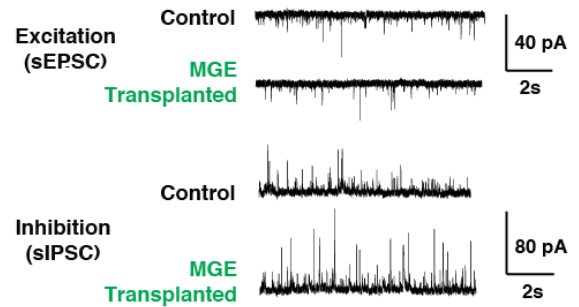
## B Low-Threshold-Spiking Interneurons



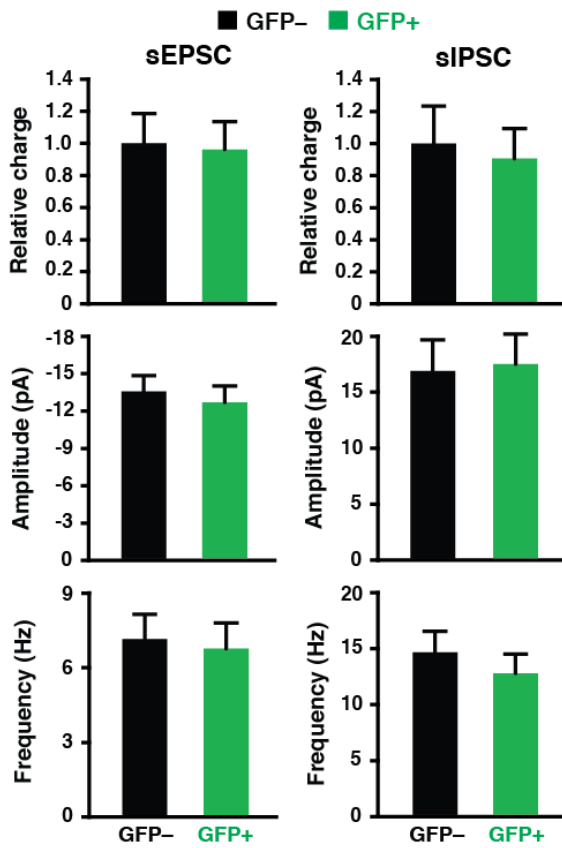
## C Hilar Interneurons



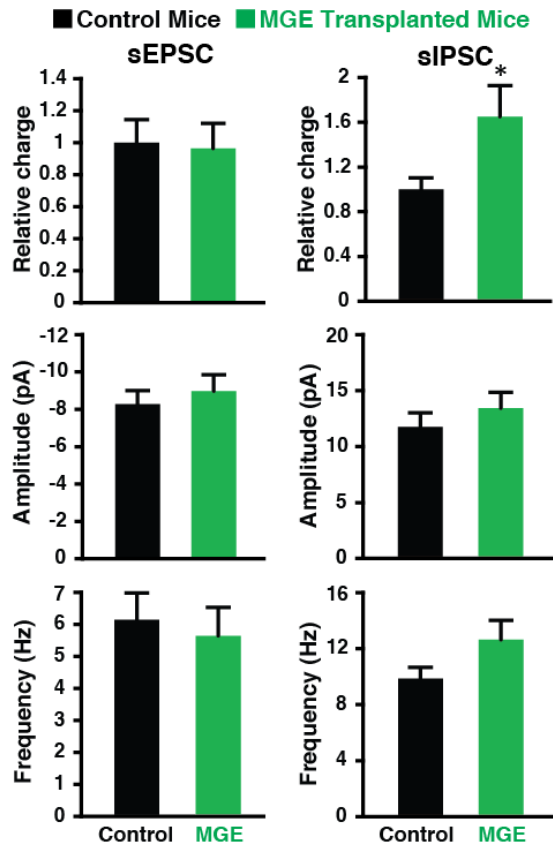
## E Dentate Granule Cells



## D Hilar Interneurons



## F Dentate Granule Cells

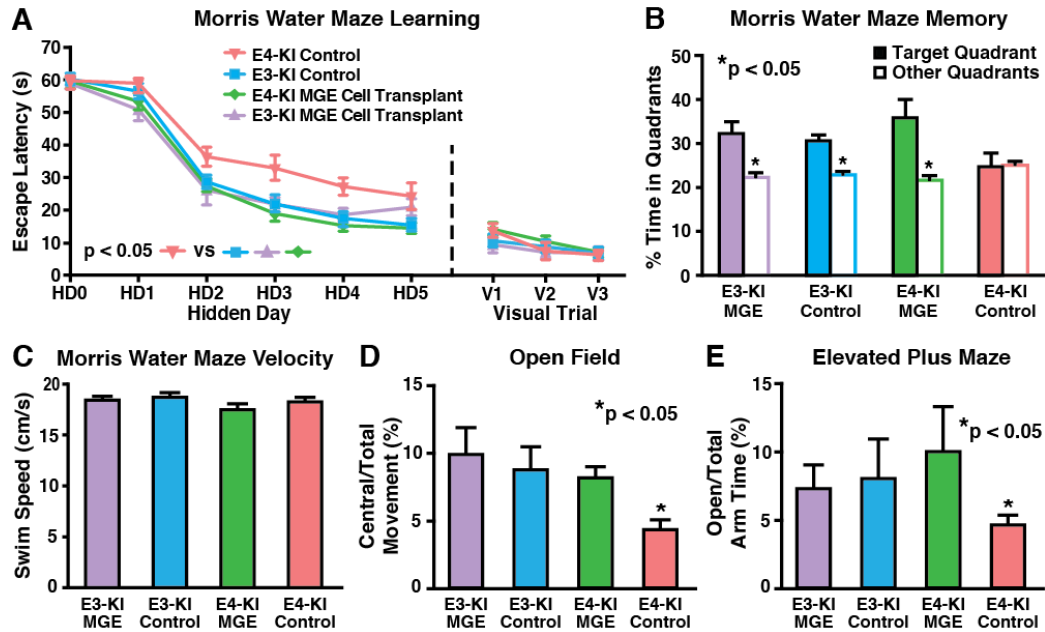


**Figure 8. Electrophysiological analyses of transplanted and endogenous cells in acute hippocampal slices from 17-month-old apoE3-KI mice at 80–90 DAT.**

(A) Fluorescent and bright field images (4x and 60x) of a GFP+ hilar cell during electrophysiological recording. (B) Representative voltage traces from a GFP+ hilar cell are shown. The intrinsic excitability study (frequency of firing versus stimulation intensity) of GFP+ hilar cells revealed firing properties indistinguishable from endogenous (GFP-) hilar cells. (C-D) Transplanted GFP+ cells displayed spontaneous excitatory (sEPSC) and inhibitory (sIPSC) post-synaptic currents (C) at frequencies and amplitudes (D) comparable to endogenous (GFP-) hilar interneurons. (E-F) Current traces (E) from dentate granule neurons in control and MGE cell-transplanted apoE3-KI mice show no quantifiable changes (F) in sEPSC, but a significant increase in inhibitory charge transfer. Values are mean  $\pm$  SEM. \* $p < 0.05$  *t-test*. n=8–15 cells per group.

## **Transplantation of MGE-derived GABAergic Interneurons Rescues ApoE4-induced Cognitive Deficits in Aged Mice**

To determine the behavioral effects of MGE cell transplantation, mice were evaluated for cognitive function in the Morris water maze (MWM). MGE cell transplantation restored normal learning in aged apoE4-KI mice at 70–80 DAT, matching the performance of apoE3-KI mice (Fig. 9A). MGE cell transplantation also rescued apoE4-induced memory deficits in a probe trial (Fig. 9B). No vision or motor deficits were apparent, as determined by visible trials (Fig. 9A) and swim speed (Fig. 9C). The results of open field and elevated plus maze assays provided additional evidence that MGE cell-transplantation corrected the abnormal activity, anxiety, and exploratory behaviors of apoE4-KI mice (Fig. 9D,E). In all behavioral tests, no significant differences were observed between control and MGE cell-transplanted apoE3-KI mice (Fig. 9), suggesting that moderately increasing the numbers of hilar inhibitory interneurons in unimpaired brains does not alter learning, memory, or other behaviors.

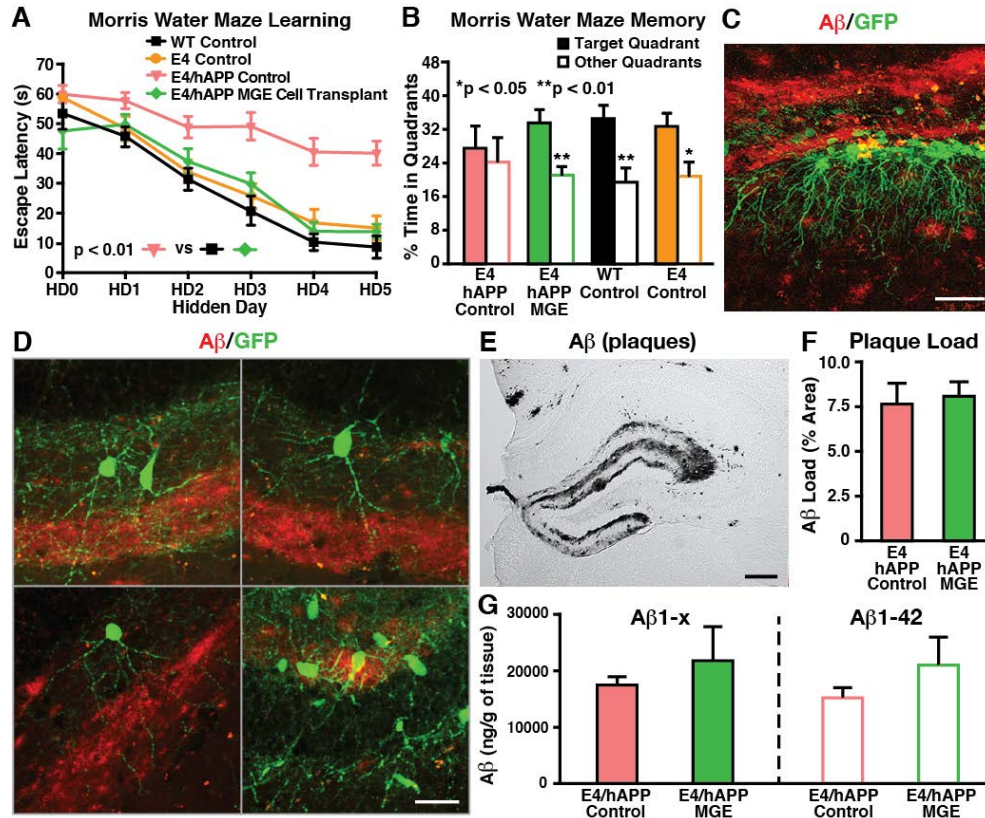


**Figure 9. MGE cell-transplantation rescued behavioral deficits of 17-month-old apoE4-KI mice at 70–80 DAT.**

(A) Morris water maze (MWM) test showed a rescue of learning over 5 days of hidden platform trials in MGE-cell transplanted apoE4-KI mice, with no differences in visual trials. Points represent averages of daily trials. HD, hidden platform day (two trials/session, two sessions/day); HD0, first trial on HD1; V, visible platform day (two trials/session, two sessions/day). Y-axis indicates time to reach the target platform (escape latency, mean  $\pm$  SEM). (B) The probe trial of MWM test showed a rescue of memory 120 hours after the last learning trial in MGE-cell transplanted apoE4-KI mice. (C) Swim speeds did not differ significantly among all groups. (D-E) MGE cell transplantation ameliorated apoE4-induced behavioral deficits in the open field (D) and elevated plus maze (E) tests. Values are mean  $\pm$  SEM. \* $p < 0.05$  repeated-measures ANOVA (A), one-way ANOVA (C), and *t*-test (B,D,E).  $n = 9-13$  mice per group.

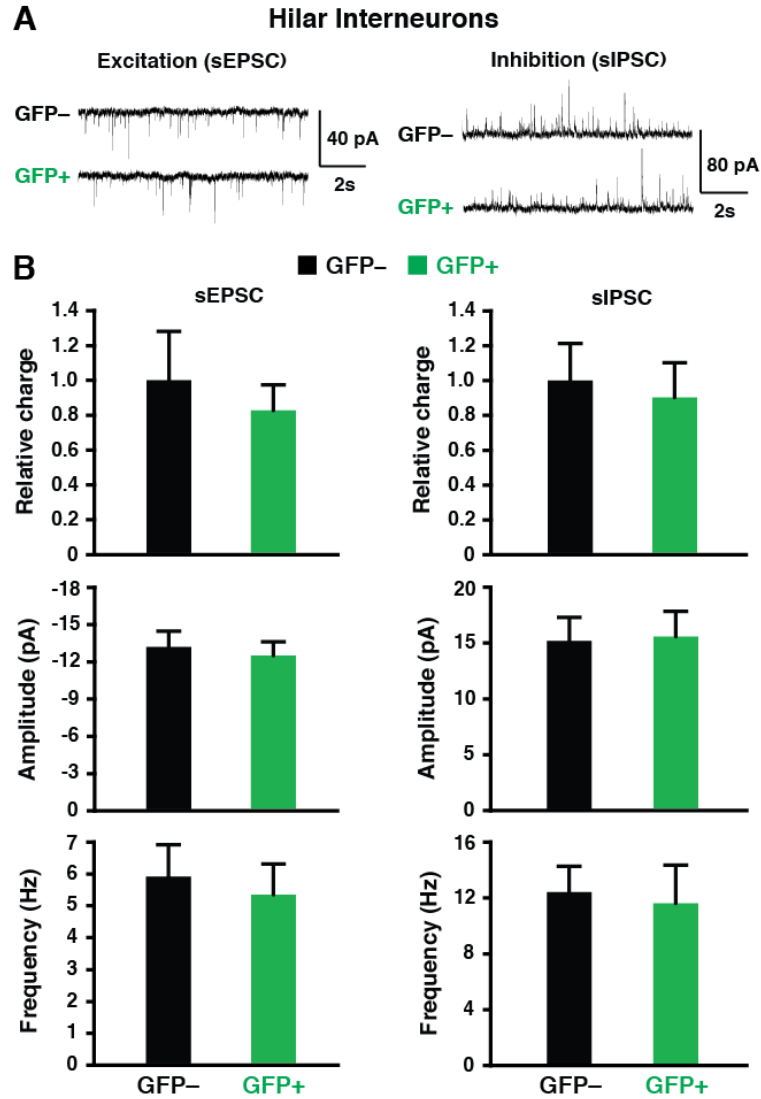
## **Transplantation of MGE-derived GABAergic Interneurons Rescues Cognitive Deficits in the Presence of A $\beta$ Accumulation in ApoE4-KI Mice**

In AD patients, apoE4 is often associated with enhanced brain A $\beta$  accumulation (Huang and Mucke, 2012); A $\beta$  also impairs GABAergic interneurons (Verret et al., 2012). ApoE4-KI mice expressing human APP with familial AD (FAD)-causing mutations (apoE4-KI/hAPPFAD) accumulate high levels of A $\beta$  (Bien-Ly et al., 2012) and exhibit severe learning and memory impairments as early as 3 months of age (Palop et al., 2007; Verret et al., 2012). To explore whether MGE cell-transplantation rescues learning and memory deficits in the presence of both A $\beta$  accumulation and apoE4, we transplanted MGE cells into the hilus of the dentate gyrus of 10-month-old apoE4-KI/hAPPFAD mice that displayed severe learning and memory deficits (Fig. 9Fig. 10A,B). Age-matched wild-type mice without transplantation were used as controls. ApoE4-KI mice without hAPPFAD expression, which did not develop learning and memory deficits at 12 months of age (Leung et al., 2012), were also included as controls. Strikingly, MGE cell-transplantation completely rescued both learning and memory deficits in apoE4-KI/hAPPFAD mice to levels indistinguishable from wild-type mice when tested two months later (Fig. 10A,B). MGE cell transplantation did not significantly alter hippocampal plaque loads or A $\beta$  levels (Fig. 10E–G). Histological analysis revealed that transplanted GFP+ MGE cells survived well in the hippocampal circuitry in the presence of A $\beta$  plaque buildup (Fig. 10C,D). Electrophysiological analyses confirmed that transplanted GFP+ MGE cells functionally integrated in the hippocampal circuitry in the presence of A $\beta$  plaques (Fig. 11).



**Figure 10. MGE cell-transplantation rescued learning and memory deficits of 12-month-old apoE4-KI/hAPP<sub>FAD</sub> mice at 70–80 DAT.**

(A-B) MWM test of learning (A) and memory (B) for transplanted and control apoE4-KI/hAPP<sub>FAD</sub>, wild-type, and apoE4-KI mice (n=7–12 mice per group). (C-D) Transplanted GFP<sup>+</sup> cells survived and integrated in the dentate gyrus in the presence of plaques (C), with mature morphologies and processes extending through the plaques (D). (E-G) DAB-immunostaining of hippocampal Aβ (E) as well as quantification of plaque load (F) and Aβ levels (G) showed no significant effects of MGE cell transplantation on Aβ accumulation. n=5–8 sections per brain, 5 mice per group. Values are mean ± SEM. \*\*p<0.01 repeated-measures ANOVA (A) and t-test (B). Scale bars are 50 μm in C, 20 μm in D, and 250 μm in E.



**Figure 11. Electrophysiological analyses of transplanted and endogenous cells in acute hippocampal slices from 12-month-old apoE4-KI/hAPP<sub>FAD</sub> mice at 80–90 DAT.**

(A) Recorded sEPSCs and sIPSCs traces in transplanted GFP+ and endogenous GFP- hilar interneurons. (B) Quantification of post-synaptic currents showed similar sEPSC and sIPSC relative charge, amplitude, and frequency in transplanted GFP+ and endogenous GFP- hilar interneurons (n=12–18 cells per group).



## CHAPTER 4

### Discussion and Conclusion

Inhibitory interneuron impairments are a feature of both AD-related mouse models and human AD patients, and interneurons are impaired by both apoE4 and A $\beta$  (Huang and Mucke, 2012). Our lab has previously shown that apoE4-KI mice have a significant age-dependent decrease in hilar GABAergic interneurons that correlates with the extent of apoE4-induced learning and memory deficits in aged mice (Li et al., 2009; Andrews-Zwilling et al., 2010; Leung et al., 2012). Similarly, A $\beta$  overproduction or accumulation impairs interneuron function, leading to aberrant dentate gyrus activity and learning and memory deficits (Palop et al., 2007; Verret et al., 2012). The current study highlights the importance of inhibitory interneuron impairments in age-related cognitive decline in mouse models of AD. We demonstrate that lost cells can be directly replaced by transplantation of embryonic MGE-derived inhibitory interneuron progenitors into the hippocampal hilus of aged mice. In all measures examined, these cells restore neuronal network function and behavior in two widely used AD-related mouse models—apoE4 expression without and with A $\beta$  accumulation (Huang and Mucke, 2012).

#### **The Detrimental Effects of ApoE4 on Hilar GABAergic Interneurons Are Cell-autonomous**

Strikingly, the grafted MGE-derived inhibitory interneurons not only survive, but functionally integrate in the hippocampus of aged mice (12–17 months) despite an apparently toxic environment. Since wild-type mouse MGE-derived GABAergic interneuron progenitors, which express wild-type mouse apoE, survive and integrate equally well in the hippocampal hilus of apoE3-KI and apoE4-KI mice (including those

with significant A $\beta$  plaque buildup) for at least three months, this provides further support that the detrimental effects of apoE4 on hilar GABAergic interneurons are cell-autonomous. As described in the Introduction (Knöferle et al., 2014), the apoE4 produced in interneurons themselves appears largely responsible for learning and memory deficits in these mice (Knöferle et al., 2014). This was determined by using cell-type specific cre-driver lines crossed with the apoE-fKI mice described previously (Bien-Ly et al., 2012). Mice with apoE4 removed from astrocytes showed no improvement to learning and memory nor interneuron loss, whereas pan-neuronal apoE4 removal or interneuron-specific apoE4 removal was sufficient to rescue both deficits (Knöferle et al., 2014). Earlier studies, such as transgenic neuron-specific expression of apoE4, also showed that apoE4 expressed by neurons increased phosphorylated tau and severe impairments to cognitive behavioral performance (Brecht et al., 2004). In contrast, astrocyte promoter-driven apoE4 expression alone caused neither phosphorylated-tau increases nor behavioral impairment (Brecht et al., 2004).

This study and others (Alvarez-Dolado et al., 2006; Zipancic et al., 2010; Tanaka et al., 2011; Hunt et al., 2013; Valente et al., 2013) have demonstrated that host environment influences the eventual ratios of mature interneuron subtypes that arise from the grafted cells. Typically, they reflect ratios comparable those present in the endogenous anatomical structure (Alvarez-Dolado et al., 2006; Tanaka et al., 2011; Hunt et al., 2013; Valente et al., 2013).

Taken together, the cell-autonomous aspects will be important for any potential stem cell-based therapy of AD in the future, indicating that transplanted human MGE-like cells

without apoE4 expression or A $\beta$  overproduction might have a good chance to survive and functionally integrate in brains of AD patients.

### **The SOM Subtype of Hilar Interneurons Are Particularly Vulnerable to ApoE4 and May Play a Critical Role in AD Pathogenesis**

When transplanted into the dentate of adult mice, the current study and others (Alvarez-Dolado et al., 2006; Hunt et al., 2013) demonstrate that the predominant interneuron subtype derived from MGE cells is SOM positive. The age-dependent decline in hilar interneurons is predominantly for the subtype positive for SOM in both normal aging and in apoE4-KI females, which has the strongest correlation with cognitive impairments (Andrews-Zwilling et al., 2010; Leung et al., 2012; Stanley et al., 2012; Spiegel et al., 2013; Koh et al., 2014). It has also long been known that SOM levels are decreased in human AD patients (Davies et al., 1980). SOM itself plays a variety of roles throughout the brain, especially in the hypothalamus where it is involved in suppressive metabolic activities such as inhibition of growth hormone secretion (Epelbaum, 1986; Epelbaum et al., 2009). It is also a prominent neuropeptide in a subset of interneurons where it is typically co-released upon action potential firing, but not all of its roles are clear (Epelbaum, 1986; Mattis et al., 2014). The SOM cells and their circuits have historically received much less attention than the cholinergic neurons in the study of AD pathogenesis and in the development of AD treatments (Epelbaum et al., 2009). Many SOM+ projections exist between the dentate gyrus and the cholinergic nuclei-containing septum of the basal forebrain (Bassant et al., 2005; Amaral et al., 2007). It is conceivable that the observed loss of cholinergic neurons in AD patients is due in part to the decline in SOM+ projecting neurons.

## **Stabilizing the Network and AD Pathogenesis and Treatment**

Recent research has highlighted the changes of the brain network activity in AD patients (Palop and Mucke, 2010; Huang and Mucke, 2012). Dysfunction of the GABAergic system is likely a substantial contributor to cognitive impairment in humans in a way similar to that seen in rodents. Natural progressive inhibitory interneuron dysfunction or loss (Stanley et al., 2012; Spiegel et al., 2013; Koh et al., 2014) is almost certainly involved in the memory decline during healthy aging as it leads to slight imbalances in brain activity (Hardy et al., 1987; Huang and Mucke, 2012). Furthermore, GABA levels in human CSF decrease with age (Bareggi et al., 1982)—the strongest risk factor for AD. Pathological conditions may be a reflection of extreme cases of GABAergic system decline. AD patients have decreased GABA and SOM levels in the brain and CSF (Davies et al., 1980; Bareggi et al., 1982; Zimmer et al., 1984; Hardy et al., 1987; Sunderland et al., 1987; Reinikainen et al., 1990; Seidl et al., 2001; Burgos-Ramos et al., 2008; Epelbaum et al., 2009; Martel et al., 2012) and these alterations were more severe in apoE4 carriers (Grouselle et al., 1998).

As the disease progresses, overall brain shrinkage is in part due to the death of neurons themselves, but shrinkage and loss of neuronal processes may actually be the major contributing factor to gross volume decline. Furthermore, the loss of synapses and spines, rather than cell death, may account observed patient behavior, which is often described as day-to-day fluctuations (“good days” and “bad days”), but gradually declining on average overall (Palop et al., 2006). This could be a reflection of impaired GABAergic inhibitory control, which initially appear most severe in the dentate (Palop and Mucke, 2010; Huang and Mucke, 2012). It has also been observed that some of the

earliest functional changes in the brains of early AD patients and patients-to-be, including those with amnesic mild cognitive impairment (aMCI), are the increased activity in the default mode network (DMN) and hyperactivation of hippocampal region in the medial-temporal lobe when challenged with memory tasks (Sperling et al., 2009; Yassa et al., 2011; Bakker et al., 2012). The DMN is evaluated with fMRI and is described to have higher activity in healthy individuals when they are not thinking about anything in particular, and its activity goes down under a cognitive task (DMN deactivation) (Sperling et al., 2009). High-resolution fMRI points the hyperactivity in MCI patients to the dentate gyrus-CA3 region of the hippocampus (Yassa et al., 2010), which parallels the findings of GABAergic hypofunction in the dentate in aged apoE4-KI mice (Li et al., 2009; Andrews-Zwilling et al., 2010; Leung et al., 2012). Even at a young age, in the absence of any clinical behavioral symptoms, apoE4-carriers also display increased brain activity in the DMN both at rest and in response to memory tasks, suggestive of inherent vulnerability and/or early changes that take place within the brain decades before the onset of AD (Filippini et al., 2009; Dennis et al., 2010).

Epileptiform activity in patients with mild cognitive impairment and early AD is associated with poorer prognosis and faster cognitive decline (Vossel et al., 2013). Epileptiform brain activity and abnormal EEG network signal has also been characterized in the hAPP<sub>FAD</sub> mice and attributed to deficits in voltage-gated sodium channel, NaV1.1 expressed in the PV-positive subtype of interneurons (Verret et al., 2012). Other AD models generated with both amyloidosis and tauopathy also display selective degeneration of hippocampal GABAergic neurons (Loreth et al., 2012). Recently, two groups have shown that GABA is aberrantly released from dentate gyrus astrocytes in

AD mouse models with over-expressed mutant APP (Jo et al., 2014; Wu et al., 2014). These observations are not necessarily incompatible with the decreases in GABAergic signaling and immunohistochemistry seen by many other groups in various models. One possible explanation is that this model has a different mechanism that leads to network imbalance, involving aberrant release of GABA from astrocytes. Another possibility is the models used might reflect later stages of disease progression. Another recent study has postulated that APP physiological function may actually be to regulate phasic and tonic GABAergic inhibition in the dentate (Wang et al., 2014).

The conclusions that pathological decreases in dentate GABAergic signaling versus aberrant excessive hippocampal astrocytic GABA release are seemingly opposite. However, they are not necessarily mutually exclusive considering the use of different animal models, which might reflect different states of the disease. They both indicate that network balance is severely disrupted in AD. This supports the call to develop novel therapeutics that aim to balance this activity may lead to a functional treatment for the disease (Cui et al., 2008; Morellini et al., 2010; Andrews-Zwilling et al., 2012). As demonstrated in this proof-of-concept study, embryonic-derived inhibitory interneurons can indeed provide some degree of network re-balancing in order to restore learning and memory behaviors.

### **Unique Properties of Interneurons Are Well-suited for Cell-based Therapeutics**

MGE-born interneurons are unique in their remarkable properties to migrate through and functionally integrate into pre-existing brain tissue and neuronal networks (Pleasure et al., 2000; Marín and Rubenstein, 2001; Wonders and Anderson, 2006; Kriegstein and Alvarez-Buylla, 2009; Bartolini et al., 2013). Perhaps these migratory properties exist

because they originate from a concentrated location in the developing embryonic brain. This, combined with their very nature being interneurons, receiving feedback to fine tune and regulate a signaling system, make them ideal candidates for cell transplantation therapeutics. A key aspect of hilar GABAergic interneurons is that one such inhibitory neuron connects to and thus influences thousands of excitatory granule neurons in the dentate gyrus (Amaral et al., 2007; Morgan et al., 2007; Myers and Scharfman, 2009) . This suggests that, even if a small number of the transplanted therapeutic cells survive and functionally integrate, they could make a significant contribution to the dentate gyrus function and thus learning and memory.

Interestingly and importantly, the transplanted MGE cells appear to have minimal effect on learning and memory in apoE3-KI recipients with normal cognition. We previously reported a threshold number of hilar GABAergic interneurons (~2500–3000) that appeared to distinguish normal versus impaired learning and memory in female apoE4-KI mice at 16 months of age (Andrews-Zwilling et al., 2010; Leung et al., 2012). At this age, all female apoE3-KI mice had >3000 hilar GABAergic interneurons (~3000–5200), whereas a substantial portion of the female apoE4-KI mice had <3000 interneurons in the hilus, and those individuals typically showed the greatest learning deficits. Thus, this may explain why adding ~1000 functionally integrated hilar interneurons by MGE cell transplantation significantly improved learning and memory performance in female apoE4-KI mice. Once above this potential threshold of hilar interneuron numbers, we observed no apparent correlation between their numbers and learning and memory performance. In apoE3-KI mice, for instance, hilar interneuron numbers varied between 3000 and 5200 (Andrews-Zwilling et al., 2010; Leung et al.,

2012). Above the threshold, the normal network activity is unaffected by additional GABAergic neurons, and not surprisingly, hilar MGE cell transplantation, which adds ~1000 interneurons, has minimal effect on normal learning and memory in apoE3-KI mice.

Other studies have demonstrated that transplanted MGE-derived cells undergo apoptosis at stereotypical rates and ratios (about 40%) regardless of total transplanted cell number, with transplant ranges varying 200-fold (Southwell et al., 2012). This is further indication that the brain can support large amounts of additional interneurons without any substantial effects observed on behavior or otherwise (Southwell et al., 2012 and unpublished data). The reason for this may lie in the inherent capability of these inhibitory cells to bring fine-tuned homeostasis to the network via negative feedback.



## CHAPTER 5

### Future Directions

To date, among all efforts to develop therapies to prevent, slow, or reverse the pathology and cognitive decline in AD, a few of them have yielded treatments with minimal effects and unpleasant side effects, and the vast majority, including all those targeting the APP-processing pathway, have outright failed in human clinical trials (Golde et al., 2011; Huang and Mucke, 2012). As a result, there is emerging consensus that our current treatments are given too late in the disease stage. Thus, we urgently need new approaches for combatting the disease (Huang and Mucke, 2012). In this regard, strategies that target network imbalance, including cell-replacement therapies, may hold potential for treating AD patients. A major constraint with the use of embryonic inhibitory interneuron progenitors is the limited availability of human cells and the obvious ethical concerns associated with their collection and usage. This also limits the ability to study and characterize the human MGE. It is not even completely clear that human MGE-derived cells will behave the same as their mouse counterparts (Hansen et al., 2013).

The work presented here is a proof-of-concept study that provides support for efforts to develop inhibitory interneuron from alternative sources before even attempting pre-clinical studies to develop replacement therapies for AD. A readily available source of interneurons that behave in humans the way mouse MGE-derived cells behave in mice needs to be developed first. Such a population could be immensely useful in treating a wide range of diseases that have been previously studied in other mouse MGE-transplants (Southwell et al., 2010; Tyson and Anderson, 2014). Potential sources could include fetal

tissue that has been manipulated to become self-renewing or cells from directed in vitro differentiation of embryonic stem cells (ESCs). Perhaps most ethnically attractive and most immunologically compatible for transplant would be interneurons derived from patient-specific induced pluripotent stem cells (iPSCs) or from direct transdifferentiation of somatic cells.

It is also unclear if patient-derived cells would require genome editing. In this study, the transplanted MGE-cells contained mouse apoE rather than human apoE3 or apoE4. Before advancing to the clinic, it will be important to determine cell autonomous effects of apoE isoform in the transplanted cells. This biology could be dissected by comparing survival, integration, and behavior in apoE4-KI recipients by using MGE cells taken from apoE3-KI and apoE4-KI mice. This could also be compared to survival and integration in a wildtype recipient to represent a more neutral host brain environment.

Several groups have already taken the first steps toward developing cortical interneuron progenitors from both human ESCs and iPSCs (Liu et al., 2013; Maroof et al., 2013; Nicholas et al., 2013; Kim et al., 2014). One even demonstrated that transplantation of these cells can rescue learning and memory deficits induced by acute hippocampal lesion in mice (Liu et al., 2013). Another has shown the amelioration of seizures in epileptic mice (Cunningham et al., 2014). Additional studies are needed to determine the extent to which these ESC-derived human cells are MGE-like and what can be done to improve upon their derivation.

We have conducted preliminary experiments and have planned further study examining the effects of transplanted human embryonic stem cell-derived MGE-like cells in apoE4-KI mice in collaboration with Drs. Nicholas and Kriegstein at UCSF, who

published one of the promising protocols for human MGE-like cell generation. For the transplantation and behavior experiments, we are using the same methods and line of mice, aged apoE4-KI female mice, described earlier. The pilot results (not shown here) have been encouraging in that the grafted human MGE-like cells can clearly survive at least 6 months after transplantation with only limited immune suppression, but it is yet to be seen if the grafted cells have significant effects on physiology or learning and memory behavior.

Beyond function in mouse models, the road to the clinic is clearly a lengthy one and undoubtedly filled with yet unconsidered hurdles. Eventually, good manufacturing practices will need to be applied in creating cells intended to be transplanted for clinical use. It would also be advantageous to advance genome editing technology to manipulate the cells *in vitro*, for instance with tools such as zinc fingers, TALENs, or CRISPR/Cas9s (Gaj et al., 2013). All grafted cells would ideally be transgenically equipped with a molecular “kill switch” that could be easily activated in the event of adverse effects. Ideally, when more reliable and efficient protocols are developed, therapies would involve patient-specific, isogenic genome-modified cells. Interestingly however, a transplant case of fetal midbrain dopaminergic cells survived in a Parkinson’s disease patient for over 14 years with only a six month-long daily regimen of cyclosporin A, showing that this limited immune suppression might be sufficient for prolonged survival (Mendez et al., 2005; Hallett et al., 2014). This is further supported by our preliminary observation of xenografted human ESC-derived MGE-like cells into the brains of immunocompetent adult mice. Nevertheless, unless patient-derived cells can be used for each case, approaches to enhancing donor cell and patient compatibility and immune

suppression to prevent rejection of transplanted cells would be needed for future stem cell-based therapies in AD.

Furthermore, because AD can be a relatively slow-progressing disease, clinical trials will likely take many years to demonstrate success for cell therapies in halting or reversing disease progression. The safe and ethical future of stem cell therapies, especially for AD, will likely be slow, expensive, and tightly controlled (Dunnett and Rosser, 2014). However, due to the uniqueness of stem cell-based therapies, the adaptation of existing regulatory standards and policies will likely be needed to develop new regulatory policies to foster their appropriate development and success.

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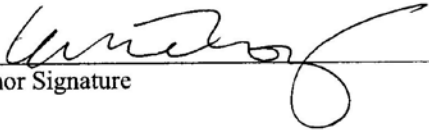


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