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Human Apolipoprotein E Genotype Differentially Affects Olfactory Behavior and Sensory Physiology in Mice

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Abstract—Apolipoprotein E (ApoE) is an important lipid carrier in both the periphery and the brain. The ApoE ε4 allele (ApoE4) is the single most important genetic risk-factor for Alzheimer's disease (AD) while the ε2 allele (ApoE2) is associated with a lower risk of AD-related neurodegeneration compared to the most common variant, ε3 (ApoE3). ApoE genotype affects a variety of neural circuits; however, the olfactory system appears to provide early biomarkers of ApoE genotype effects. Here, we directly compared olfactory behavior and olfactory system physiology across all three ApoE genotypes in 6-month- and 12-month-old mice with targeted replacement for the human ApoE2, ApoE3, or ApoE4 genes. Odor investigation and habituation were assessed, along with, olfactory bulb and piriform cortical local field potential activity. The results demonstrate that while initial odor investigation was unaffected by ApoE genotype, odor habituation was impaired in E4 relative to E2 mice, with E3 mice intermediate in function. There was also significant deterioration of odor habituation from 6 to 12 months of age regardless of the ApoE genotype. Olfactory system excitability and odor responsiveness were similarly determined by ApoE genotype, with an ApoE4 > ApoE3 > ApoE2 excitability ranking. Although motivated behavior is influenced by many processes, hyper-excitability of ApoE4 mice may contribute to impaired odor habituation, while hypo-excitability of ApoE2 mice may contribute to its protective effects. Given that these ApoE mice do not have AD pathology, our results demonstrate how ApoE affects the olfactory system at early stages, prior to the development of AD.

Key words: olfaction, apolipoprotein E, piriform cortex, olfactory bulb, Alzheimer's disease, odor habituation.

INTRODUCTION

Apolipoprotein E (ApoE) is the primary carrier of cholesterol within the brain, and ApoE genotype is an important determinant of an individual's risk for developing Alzheimer's disease (AD) (Corder et al., 1993; Farrer et al., 1997; Bu, 2009; Liu et al., 2013). Three alleles of ApoE occur in humans: ε2 (cys112, cys158; ~6% of the ApoE alleles in the population), ε3 (cys112, arg158; the most abundant allele at ~80%), and ε4 (arg112, arg158; ~14%) (Mahley, 1988; Mahley and Rall, 2000). ApoE4, in a dose-dependent manner, is the single most important genetic risk-factor for AD (Corder et al., 1993; Farrer et al., 1997; Bu, 2009; Liu et al., 2013). ApoE3 is viewed as the neutral allele in terms of neurodegenerative risk (Corder et al., 1993; Mahley and Rall, 2000; Liu et al., 2013) (and most closely resembles murine ApoE (Raffai et al., 2001)). ApoE2 is associated with a lower risk of AD-related neurodegeneration (Corder et al., 1994; Liu et al., 2013), delayed age of onset of AD, and a greater likelihood of survival to advanced age compared to the ApoE3 and ApoE4 alleles [reviewed in (Suri et al., 2013)]. In addition to AD, there is

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Abbreviations: AD, Alzheimer's disease; aPCX, anterior piriform cortex; ApoE, apolipoprotein E; FFT, Fast-Fourier Transform; LFP, local field potential; NKI, The Nathan S. Kline Institute for Psychiatric Research; OB, olfactory bulb; PCX, piriform cortex.

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increasing evidence that ApoE is involved in several other disorders, with ApoE4 often exerting a deleterious and ApoE2 a protective effect; while not all studies have found a difference between ApoE2 and ApoE3 carriers [reviewed in (Suri et al., 2013)], both ApoE4 and ApoE2 appear to be associated with hemorrhagic and ischemic cerebrovascular disease [reviewed in (Liu et al., 2013; Suri et al., 2013; Lopez et al., 2014)], with a high risk of argyrophilic grain disease and frontotemporal dementia [reviewed in (Suri et al., 2013)]. ApoE2 also appears to confer an increased incidence and severity of posttraumatic stress disorder (PTSD) (Freeman et al., 2005; Kim et al., 2013; Johnson et al., 2015). While the effects of ApoE4 have been extensively studied, the inconsistencies among a small number of studies regarding the effect of ApoE2 may in part be due to challenges related to its low frequency allele.

While ApoE genotype may affect a myriad of neural circuits and functions, the olfactory system and olfactory perception appear to be a unique and early biomarker of ApoE genotype. In humans, ApoE4 carriers show early emergence of olfactory dysfunction (Price et al., 1991; Bacon et al., 1998; Mesholam et al., 1998; Murphy et al., 1998; Graves et al., 1999; Gilbert and Murphy, 2004; Josefsson et al., 2017; Peng et al., 2017), show impaired odor identification (Murphy et al., 1998; Olofsson et al., 2010, 2016), and modified olfactory related evoked potentials (Kowalewski and Murphy, 2012; Morgan and Murphy, 2012) prior to other forms of cognitive impairment, including AD impairment associated with amyloid β and tau pathology. In mice, ApoE4 impairs short-term odor memory and induces olfactory system hyperexcitability including in the olfactory bulb (OB) and piriform cortex (PCX) (Peng et al., 2017) as well as the lateral entorhinal cortex (Nuriel et al., 2017) The olfactory system is suitable for assessing links between cell biology, circuit function and behavior given its relatively simple circuitry and the availability of reliable, robust behavioral assays in both humans and non-human animals. ApoE is important for neuroregeneration of the rodent olfactory system (Nathan et al., 2005), and ApoE knock-out mice show impaired olfactory detection (Nathan et al., 2004). This olfactory dysfunction is associated with neurophysiological (Mesholam et al., 1998; Corby et al., 2012; Kowalewski and Murphy, 2012; Peng et al., 2017), structural (Tanaka et al., 1998; Hashimoto et al., 2001) and cellular changes (Tsuboi et al., 2003; Nathan et al., 2005; Hussain et al., 2013) in olfactory regions of the brain. Understanding how ApoE isoforms may influence olfactory system function and perception would provide important insights into ApoE4 as a major risk factor for olfactory and cognitive decline.

Here, we directly compared olfactory behavior and olfactory system physiology across all three ApoE genotypes in mice that are homozygous for human ApoE2, ApoE3, or ApoE4. We hypothesized a genotype-dependent gradient in odor habituation and olfactory system excitability, such that, for example, ApoE4 mice would display hyper-excitability and impaired behavioral habituation, and ApoE2 mice hypo-excitability and increased behavioral habituation, relative to ApoE3 mice. The results were in line with our hypotheses and they extend previous work (Peng et al., 2017).

EXPERIMENTAL PROCEDURES

Study approval

All animal procedures were performed in accordance with the Nathan S. Kline Institute (NKI) for Psychiatric Research Institutional Animal Care Committee's approval.

Mice

Mice used in this experiment were homozygous for human ApoE2, ApoE3, and ApoE4 genes on a C57BL/6 background which are from long-standing colonies at NKI. These targeted-replacement mice express human ApoE under the control of the endogenous murine promoter (Sullivan et al., 1997), which allows for the expression of human ApoE at physiologically regulated levels in the same temporal and spatial pattern as endogenous murine ApoE. The native mouse ApoE protein has structural similarities to human ApoE4, but functional similarities to human ApoE3 (Raffai et al., 2001). Thus, a direct comparison between human and mouse ApoE genotypes would be, at best, difficult to interpret. A total of 40 mice were used for the habituation component of this study (6-month, ApoE2 = 9; 6-month, ApoE3 = 6; 6-month, ApoE4 = 6; 12-month, ApoE2 = 6, 12-month ApoE3 = 9; 12-month, ApoE4 = 4). A total of 46 mice were used for the electrophysiology component of this study (6-month ApoE2 = 6; 6-month ApoE3 = 13; 6-month ApoE4 = 10; 12-month ApoE2 = 6, 12-month ApoE3 = 6; 12-month, ApoE4 = 5).

Odor habituation

To investigate for simple behavioral odor memory deficits, mice were screened using an odor habituation test. Prior to behavioral assessment (24–48 h), mice were single-housed in new home cages with fresh corncob bedding. Test odors (2-heptanone, isoamyl acetate, (+) enantiomer of limonene, and ethyl valerate; Sigma Aldrich, St. Louis, MO, USA) were diluted in mineral oil to a concentration of 100 ppm. The dilution was then applied to a cotton-tipped applicator that was subsequently enclosed in a piece of odorless plastic tubing. The tubing allowed for volatile odor delivery while preventing the liquid from coming in direct contact with either the chamber or animal. Each trial consisted of a 20-s odor presentation (inserting the applicator into a port on the side of the animal's home cage) followed by a 30-s intertrial interval with four presentations of a single odor in each block. Each odor was presented four times and odor sequence was the same for all mice. The duration of time spent investigating, defined as snout-oriented sniffing within 1 cm of the odor presentation port, was recorded by a single observer blind to animal genotype. Data were averaged across odors to provide a single odor habituation curve for each mouse. All testing took place during the light

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