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APOLIPOPROTEIN E4 CAUSES EARLY OLFACTORY NETWORK ABNORMALITIES AND SHORT-TERM OLFACTORY MEMORY IMPAIRMENTS

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Abstract—While apolipoprotein (Apo) E4 is linked to increased incidence of Alzheimer's disease (AD), there is growing evidence that it plays a role in functional brain irregularities that are independent of AD pathology. However, ApoE4-driven functional differences within olfactory processing regions have yet to be examined. Utilizing knock-in mice humanized to ApoE4 versus the more common ApoE3, we examined a simple olfactory perceptual memory that relies on the transfer of information from the olfactory bulb (OB) to the piriform cortex (PCX), the primary cortical region involved in higher order olfaction. In addition, we have recorded *in vivo* resting and odor-evoked local field potentials (LPF) from both brain regions and measured corresponding odor response magnitudes in anesthetized young (6-month-old) and middle-aged (12-month-old) ApoE mice. Young ApoE4 compared to ApoE3 mice exhibited a behavioral olfactory deficit coinciding with hyperactive odor-evoked response magnitudes within the OB that were not observed in older ApoE4 mice. Meanwhile, middle-aged ApoE4 compared to ApoE3 mice exhibited heightened response magnitudes in the PCX without a corresponding olfactory deficit, suggesting a shift with aging in ApoE4-driven effects from OB to PCX. Interestingly, the increased ApoE4-specific response in the PCX at middle-age was primarily due to a dampening of baseline spontaneous activity rather than an increase in evoked response power. Our findings indicate that early ApoE4-driven olfactory memory impairments and OB network abnormalities may be a precursor to later network dysfunction in the PCX, a region that not only is targeted early in AD, but may be selectively vulnerable to ApoE4 genotype.

Key words: apolipoprotein E, olfaction, local field potential, olfactory bulb, piriform cortex, memory deficits.

INTRODUCTION

The human APOE gene exists as three alleles – ε2, ε3, and ε4 – which have a worldwide prevalence of 10%, 70%, and 20% respectively (Mahley et al., 2006). Compared to the more common apolipoprotein ε3 allele (ApoE3), the apolipoprotein ε4 allele (ApoE4) is strongly associated with several adverse clinical outcomes, including cardiovascular disease and Alzheimer's disease (AD) (Farrer, 1997; Mahley et al., 2006; Mahley, 2016). Additionally, ApoE4 has been shown to influence cognitive deficits that arise prior to detectable AD pathology and in healthy older adults (Small et al., 2004; Caselli et al., 2009; Wisdom et al., 2011), suggesting that ApoE4 expression adversely impacts brain pathways independently of AD pathology. In fact, a growing body of neuroimaging research has reported functional differences in human ApoE4 carriers versus noncarriers prior to the onset of cognitive decline within diverse brain regions (such as in hippocampi and specific regions of cortex) (Small et al., 2000; Reiman et al., 2004; Bookheimer and Burggren, 2009; Filippini et al., 2009; Dennis et al., 2010; Sheline et al., 2010; Brown et al., 2011). However, ApoE4-driven functional differences within regions that might specifically explain olfactory dysfunction have largely been ignored.

Prior to broad cognitive decline, human ApoE4 carriers exhibit odor identification impairments (Graves et al., 1999; Murphy, 1999; Wilson et al., 2007; Schubert et al., 2008; Olofsson et al., 2016) that may precede later cognitive deficits (Graves et al., 1999; Wilson et al., 2007; Schubert et al., 2008). Normal olfactory activ-

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Abbreviations: AD, Alzheimer's disease; ANOVA, analysis of variance; ApoE, apolipoprotein E; ApoE3, apolipoprotein E3; ApoE4, apolipoprotein E4; FFT, Fast-Fourier transform; LPF, local field potentials; OB, olfactory bulb; OERP, olfactory event-related potential; PCX, piriform cortex.

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ity relies on proper functioning within the olfactory network, the neural mechanisms of which are increasingly well established (Wilson, 2009): central odor processing initiates within the olfactory bulb (OB), which then transmits information directly to the piriform cortex (PCX), a region that is crucial for procedures involved in odor habituation and olfactory learning (Barnes et al., 2008; Wilson and Linster, 2008; Wilson, 2009; Gottfried, 2010). Olfactory information ultimately enters the entorhinal cortex and finally the hippocampus – a site of memory storage and retrieval (Staubli et al., 1984; Sosulski et al., 2011; Kay, 2014). Olfactory event-related potential (OERP) recordings during olfactory memory tasks in humans have demonstrated differences in peak latencies and amplitudes that distinguish ApoE4 carriers from non-carriers (Corby et al., 2012; Green et al., 2013). However, interpreting the relationship between brain activity and OERP signals measured on the scalp remains challenging (He et al., 2011). Electrode recordings of local field potentials (LFPs) in animal models allow more direct assessment of local activity in circuits directly involved in specific behaviors (Buzsaki et al., 2012). Additionally, odor-evoked LFP oscillations of the rodent olfactory system in the frequencies of 15–40 Hz (beta band; reflects long-range communication) and 40–80 Hz (gamma band; may reflect more local processing) have been shown to be especially indicative of behaviorally relevant odor processing (Neville and Haberly, 2003; Wesson et al., 2011; Kay, 2014; Martin and Ravel, 2014; Sadrian et al., 2014) and are sensitive to neuropathology related to aging (Wesson et al., 2011; Xu et al., 2015). Using LFP recordings from mice that express human ApoE, we have demonstrated within distinct olfactory regions that both ApoE4 genotype and aging play a factor in influencing the signal-to-noise ratio of an odor-evoked response, which may contribute to olfactory memory impairment.

EXPERIMENTAL PROCEDURES

Study approval

All experimental procedures involving animals in this study were approved by and complied with the guidelines of the Institutional Animal Care and Use Committee of the Nathan Kline Institute.

Mice

The mice in this study were purchased from Taconic farms (Germantown, NY, USA) and were homozygous for ApoE4 and ApoE3 on a C57BL/6 background. These targeted-replacement mice were developed to 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. Mice were investigated at 6 or 12 months of age and both sexes were examined. Separate cohorts of mice were tested for odor habituation and physiology.

Odor habituation

For odor habituation testing (done as described previously in detail (Wesson et al., 2010)), monomolecular odors (2-heptanone, isoamyl acetate, (+) enantiomer of limonene, ethyl valerate; Sigma Aldrich, St. Louis, MO, USA) were diluted in mineral oil to a concentration of 100 ppm based on vapor pressure and applied to a cotton-applicator stick which was then enclosed in a piece of odorless plastic tubing to prevent contact of the liquid odor with the testing chamber or animal, yet allowing for volatile odor delivery. In the listed order, each odor was delivered over 4 trials of 20 s each separated by a 30-s interval, by inserting the odor stick into a port on the side of the animal's home cage. 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 blinded to animal genotype. Home cages were cleaned with fresh corn-cob bedding added 24 h prior to behavioral testing, and the food bin and water bottle were removed from cages immediately prior to testing.

In vivo electrophysiology

Mice were anesthetized with urethane (1.25 g/kg, i.p.), and positioned in a stereotaxic apparatus for *in vivo* LFP recordings. The stereotaxic frame was outfitted with a water-filled heating pad to maintain core body temperature (37 °C). Skin was removed to expose the dorsal skull, and 1.5-mm diameter ipsilateral holes were drilled over the anterior PCX and the OB (as previously described (Wesson et al., 2011)). Monopolar tungsten recording electrodes were lowered into the PCX and into the OB for data acquisition. Proper electrode placement in the PCX was confirmed by appropriate evoked responses following direct stimulation in the OB.

To assess odor-evoked LFPs, odors were presented to anesthetized mice using a flow-dilution olfactometer positioned 2 cm from the animal's nose. Odor vapor was introduced with a computer-controlled pinch valve at a rate of 0.1 liter per minute to a constant 1 liter per minute flow of nitrogen gas, as we have done previously (Wesson et al., 2011; Xu et al., 2015). Stimuli included three monomolecular odorants (mesital oxide, ethyl valerate, and isoamyl acetate; Sigma Aldrich, St. Louis, MO, USA). Stimuli were introduced for 2 s per trial with at least a 30-s inter-stimulus interval. Each odor was presented for four trials. These odors were chosen based on previous experience to evoke strong oscillatory responses in the mouse olfactory system and to overlap with those used in the behavioral tests.

Data and statistical analyses

For the analysis of behavior data, all raw investigatory values (in s) were organized within animals according to odor presentation number (trial number; as described previously (Wesson et al., 2011)). As a measure of odor habituation, raw investigatory values (trials 1–4) were pooled within each group and analyzed following normalization to the initial investigation duration/animal for each odor (trial 1). The initial investigation duration was

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