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Functional consequences of hippocampal neuronal ectopia in the apolipoprotein E receptor-2 knockout mouse

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ABSTRACT

Little is known about the impact ectopically located neurons have on the functional connectivity of local circuits. The ApoER2 knockout mouse has subtle cytoarchitectural disruptions, altered prepulse inhibition, and memory abnormalities. We evaluated this mouse mutant as a model to study the role ectopic neurons play in the manifestation of symptoms associated with brain diseases. We found that ectopic CA1 pyramidal and inhibitory neurons in the ApoER2 knockout hippocampus are organized into two distinct stratum pyramidale layers. *In vitro* analyses found that ApoER2 is not required for neurons to reach maturity in regard to dendritic arborization and synaptic structure density, and electrophysiological testing determined that neurons in both strata pyramidale are integrated into the hippocampal network. However, the presence of these two layers alters the spatiotemporal pattern of hippocampal activity, which may explain why ApoER2 knockout mice have selective cognitive dysfunctions that are revealed only under challenging conditions.
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Introduction

Several human brain diseases, such as schizophrenia, autism spectrum disorders, lissencephaly, and dyslexia are associated with altered synaptic connectivity (Mirnics et al., 2001; Selkoe, 2002). Although diseases of brain connectivity are generally associated with abnormalities in cytoarchitecture (Ayala et al., 2007), little is known about the role ectopically located neurons play in the etiology of altered connectivity. Thus, animal models with defined cytoarchitectural abnormalities are needed to study the functional consequences of neuronal ectopia on circuitry.

Transgenic and spontaneously occurring mutant mice have been extremely valuable for elucidating the roles of different proteins in neuronal development. For example, the naturally occurring mutant mouse *reeler* was used to show that a deficiency in the expression of the large extracellular matrix protein Reelin results in developmental deviations in neuronal positioning and circuitry formation in laminated brain regions (e.g. cortex and hippocampus) that are reminiscent of those hypothesized to occur in humans with connectivity disorders, such as schizophrenia and autism (D'Arcangelo et al., 1995; Fatemi et al., 2001; Howell et al., 1997; Ogawa et al., 1995; Sheldon et al., 1997). Unfortunately, similar to the functional deletion of other proteins essential for neuronal development, deletion of Reelin results in severe neuronal cytoarchitectural changes making *reeler* an unrealistic model system to study altered brain connectivity. However, subtle disruption in neuronal cytoarchitecture can be achieved by functional attenuation of essential proteins, heterozygosis of essential genes, or by knocking out genes that play minor roles in neuronal development.

An animal model in which subtle cytoarchitectural disruptions occur is the apolipoprotein E receptor-2 (ApoER2) knockout mouse. ApoER2 is a receptor for Reelin, and the transmission of the Reelin signal via this receptor and/or the very-low-density lipoprotein receptor (VLDLR) to the cytoplasmic adaptor protein disabled-1 (Dab1) during neuronal migration is required for the establishment of normal brain architecture (Sheldon et al., 1997; Trommsdorff et al., 1999). Although brain development in ApoER2 and VLDLR double receptor knockout mice is indistinguishable from that found in the *reeler* mouse (Trommsdorff et al., 1999), in ApoER2 knockout mice ectopic neurons are found mostly in the hippocampus, while in VLDLR mutants the neuroarchitecture of the cerebellum is mostly altered (Trommsdorff et al., 1999). Thus, the distinct neuroanatomical phenotypes associated with the individual receptor knockouts suggest that their functions and/or brain expression patterns only partially overlap.

ApoER2 knockout mice have been recently found to have deficits in certain aspects of prepulse inhibition that mirror those in psychotic disorders (Barr et al., 2007a). In addition, they have complex deficits in memory (Barr et al., 2007b; Dowell et al., 2004; Weeber et al., 2002)

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and deficits in CA1 hippocampal long-term potentiation (LTP) (Weeber et al., 2002). These findings taken together with those reporting the loose packing of neurons in CA1 of the ApoER2 knockout pyramidal layer (Trommsdorff et al., 1999) suggest that the hippocampus of the ApoER2 knockout mouse is a good candidate model system for studying the functional consequences of neuronal ectopia on local circuitry.

To assess local circuitry in ApoER2 knockout mice we have performed a thorough electrophysiological assessment of its hippocampal CA1. Our results show that ectopic neurons integrate into the hippocampal network, altering the temporal and spatial tone of inhibition. Our model suggests that these changes may contribute to the behavioral deficits these animals have in sensory motor gating and memory.

Materials and methods

Antibodies and immunostaining

Mouse anti-parvalbumin (1:1000), rabbit anti-calretinin (1:1000), mouse anti-synaptophysin (1 μg/ml) and rabbit anti-MAP2 (1:1000) antibodies were purchased from Millipore (Billerica, MA). The rabbit anti-Dab1 antibody was kindly provided by Dr. Jonathan Cooper (Fred Hutchinson Cancer Research Center, Seattle, WA). Primary and secondary antibodies were incubated for 45 min (dissociated cultures) or overnight (tissue sections). Species specific secondary antibodies conjugated to Alexa Fluor fluorophores were purchased from Invitrogen. NeuroTrace fluorescent Nissl stain and fluorescently conjugated phalloidin were purchased from Invitrogen (Carlsbad, CA) and used as per the manufacturer's recommendations. Tissue sections, tissue slices, and dissociated cultures were mounted using Fluormount-G purchased from Electron Microscopy Sciences (Hatfield, PA).

Animals

ApoER2 knockout (B6;129S-Lrp8^{tm1Her}), VLDLR knockout (B6;129S7-Vldlr^{tm1Her}), *reeler* mice (B6C3Fe *ala-Reln*^{rl}/+), and wild type (wt; B6129SF2/J) mice were obtained from The Jackson Laboratory (JAX; Bar Harbor, ME). For all studies, littermates of ApoER2 het/VLDLR het breeders were the source of ApoER2 knockout, ApoER2 knockout/VLDLR knockout, and wt mice. In addition, mice of the same genotype used in the histological and electrophysiology experiments were from different litters. Colony maintenance was performed using dietary information provided by JAX and when necessary, wt mice of the same background (also from JAX). Genotyping was performed by PCR using protocols provided by JAX. Animal care was in accordance with institutional and NIH guidelines.

Dissociated cultures

Neurons were cultured at low density from embryonic day (E) 15 control and mutant mice as previously described (Aridor et al., 2004; MacLaurin et al., 2007). Briefly, hippocampal neurons were plated onto poly-L-lysine-coated glass coverslips that are inverted over a monolayer of glial cells after 2 h incubation. Cells were plated at a density of 2700 cells/cm^2 to achieve low-density cultures, which were required in order to accurately measure individual dendritic branch lengths. Importantly, several precautions were taken to ensure that we had highly pure cultures of hippocampal pyramidal neurons for our study. Neurons were isolated from E15 embryos to decrease the possibility of contaminating cultures with dentate granule cells. Paraffin dots attached to the coverslips were used to keep the neurons separated from the cells making up the glial feeder. Furthermore, since the quality of the glial feeder layer, which supplies neurotrophic substances, determines how well the neurons differentiate, the feeder layer used in all experiments was generated at least one week prior to the day of the experiment from the cortices of P1 wt pups. Using glial cells derived from wt animals assured that neurons in all experiments developed in a similar environment. Most neurons, >90%, developed the characteristic mature morphology of spiny neurons between 16–21 days *in vitro* (DIV). As a measure of culture maturity in experiments using 20 DIV neurons we confirmed that the majority of the cells in the wt culture had a relatively high density of dendritic spines (0.5–1.5 puncta/μm of dendrite) using rhodamine-phalloidin.

Microscopy

Images were collected on an Olympus FV500 confocal microscope or an Olympus IX-70 microscope (Olympus America Inc., Melville, NY) equipped with a Hamamatsu C4742-98 CCD camera (Hamamatsu Corporation, Bridgewater, NJ) and a Ludl motorized XYZ stage (LEP Ltd., Hawthorne, NY). Data was deconvolved using the Agard/Sadat inverse matrix algorithm.

To quantify dendritic characteristics 5 sequential confocal slices taken 0.1 μm apart through the midplane of the dendrites were collected with fixed laser illumination and pinhole size using a 60× 1.4 NA plan apochromat objective or a 40× 1.3 plan fluorite objective on an Olympus FV500 confocal. Using SlideBook 4.1 Imaging software (Intelligent Imaging Innovations, Inc; Denver, CO) three-dimensional images were constructed of each dendrite. The reconstructed images were then delineated and the threshold set. The number, size, and signal intensity were then measured. No significant difference was detectable in the grayscale values for each voxel within the area of interest in any of the cells or any of the conditions. Using the program ImageJ with the semiautomatic neurite tracing plugin NeuronJ (Meijering et al., 2004) we measured dendritic length based on threshold segmentation. Using these experimental parameters, the dendrites from a minimum of 21 neurons from at least three different experiments per condition were measured.

Electrophysiology

P80–P120 mice were used for electrophysiology experiments. Our detailed methods described previously (Krucker et al., 2002; Krucker et al., 1998) were slightly modified. Briefly, mice were anesthetized with 3% halothane and decapitated. Brains were rapidly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 130; KCl, 3.5; KH2PO4, 1.25; MgSO4, 1.5; CaCl2, 2.0; NaHCO3 24 and glucose 10; with a pH of 7.4 and saturated with carbogen (95% O2+5% CO2). We dissected both hippocampi and cut transverse slices (400 μm thick) using a McIlwain tissue chopper. All experiments were done at 32±0.2 °C in a submerged-type slice chamber. Stimulation was delivered as indicated by square current pulses of 0.05 ms duration at 0.02 Hz through an isolated tungsten bipolar monocentric electrode. We recorded compound field potentials with glass electrodes (tip resistance 1–4 MΩ) filled with 3 M NaCl and positioned either two electrodes simultaneously in cell layers or made consecutive recordings moving one electrode along a parallel line to the dendrosomatic axis of the stratum pyramidale. The evoked responses were recorded, amplified (Axoclamp-2A or -2B, Axon Instruments, Foster City, CA), digitized at a sampling rate of 10 kHz, and stored on a PC using pClamp software (Axon Instruments, Foster City, CA). We measured the pEPSP amplitude as the *y*-axis difference between its most negative and/or positive peak and the baseline value 1 ms preceding the stimulation; the slope was calculated as the initial negative linear segment of the trace using least-squares regression analyses. We calculated PS amplitude as the difference between two positive and the most negative peaks of the trace. We evoked PPF by delivering two stimuli of the same intensity separated by inter-pulse intervals (IPIs) as indicated. The ratio of the slopes of fEPSP2/fEPSP1 was an index of PPF. For PPI we evoked maximal somatic PS amplitude

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