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PREFERENTIAL REDUCTION OF SYNAPTIC EFFICACY IN THE DENTATE GYRUS OF HIPPOCAMPAL SLICES FROM AGED RATS DURING REDUCED GLUCOSE AVAILABILITY

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Abstract—Glutamatergic synaptic activity entails a high energetic cost. During aging, a variety of neural metabolic changes have been reported that could compromise the capacity of neural circuits to maintain synaptic transmission during periods of reduced extracellular glucose. Indeed, a preferential compromise in evoked synaptic activity has been observed in hippocampal CA1 with age during exposure to low-glucose solutions. Whether this aging-related compromise in synaptic activity is regionally specific is unclear, however. Data suggest that the dentate gyrus (DG) preferentially exhibits hypometabolism with age and this region plays a critical role in spatial pattern separation, which is compromised with age. Therefore, we assessed whether synaptic activity is also preferentially affected in the DG with age. *In vitro* extracellular field potential recordings were used to monitor orthodromic and antidromic evoked activity in the DG granule cell layer in hippocampal slices from adult (8–12 months) and aged (22–27 months) rats in aCSF containing 10 mM glucose, followed by a reduced glucose aCSF containing 1 mM glucose. In 10 mM glucose-aCSF, orthodromic- and antidromic-evoked field potential activity was comparable between age groups. However, orthodromic-evoked population spike amplitude and field excitatory post-synaptic potential (EPSP) slope were preferentially decreased in slices from aged rats during exposure to 1 mM glucose-aCSF. Antidromic population spike amplitude was not differentially affected in slices from aged versus adult rats, however. These data suggest that synaptic efficacy is preferentially compromised with age under reduced glucose availability and, combined with a decreased capacity of the periphery to provide glucose to the central nervous system (CNS) during metabolically challenging conditions, could contribute to aging-related hippocampal dysfunction and cognitive decline.

Key words: aging, metabolism, glucose, synaptic transmission, dentate gyrus, *in vitro*.

INTRODUCTION

Central nervous system (CNS) function depends upon the efficacy and plasticity of ionotropic glutamatergic and GABAergic synaptic neurotransmission, which entails a high energetic demand (Ames, 2000; Attwell and Laughlin, 2001; Lennie, 2003). Glucose, the predominant energy source of the brain, and its associated metabolites are either directly or indirectly required for the synthesis (i.e., glutamate-glutamine cycling), vesicular packaging, synaptic release, post-synaptic receptor affect, and reuptake of glutamate and GABA, as well as the maintenance of ionic gradients (Hoyer 1990; Schousboe et al., 2007; Boumezbeur et al., 2010; Howarth et al., 2012; Harris et al., 2012; Khatri and Man, 2013).

Previous studies have demonstrated that synaptic activity and plasticity are compromised in the CNS *in vitro* during cellular energy deprivation, specifically upon reducing the extracellular glucose concentration from 10 mM, which is typically used *in vitro* (Yamamoto and McIlwain, 1966; An et al., 2008), to lower levels (Cox and Bachelard, 1982; Fan et al., 1988; Schurr et al., 1989; Shoji, 1992; Izumi et al., 1994; Izumi and Zorumski, 1997; Wada et al., 1998; Alici et al., 1998; Sadgrove et al., 2007; Galow et al., 2014). Further, two studies suggest that this occurs preferentially during aging (Tekkök et al., 1998; Galeffi et al., 2014). This observation is intriguing since a decrease in local cerebral glucose use (hypometabolism) in the hippocampus *in vivo* is correlated with cognitive decline in aged rats and humans (Gage et al., 1984; Small et al., 2004). Several potential mechanisms that could underlie this age-related decrease in glucose utilization include: (1) a decreased capacity of the periphery to provide sufficient blood glucose to the hippocampus during cognitive tasks due to a blunted peripheral epinephrine response (McNay and Gold, 2001; Gold, 2005), (2) a decrease in glucose transport across the blood–brain barrier due to alterations in glucose transporter type 1 expression levels

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Abbreviations: DG, dentate gyrus; EC, entorhinal cortex; EPSP, excitatory post-synaptic potential; GCL, granule cell layer.

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(Mooradian et al., 1991; Vorbrodt et al., 1999), (3) a decrease in vascularization within the aged brain that results in a reduction in local blood flow (Sonntag et al., 1997; Buée, 1999; Lynch et al., 1999; Wang et al., 2004), and/or (4) a decreased capacity of neural tissue itself to utilize available glucose (Galeffi et al., 2014). Regarding an aging-related compromise in neural metabolic capacity, several changes have been reported that could contribute to this including: (1) changes in both neuronal and glial mitochondrial function (Liu et al., 2002; Sandhu and Kaur, 2003; Boumezbeur et al., 2010), (2) a decrease in neuronal glucose transporters (Fattoretti et al., 2001,2002), (3) a change in glial/neuronal glutamate-glutamine cycling (Boumezbeur et al., 2010), (4) a change in NADH formation (Galeffi et al., 2014), and (5) a decrease in insulin-sensitivity (e.g., Cholerton et al., 2011; Ketterer et al., 2011), which can exert a variety of negative effects on downstream targets including the translocation of the insulin-sensitive glucose transporter four into the neuronal plasma membrane (Grillo et al., 2009).

To date, the two studies that have demonstrated a preferential aging-related compromise in synaptic activity were exclusively performed in hippocampal region CA1, and did not assess whether this aging-related change could be due, in part, to an altered capacity of neurons to fire action potentials. In this study, we examined whether a similar compromise in synaptic efficacy occurs in the dentate gyrus (DG) with age during reduced glucose availability (i.e., is this a regionally specific phenomenon?) and whether a decreased capacity of neuronal excitability could be involved (i.e., is the capacity of post-synaptic neurons to fire action potentials compromised?). The DG was selected for this study for several reasons. Foremost, 2-deoxyglucose and imaging data suggest that this region is preferentially hypometabolic with age (Gage et al., 1984; Small et al., 2004, respectively). This finding is intriguing given that the DG is critically involved with pattern separation (O'Reilly and McClelland, 1994; Rolls, 1996; Gilbert et al., 2001; Kesner, 2013a,b) and this process is compromised with age (Wilson et al., 2006; Holden and Gilbert, 2012). Secondly, dentate granule cells and hippocampal pyramidal neurons can exhibit differential responses to experimentally induced insults/manipulations (e.g., Freund et al., 1990,1992; Cavazos et al., 1994; Keyser and Pellmar, 1994; Wada et al., 1998; Gary et al., 2000; Tanaka et al., 2008; however also see Tasker et al., 1992). Finally, the DG plays a key role in regulating the spread of activity from extra hippocampal regions into the seizure-sensitive hippocampal network (Lothman et al., 1992; Behr et al., 1998; Jandová et al., 2006). Patrylo and colleagues have shown that the DG exhibits proconvulsant changes during aging (Patrylo et al., 2007; Patrylo and Williamson, 2007). Given the metabolic deficits that have been described previously in the CNS during aging and the potential role of the DG in several aging-related pathologies, this study investigated whether reducing the extracellular glucose concentration from 10 to 1 mM would differentially affect *in vitro* synaptic transmission in the DG of acute slices prepared from adult and aged rats. Since extracellular glucose can been precisely controlled in this reduced preparation and is independent of peripheral glucose supply and CNS vascularization, differential age effects of synaptic transmission can be attributed to direct deficits in astrocytic or neuronal glucose utilization.

EXPERIMENTAL PROCEDURES

Animals

Male Fischer 344 rats (adult: 8–12, and aged: 22–27 months old) were used in all experiments and were purchased from Harlan Laboratories (Indianapolis, IN, USA). Animals were housed in the Southern Illinois University Carbondale (SIUC) vivarium, kept on a 12-h/12-h light dark cycle, and provided with standard rat chow and water *ad libitum*. All experiments were approved by SIUC's Institutional Animal Care and Use Committee (IACUC) and comply with the guidelines set forth by the National Institutes of Health. Brains of animals that were found to have pituitary tumors upon sacrifice were excluded from the study.

Preparation and maintenance of hippocampal slices

Rats were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and then decapitated. Their brains were rapidly removed and placed in cold artificial cerebrospinal fluid (aCSF; 1–2 °C) for 1 min. The aCSF was composed of (in mM): 124 NaCl, 3 KCl, 2 $CaCl_2$, 26 $NaHCO_3$, 1.3 $MgSO_4$, 1.25 NaH_2PO_4, 10 glucose and equilibrated with 95/5% O_2/CO_2 (pH 7.2–7.4; 290–295 mOsm). Using a vibratome, 400-μm-thick horizontal slices were sectioned in ice-cold aCSF perpendicular to the longitudinal axis of the hippocampus and were micro-dissected for electrophysiological recording experiments. Slices were then placed in an interface recording chamber, perfused with aCSF (1.5–2 ml/min; 31–33 °C), humidified with 95/5% O_2/CO_2, and allowed to recover $\geqslant$90 min before recordings commenced.

In vitro electrophysiology

Recording electrodes were made with a Flaming/Brown puller (Sutter Instruments) and were filled with 1 M NaCl (resistance 4–10 MOhms). Electrodes were placed in the granule cell layer (GCL) of the DG and routinely positioned within 100 μm of the top surface of the slices. Since data suggest the perforant path afferent input into the DG from the medial vs. lateral entorhinal cortex (EC) is anatomically and physiologically distinguishable (Hjorth-Simonsen, 1972; Steward, 1976; Wyss, 1981; Abraham and McNaughton, 1984) and are believed to play different roles in hippocampal-dependent learning and memory (Biella and de Curtis, 2000; Ewell and Jones, 2010) the current experiments focused exclusively on afferent input from the lateral EC. To specifically target lateral perforant path inputs, stimulating electrodes, made of two twisted teflon-insulated platinum-iridium wires, were placed in the outer molecular layer of the DG and activation of the lateral perforant path was verified by paired-pulse facilitation in the GCL when a 50–75-ms