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Research report

Age-dependent changes in brain hydration and synaptic plasticity

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ABSTRACT

Aging in humans and animals is associated with gradual and variable changes in some cognitive functions, but what causes them and explains individual variations remains unclear. Hydration decreases with aging but whether dehydration contributes to cognitive dysfunction is not known. The brain hydration of aging mice was determined by colloidosmotic-pressure titration. Dehydration increased with age from ~76 mmHg at 6 weeks to ~105 mmHg at 40 weeks, or a progressive ~10 percent loss of brain water but seemed to level off afterward. When we adjusted dehydration in hippocampal slices of <8-week-old mice to the levels seen in mice 40 weeks and older, their basal synaptic responses were amplified at all stimulus voltages tested, but induction of late-phase long-term potentiation was impaired. Our results document progressive brain dehydration with age in inbred mice to levels at which *in vitro* synaptic plasticity appears dysregulated. They also suggest that dehydration contributes to some of the changes in synaptic plasticity observed with aging, possibly due to adjustments in neuronal excitation mechanisms.

1. Introduction

With age, some biological mechanisms thought to underlie cognition change at the cellular level; for example, dysregulation of excitation and of signaling pathways in hippocampal neurons have been documented (Hansen and Zhang, 2013; Oh et al., 2016; Whalley, 2001). At the same time, the proportion of body water (Hooper et al., 2014) and brain parenchyma volume decrease, but no mechanistic relationship has been established between these cognitive and hydration changes.

The human brain progressively shrinks. The ventricular volume increases, while certain areas, including the caudate, cerebellum, hippocampus, and the association cortices, show substantial, albeit localized, diminution, which could be due, in part, to a decrease in gray matter, perhaps from neuronal loss or shrinkage, reduced synaptic spines, or fewer synapses (Deary et al., 2009; Fjell and Walhovd, 2010; Kempton et al., 2011; Raz and Rodrigue, 2006; Walhovd et al., 2005). However, neuronal loss, in particular in the hippocampus and neocortex, is not found in healthy aging and cannot account for the reduced volume measured in these areas (Burke and Barnes, 2006; Rapp and Gallagher, 1996). Water loss from cells and the interstitial spaces might be a factor, but we do not know its precise contribution to brain volume.

Because many forces balance interstitial hydration, the volume and/or total weight of brain could change, at least in theory, without concomitant changes in water content and vice versa. Our earlier work developed the concept of *hydration potential* as a useful parameter to explore hydration changes in interstitial microenvironments (McGee et al., 2009, 2011, 2012, 2014). The present study measured it in the aging brain to document any age-related changes in hydration that might explain the previously documented volumetric reduction and the physiologically relevant ranges. We must first know the ranges typical for healthy aging brain to test whether physiologic ranges of brain dehydration influence neural plasticity.

Aging has been associated with dysregulated excitability in hippocampal pyramidal neurons mainly due to enhanced hyperpolarization (Foster and Kumar, 2002). In addition to changes in the intrinsic excitability of hippocampal neurons, a variety of differences between old and young brain function have been described (Burke and Barne, 2006). They include both low-level cognitive functions, such as attention, working memory, long-term memory, and perception, and high-level cognitive functions, such as speech and language, decision making, and executive control (Glisky, 2007; Whalley, 2001). Animal studies have associated aging with certain apparent deficits in consolidating short-term into long-term memory. Synaptic function in L-LTP and LTD (late-phase long-term potentiation and long term depression, respectively; *ex-vivo* models of neuronal plasticity) appear impaired in old compared to young rodents and contribute to cognitive changes (Disterhoft and Oh, 2006; Bach et al., 1999; Huang and Kandel,

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2006). LTP impairments and accelerated decay (Rosenzweig and Barnes, 2003, Rogers et al., 2017) as well as reduced synaptic strength, paired pulse facilitation, and spatial memory have also been reported (Weber et al., 2015).

This study tested the hypothesis that changes in hydration influence neuronal pathways. To determine the physiologic range of hydration we measured hydration potential in brain tissue from mice aged 6 to 60 weeks. To determine whether hydration influences neuronal function we modified the hydration potential of hippocampus slices from young inbred mice and measured the resultant changes in basal synaptic transmission and L-LTP induction.

2. Results

2.1. Water transfers from ACSF to brain interstitium

The *hydration potential* of mouse brain tissue was derived *ex vivo* from changes in the explants' weight. In all age groups, water flowed into the brain tissue when it was immersed in ACSF (artificial cerebrospinal fluid) without colloids. Influx rates were faster initially, approaching a maximum in approximately 30 min, and remaining stable for at least 1 h. Routinely, initial rates, *Ri*, were calculated from the first derivative of second-degree polynomials fitted to the progress curves. When the colloidosmotic pressure in ACSF was varied, R_i changed in proportion; $\Delta R_i = K \cdot \Delta P_i$ where ΔP_i is the change in fluid-driving pressure induced at each colloid concentration (Fig. 1A–D). In regression analyses, plots of the initial rate versus pressure were consistent with a linear model giving $r^2 > 0.8$.

The slope K of fitted lines, $\Delta R_i = K\ \Delta P_i$, with units of μl/min/mmHg/g, reflects the *hydraulic conductance* of the brain tissue. The ΔP_i in mmHg when $Ri = 0$ is the fluid driving pressure and numerically equals the *hydration potential* (Fig. 2A–F). This value depends on all the hydrating interactions when fluid-driving forces are balanced and reflects the competition for water in brain tissue. The higher the potential, the more dehydrated the tissue and the stronger the interstitial suction forces generated by the matrix.

In the 6-week-old animals used as a reference, the hydration potential was 75 ± 5 mmHg relative to ACSF, and the hydraulic conductance was 0.060 ± 0.007 μl/mmHg/min/g. The hydration potential increased with age up to 40 weeks (Fig. 2E summarizes mean values and statistical analyses). Compared to the 6-week-old mice, the change in hydration potential observed in 40- and 60-week-old mice corresponds to an approximately 10 percent decrease in relative water content (dehydration). Since the literature reports that the total brain weight of the C57BL/6 strain changes little with age, and the hippocampal weight decreases slightly only after 112 weeks (Lessard-Beaudoin et al., 2015), the dehydration we detected may be associated to relative increases in the weight of some other tissue component.

In contrast to these hydration potential changes, no significant changes in the hydraulic conductance were detected in aging mice. The hydraulic conductance, *K*, reflects the resistance to, and power for, fluid transfer across the brain extracellular matrix. Since it depends on matrix structure and cell mechanical activity, physiologic variations in its value are expected to be small under the conditions of these experiments. Statistical analyses comparing hydration parameters among all age groups tested are summarized in Fig. 2E and F.

The observed increase in hydration potential implies decreased water activity relative to ACSF and therefore increased activity of all hydrated molecules and surfaces in the brain tissue. It also indicates an increase in the interstitial suction that must be balanced in the intact brain by blood and lymphatic hydrostatic and colloidosmotic potentials.

2.2. Basal synaptic transmission increases in dehydrated brain tissue

Hydration levels in the brains of young mice were manipulated *ex vivo* by equilibration in baths at potentials set from Δ0 to Δ200 mmHg, which includes the physiologic 75–102 mmHg range found in mice 6–60 weeks old. After equilibration, the basal synaptic transmission strength increased with the osmotic pressure of the equilibrating bath, indicating that the level of hydration in the micro-environment influenced neuronal responses to a single stimulus. The fEPSP (field excitatory postsynaptic potentials) recorded in response to stimulating amplitudes from 10 to 35 V were higher in dehydrated slices than those at the physiologic hydration level; dehydration induced no apparent saturation. Fig. 3B shows the changes in fEPSP/voltage with changes in hydration, summarizing the results and statistics from linear regression analyses.

The changes in synaptic strength were further analyzed using the amplitudes and parameters derived from Eq. (2) (Section 4.4, Fig. 3C and D), including apparent stimulation threshold (i.e., the x-axis intercept), the A_{max}, and the B factor. While the apparent stimulation threshold did not change with hydration level, both A_{max} and B increased with dehydration. Fig. 3C illustrates the convergence of thresholds, and Fig. 3D, the nonlinear relationships between the increases in basal A_{max} and B with dehydration. Large changes in synaptic transmission strength are detected when hydration is below the physiologic levels of 6 weeks-old mice, but little change is noted when it is above.

Overall, the relationships between the hydration potential and either the A_{max} or B parameter are better described by exponential than linear equations, which suggests that the measured variations in neuronal responses depend on the a_w reached at equilibrium rather than the potentials driving water flow during equilibration (Eq. (1)). If that is the case, in the slices equilibrated at increasing levels above physiologic, the functional changes proportional to Δa_w are expected to become progressively smaller. Alternatively, sensing may be linear with the potential, but the observed nonlinear response reflects other intervening processes; for example, changes in the velocity of reactions with hydration/dehydration rate-limiting steps (McGee et al., 2002).

The increase in synaptic transmission was unrelated to the physicochemical characteristics of the polymer used to decrease water activity in the bath. The observed change was similar with dextran 10 or PEG, both adjusted to a colloidosmotic pressure of ~100 mmHg (Fig. 4A). These inert polymers are similar in size and molecular weight, but their very different structure and chemistry impart different properties in solution, including their viscosity. Furthermore, increases in basal synaptic transmission were fully reversible, excluding the possibility of permanently altered neuronal function, as shown in experiments where slices equilibrated at Δ100 mmHg colloidosmotic pressure returned to the expected basal levels after re-equilibration in the ACSF solution at Δ0 mmHg (Fig. 4B).

2.3. Dehydration inhibits induction of late-phase, long-term potentiation

Late-phase, long-term potentiation was induced in slices equilibrated and perfused with physiologic ACSF solution at 0 mmHg colloidosmotic pressure (Dong et al., 2008). After 30 min of stable baseline recording, when stimulated by 4 × 100 Hz trains spaced 5 min apart, L-LTP was induced and maintained for three hours. When the procedure was repeated in slices equilibrated within a 54–196 mmHg pressure range, L-LTP induction was significantly impaired at 100 and 196 mmHg compared to that in slices at 0 and 54 mmHg (0 mmHg: 159.80 ± 5.08%, 54 mmHg: 155 ± 8.96%, 101 mmHg: 112.50 ± 1.77%; $p < .001$, 196 mmHg: 97.55 ± 2.29%;